Metabolic Characteristics of Deoxynivalenol (DON) Postprint

Authors: Zhang Zhiqi, Wang Ruiguo, Zhang Wei, Peilong Wang, Su Xiaoou

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Abstract

Deoxynivalenol (DON) contamination of cereal-based food and feed represents a global issue that seriously jeopardizes human and animal health. The metabolic profile of DON in humans and animals constitutes the foundation for research on DON exposure assessment, mechanisms of toxic action, and intervention technologies. In recent years, investigations into the toxicokinetics of DON—including its absorption, distribution, metabolism, and excretion—have garnered extensive attention worldwide. This article presents a comprehensive review of recent advances in DON metabolic characteristics.

Full Text

Preamble

Metabolic Characteristics of Deoxynivalenol

ZHANG Zhiqi, WANG Ruiguo, ZHANG Wei, WANG Peilong, SU Xiaoou*
(Institute of Quality Standards and Testing Technology for Agricultural Products, Chinese Academy of Agricultural Sciences; Key Laboratory of Agrifood Safety and Quality, Ministry of Agriculture, Beijing 100081, China)

Abstract: Deoxynivalenol (DON) contamination of grain-based food and feed represents a global problem that seriously endangers human and animal health. Understanding the metabolic characteristics of DON in humans and animals is fundamental for research on exposure assessment, toxic mechanisms, and intervention technologies. In recent years, studies on the toxicokinetics of DON, including its absorption, distribution, metabolism, and excretion, have attracted widespread attention worldwide. This paper provides a comprehensive review of the latest research progress on DON metabolic characteristics.

Keywords: deoxynivalenol; toxicokinetics; metabolism

*Corresponding author: Professor SU Xiaoou, E-mail: suxiaoou@caas.cn

Deoxynivalenol (DON), also known as vomitoxin, is a secondary metabolite produced by *Fusarium graminearum* [1] that is ubiquitous in grain-based foods and feeds such as wheat, barley, oats, and corn. The contamination level of DON in grains is closely correlated with precipitation, humidity during flowering, and storage conditions [2]. DON is one of the most widely distributed and severely contaminating mycotoxins worldwide [3-10], posing a significant threat to feed and food safety.

DON can affect the activity and function of cells in the intestine, immune system, and nervous tissue, causing symptoms including vomiting, anorexia, abdominal pain, diarrhea, headache, and dizziness. This leads to malnutrition, immune dysfunction, growth inhibition, and even shock and death, thereby not only seriously harming human and animal health but also causing substantial economic losses to the livestock industry [11-14]. Analyzing DON levels in grains and feed is an effective approach to controlling DON contamination at the source, while its metabolites serve as effective biomarkers for DON exposure [15-16]. Physiological samples such as blood, urine, and tissues are important diagnostic targets for DON intoxication, which has drawn considerable attention to toxicokinetic studies of DON worldwide. This paper comprehensively reviews the latest advances in DON metabolic characteristics.

1. Contamination Distribution Characteristics of DON

DON contamination of grain-based food and feed is a global issue. European surveys of DON contamination in feed samples have found that approximately 57% of feed samples were contaminated with DON at levels ranging from 91 to 5,000 g/kg [17]. Another study investigating 82 feed samples across three different matrices (sow feed, wheat, and corn) reported that 67 types were contaminated with DON, with maximum contamination levels reaching approximately 9,528 g/kg [18]. In comparison, the European Union has set a maximum limit of 1,250 g/kg for DON in grains. Grain-based foods in Spain, the Czech Republic, South Africa, and other countries are also commonly contaminated with DON, with regional contamination details summarized in Table 1 . Furthermore, DON from food can contaminate water sources through animal and human excreta via sewage systems [19], creating potential environmental pollution risks.

2. Biochemical Properties of DON

DON, commonly known as vomitoxin, belongs to the trichothecene mycotoxin family [1]. Its chemical name is 12,13-epoxy-3,7,15-trihydroxytrichothec-9-en-8-one. It exists as colorless needle-like crystals, is a polar compound, and is readily soluble in water and polar organic solvents (Figure 1 [Figure 1: see original paper]). Due to its chemical stability even at 350°C, DON is not affected by processing and cooking procedures and thus pervades the entire food chain [2,20]. The epoxy group at positions C12 and C13 of this small sesquiterpenoid compound is critical for DON toxicity, likely interacting with amino, carboxyl,

and hydroxyl groups of cellular proteins, binding to ribosomes, causing ribosomal stress, and activating various protein kinases to exert its toxic effects [21-28].

