Blood biochemistry, histology, and metabolomic profiling of contaminated with deoxynivalenol: a repeated dose 28-day oral toxicity study in rats

Jin-Young Jeong, Min-Ji Kim, Seol-Hwa Park, Hye-Ran Kim, Hyun-Jung Jung
Animal Nutrition & Physiology Division, National Institute of Animal Science
e-mail:jeong73@korea.kr

데옥시니발레놀의 혈액 생화학, 조직학 및 대사체 분석: 쥐에 28일 동안 경구 투여 독성 연구

정진영, 김민지, 박설화, 김혜란, 정현정
국립축산과학원 동물영양생리과

Abstract

This study was conducted to estimate the effects of blood biochemistry, histology, metabolic profiles in rats fed with deoxynivalenol (DON). Histological changes, metabolomic profiling, and blood biochemical analysis were performed Masson's trichrome stain kit, liquid chromatography mass spectrometry, and VetTest chemistry analyzer, respectively. Animals were exposed to 0.9% saline and DON (0.02 and 0.2 mg/kg) for 28 days. The final body weight of rats did not differ between the control and DON-treated groups. However, biochemistry levels and fibrosis were significantly differentially expressed according to DON concentration. For metabolomic profiling, Data were analyzed using MetaboAnalyst 4.0. The DON-treated groups showed discriminating metabolites in the different concentrations. In conclusion, this finding suggest that DON exposure disturbs the histological changes and metabolites. Dietary DON in rats may better understand the physiological effects consistent with metabolism, which are commonly reported in animals exposed to DON.

Keywords: Mycotoxin, Deoxynivalenol, Rat, Metabolomic

1. Introduction

Mycotoxins, toxic secondary metabolites, produced by harmful fungi exert certain physiological effects on humans and animals [1]. Mycotoxins produced by *Fusarium* species are generally observed in food and forage [2]. The contamination of mycotoxins induces a health problem in many countries worldwide. Particularly, pigs are relatively more sensitive to mycotoxins than other organisms [3]. The representative trichothecene mycotoxin, deoxynivalenol (DON) causes vomiting, diarrhea, and body weight loss in pigs, while ruminants and poultry were observed to be less sensitive to mycotoxins [4, 5]. Feedstuffs were commonly contaminated by DON. Metabolomics is a biological systems with large-scale assessment to phenotype or environmental processes [6]. It would be interesting to determine the toxic effects in animal feed. Therefore, this study aimed to investigae the effects of mycotoxin and identify metabolome-related compounds in DON-treated rats.

2. Materials and Methods

2.1. Experimental design

Male Sprague-Dawley rats were purchased from Koatech Co. (Pyeongtaek, Korea) and were maintained in the animal facility at the National Institute of Animal Science. The animals were treated in accordance with standard guidelines for laboratory animal care. All rats were provided with normal chow and water *ad libitum*. Animals were acclimated at 22 ± 2°C for 1 week. The rats were assigned to control (n = 5) and DON-treated groups (T1 = 5, T2 = 5) for histology and metabolomic. DON was given orally at doses of 0.02 mg/kg and 0.2 mg/kg in 0.9% saline for 28 days. Control rats were

given 0.9% saline.

2.2. Histological, blood biochemical, and metabolomic profiling studies

Liver and kidney tissue samples were fixed in 10% neutral buffered (Sigma-Aldrich, St Louis, MO, USA) and were dehydrated and embedded with paraffine. The sections were stained using Masson's trichrome (MT) staining kit according to the manufacturer's protocol. All slides were assessed under the microscope at 200× magnification.

Blood biochemical tests were prepared and estimated as described previously [7]. All biochemical parameters (glucose, creatinine, blood urea nitrogen, phosphate, calcium, total protein, albumin globulin, etc.) were measured using a VetTest chemistry analyzer (IDEXX, USA).

Liver samples were analyzed by ultra-high performance LC-quadrupol time-of-flight (TOF)-MS (Waters Co. Milford, MA, USA). Both acquisition and peak integration were performed using Masslynx software version 4.1 (Waters Corporation).

2.3. Statistical analyses

The statistical significance of metabolite concentrations and blood biochemical compositions was evaluated by unpaired t-tests for DON-induced toxicity. For metabolic profiles, LC-MS data were processed using XCMS software. The binning results were imported into SIMCA 14.1 (Umetrics, Sweden). Variable importance in projection (VIP) plots were also used as potential indicators. Data analysis was performed using graphing software (e.g., Excel and GraphPad Prism 5).