3. Toxicokinetics of DON

3.1 Absorption of DON

Toxicokinetic studies have demonstrated that following oral administration, DON can rapidly cross the intestinal barrier, enter the bloodstream, and distribute to peripheral organs [24]. Notably, DON can also cross the blood-brain barrier (BBB) to distribute to the central nervous system [24].

3.1.1 DON Crossing the Intestinal Barrier The toxicity of DON stems from its ability to cross biological barriers and subsequently affect cellular activity and function in various organs. Following ingestion, DON first crosses the intestinal barrier. The gastrointestinal tracts of animals and humans harbor symbiotic microbial communities that protect the host from pathogenic microorganisms and toxins [29-30]. DON absorption in the small intestine shows substantial species-specific variation: pigs (82%) > chickens (19%) > sheep (5.9–9.9%) > cattle (1%) [31-34], which is primarily related to the distribution of commensal microbiota along the small intestine [35-37].

In ruminants and poultry, DON encounters high concentrations of microorganisms before entering the small intestine and is metabolized into the detoxified product deepoxydeoxynivalenol (DOM-1), thereby reducing the sensitivity of these animals to oral DON [38]. In humans and monogastric animals (pigs and rodents), high microbial populations are only present in the colonic region, so only a small fraction of DON reaches the colon where it can be metabolized into DOM-1 and excreted via feces [39]. Consequently, a large proportion of DON can cross intestinal epithelial cells and enter the bloodstream for rapid absorption. Therefore, pigs are more sensitive to DON compared to poultry or ruminants [31]. Studies have shown that DON can be detected in pig plasma approximately 30 minutes after oral administration, reaching peak absorption within 3–4 hours, indicating rapid and efficient absorption in the porcine proximal small intestine [40-41].

Research using Caco-2 cells and avian intestinal segments has revealed that DON is primarily absorbed through passive diffusion and paracellular pathways [42-43]. Intestinal inflammation, viral infections, pathogenic microorganisms, and toxins can all reduce tight junctions between intestinal cells, thereby promoting toxin absorption by intestinal epithelial cells [44-46]. Studies have shown that castrated male pigs exposed to DON-contaminated diets either acutely or chronically (4 weeks) exhibited absorption rates of 54% and 89%, respectively [47]. The higher bioavailability during chronic exposure may be related to incomplete elimination of DON from blood during the administration period [47] and to DON-induced damage to barrier function through altered expression of

tight junction proteins in intestinal cells [48-50]. In contrast, 7-day-old Ross broiler chickens showed decreased DON absorption after chronic exposure (4 weeks) to contaminated diets [51], possibly due to adaptation to toxin stimulation and activation of intestinal repair mechanisms at both morphological and functional levels. Additionally, DON bioavailability is related to tolerance at different developmental stages [52].

3.1.2 DON Crossing the BBB While DON may affect brain function through peripheral effects, it can also rapidly cross the BBB to directly act on brain cells and influence nervous system function [53]. The BBB is a selective barrier composed of endothelial and glial cells that inhibits the entry of exogenous molecules from plasma into cerebrospinal fluid. The rate of DON crossing the BBB varies by species, ranging from 2 to 60 minutes. Following intravenous DON administration, DON can be detected in the cerebrospinal fluid of both pigs and sheep within 2.5 minutes. In sheep, DON in cerebrospinal fluid peaks at 5–10 minutes, whereas in pigs it peaks at 30–60 minutes [53]. DON crosses the mouse BBB relatively slowly, becoming detectable in mouse cerebrospinal fluid at 5 minutes [24]. In pigs, 20–30% of plasma DON can enter the cerebrospinal fluid, with a half-life similar to that in plasma (20 hours) [53]. In mice, DON reaching the BBB accounts for approximately 10% of plasma concentration [24], while in sheep, only 5% of DON can cross the BBB [53]. However, whether DON can cross the BBB in humans and other species remains to be elucidated.

3.1.3 Mechanisms of DON Entry into Cells Currently, two possible mechanisms for DON entry into cells have been proposed. One mechanism suggests that DON does not directly enter cells but instead interacts with receptors or proteins on the cell membrane to activate various kinases and downstream signaling pathways to exert toxic effects; however, no studies have confirmed this to date. The alternative mechanism proposes that DON enters cells through lipid-soluble diffusion or endocytosis [54], with evidence indicating that DON can bind to ribosomes and trigger a series of toxic effects after entering cells [21-27]. Whether DON can exert toxic effects by binding to membrane receptors and whether DON can bind to organelles or proteins other than ribosomes after entering cells require further investigation.

3.2 Distribution of DON

The distribution and elimination rates of the parent compound and its metabolites in blood are important toxicokinetic parameters. Following a single oral dose in mice, DON rapidly enters the bloodstream and distributes to peripheral organs [24]. The peak time/concentration, distribution half-life ($t1/2\alpha$), and elimination half-life ($t1/2\beta$) of DON in plasma, liver, kidney, heart, and spleen are summarized in Table 2 , with elimination kinetics in plasma, liver, and kidney following a two-compartment model. DON enters the brain relatively slowly with lower peak concentrations (0.7–1.0 g/g) (Table 2). In pigs,

the t1/2β of DON and its metabolites in blood following intravenous DON administration or exposure to contaminated diets is 3.00–3.96 hours [33] (Table 3). However, pigs fed naturally contaminated grains show a relatively longer t1/2β. In broiler chickens administered DON via gavage, the t1/2β of free DON in blood is approximately 0.6 hours [32]. In contrast, sheep administered DON intraruminally exhibit a relatively longer t1/2β of 4.0–5.3 hours [31], indicating slower elimination of DON in ruminants.

3.3 Metabolism of DON

DON metabolism refers to the process by which DON is degraded into various products by microorganisms in the digestive tract or by intestinal mucosa, liver, kidney, and other organs. Currently, relatively few DON metabolites have been identified (Figure 2 [Figure 2: see original paper]), mainly including DOM-1, DON-glucuronide conjugates (DON-GlcA), DON-sulfonate conjugates (DON-sulfonate), and DON-sulfate conjugates (DON-sulfate). Among these, DON-GlcA serves as an effective toxicological biomarker for DON.

3.3.1 DOM-1 DOM-1 is primarily generated through microbial catalysis [59] and represents a common DON metabolite across different species (rodents, pigs, chickens, and ruminants). DOM-1 detected in blood after oral DON administration is not produced in the small intestinal lumen but is generated through bacterial detoxification in the digestive tract before being absorbed by the small intestine [60]. In vivo studies have shown that rumen microorganisms in sheep and cattle can convert DON to DOM-1, with higher conversion efficiency in dairy cows [61-63]. Additionally, microorganisms in human feces can also catalyze the formation of DOM-1 from DON [64]. Although cytochrome P450 (CYP450) enzymes isolated from deep lake bacteria can catalyze the formation of 16-OH-DON, CYP450 enzymes are not involved in DON metabolism [65]; consequently, the enzymatic mechanisms of microbial DON metabolism remain unclear. Beyond microorganisms, host tissues such as the liver may also contribute to DON de-epoxidation detoxification. For instance, DOM-1 can be detected in pigs after intravenous DON administration, with DOM-1 levels in bile being higher than in blood [40], though whether the liver can perform DON de-epoxidation requires further investigation.

3.3.2 DON-GlcA Conjugation of DON with glucuronic acid increases its water solubility, facilitating excretion via urine and bile. DON-GlcA is produced under the catalysis of UDP-glucuronosyltransferases (UGTs). Due to its lower lipophilicity (logD value), DON-GlcA has reduced efficiency in crossing cell membranes or binding to ribosomes, resulting in lower toxicity compared to DON [66]. Detoxification of xenobiotics primarily occurs in the small intestine, liver, and kidney, where UGTs are widely distributed [67]. Recent studies have demonstrated that liver microsomes can metabolize DON into DON-GlcA, mainly producing DON-3-GlcA and DON-15-GlcA [68-69]. Investigations of

DON metabolism in liver microsomes from humans, rats, cattle, pigs, and chickens have revealed that conjugation capacity at the C3 position follows the order: cattle > rat > human [68] (Figure 2). Meanwhile, both rat and human microsomes can produce DON-15-GlcA [69].