3. Results

3.1. Blood biochemistry, histology, and multivariate analysis of liver metabolites

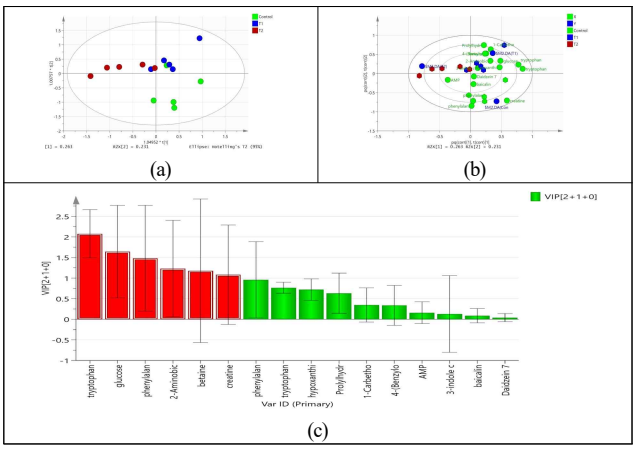
The effect of DON exposure on the biochemical properties of rat serum (Table 1). The biochemical parameters are as follows: glucose (GLU), creatine (CREA), BUN (blood urea nitrogen), blood urea nitrogen/creatinine ratio (BUN/CREA), PHOS (phosphate). CREA levels Particularly, CREA decreased significantly in the DON-treated groups compared with control ($P < 0.05$). No significant differences were detected in the other biochemical parameters (i.e., GLU, BUN, PHOS).

[Table 1] Changes of blood biochemical characteristics in rats fed with DON

Parameters	Control	T1[1]	T2[1]	SEM[2]	*P* value
GLU, mg/dL	211	166	184	13.4	ns
CREA, mg/dL	0.28	0.10	0.18	0.03	0.041
BUN, mg/dL	17.6	17.8	19.4	0.81	ns
BUN/CREA	83.2	178	122	16.7	ns
PHOS, mg/dL	13.1	11.6	11.2	0.46	ns

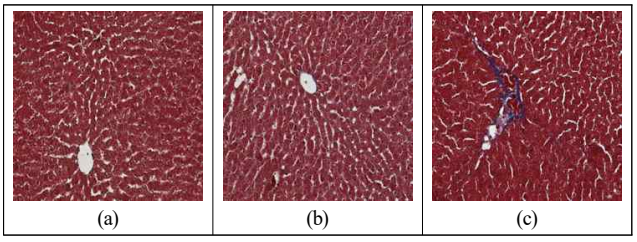
Values are mean. n = 15. [1]T1:0.02 mg/kg, T2:0.2 mg/kg. [2]SEM = standard error of the mean. ns = not significant. GLU, glucose; CREA, creatinine; BUN, blood urea nitrogen; BUN/CREA, blood urea nitrogen/creatinine ratio, PHOS, phosphate; All traits in the table were analyzed by one-way ANOVA with Tukey's multiple comparison test.

For metabolite identification, LC-MS-based metabolites were assigned to spectral peaks for the serum samples from the control and DON-treated piglets. Principal component analysis (PCA) scores and biplots were analyzed within the 95% Hotelling T2 ellipse (Fig. 1a, b). PCA score plots were developed for the three different tyreatment types, including control (0 mg/kg), T1 (0.02 mg/kg), and T2 (0.2 mg/kg) for 28 days. The PCA model characteristics were excellent: $R^2X = 0.92$; $Q^2 = 0.196$. The VIP values of these metabolites were set at > 1.0 (Fig. 1c). The liver is an important organ involved in glycogenolysis and detoxification.



[Fig. 1] Principal component analysis (PCA) of liver, serum, and urine of control (4 samples) and treated (5 samples) piglets. (a) Score plot. (b) Loading plot. $R^2X = 0.827$; $Q^2 = 0.7$. Six-week-old piglets were treated with 8 mg/kg DON for 28 days. Signals were compared after log transformation and Pareto scaling. (c) The variable importance in projection (VIP) plots from OPLS-DA showed that significant metabolites contributed to cluster separation in piglets

We further assessed the histological changes following DON-treated rat liver using MT stain kit (Fig. 2). DON groups were increased collagen fiber destruction and deteriorated fibrosis (especially, portal areas).



[Fig. 2] Histoloical cahanges of liver tissue in DON-treated rats using MT staining. Original magnification 200×.

4. Conclusion

LC-MS-based metabolomic profiling provides insights into the effects of diet-related discriminating metabolites compounds for DON-treated rats. DON exposure also regulated histological changes, fibrosis, and blood biochemical parameters. Therefore, this study suggest that rats fed with contaminated DON provide a better understanding the physiological effects consistent with immune system.

5. Acknowledgement

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