DON-7-GlcA and DON-8-GlcA are newly identified conjugation products [68]. Studies on intestinal and kidney microsomes have not indicated their involvement in DON detoxification metabolism. Approximately 75% of orally administered DON can be converted to DON-GlcA in sheep, whereas only 21% bioavailability is observed after intravenous administration, suggesting that detoxification product formation largely depends on small intestinal epithelial cells [31]. Furthermore, DON-GlcA can be detected in pig plasma after oral DON administration but not after intravenous injection, indicating that this conjugation process likely occurs prior to intestinal absorption [33,47]. However, whether intestinal cells can perform detoxification metabolism of DON remains to be further investigated.

3.3.3 Other Metabolites In addition to DON-GlcA, the formation of DON-sulfonate and DON-sulfate represents another detoxification pathway in animals. DON-sulfonate can reduce vomiting responses in pigs and shows no toxic effects on porcine peripheral blood mononuclear cells or porcine intestinal epithelial cells [71]. In chickens and rats, DON-10-sulfonate, DON-3-sulfate, and DOM-1 sulfonate conjugates (DOM-1-10-sulfonate) have been detected [72]. DON-3-sulfate has also been identified in sheep urine [73].

In summary, the generation of all DON conjugates represents important detoxification pathways. Conjugation of DON with glucuronic acid and subsequent urinary excretion constitutes the primary metabolic pathway. However, the specific UGT isoforms involved in catalyzing DON-GlcA formation remain unknown. Moreover, significant species differences exist in DON metabolism, and the regulatory mechanisms underlying these differences require further investigation.

3.4 Excretion of DON

DON is primarily excreted in the form of DON, DOM-1, DON-GlcA, and DOM-1-GlcA through urine. Studies have shown that approximately 91% of ingested DON is excreted by humans as DON-GlcA, predominantly as DON-15-GlcA [74-75]. In pigs, about 68% of ingested DON is excreted in urine as DON and DON-GlcA, while approximately 20% is excreted in feces as DOM-1 and DON [40,47]. The excretion of DON/DOM-1 and DON-GlcA/DOM-1-GlcA may occur through glomerular filtration in the kidneys and via P-glycoprotein expressed in epithelial cells of the small intestine, kidneys, and liver [54]. The excretion rate of DON and its metabolites is relatively rapid, with approximately half of the DON in plasma being eliminated within 6 hours after ingestion in pigs and sheep [31,40]. The high excretion rate of DON in animals suggests low binding affinity of its parent compound and metabolites to plasma albumin.

However, recent studies have found that DON can interact with human plasma albumin [76], indicating that DON may have a longer plasma half-life in humans, thereby increasing its potential health hazards.

3.5 Residues of DON and Its Metabolites

Detecting DON residues in animal-derived foods such as muscle, liver, kidney, milk, and eggs provides a basis for consumer risk assessment. Studies have shown that DON residues are highest in porcine edible tissues, particularly in kidney, in the form of parent DON and DOM-1 (Table 4). The residual levels in other tissues follow the order: liver > muscle > spleen > fat. The high DON residues in kidney may be related to urine concentration and are consistent with the primary urinary excretion pathway of DON in pigs. After dairy cows were fed diets containing 8.21 mg/kg DON (dry matter basis), the transfer rates to milk were 0.0001–0.0002 for DON and 0.0004–0.0024 for DOM-1 [63]. When laying hens were fed diets containing 11.9 mg/kg DON, residues of DON and DOM-1 in both yolk and albumen were below detection limits (2.5 and 1.0 g/kg, respectively) [77]. Therefore, DON residues in milk and eggs are very low.

Conclusion

DON contamination of grain-based food and feed is a global problem. Due to its stable and non-degradable properties, DON pervades the entire food chain. As a significant issue in feed and food safety, DON contamination seriously endangers human and animal health. Studies on DON absorption, distribution, metabolism, and residues in animal-derived foods provide a theoretical foundation for health risk assessment in humans and livestock. Toxicokinetic research on DON has shown that it can be rapidly absorbed into the bloodstream and distributed to peripheral organs, with the ability to cross the BBB. After absorption, DON can be metabolized into less toxic products, with glucuronidation and urinary excretion representing the primary metabolic pathway. Although progress has been made in understanding DON toxicokinetics across species, the metabolic pathways and their application in clinical diagnosis and livestock safety risk assessment require further investigation. For example, the metabolic pathways and resulting metabolites of DON show clear species differences between ruminants and monogastric animals, yet the regulatory mechanisms underlying these differences remain unclear. Compared to T-2 toxin, another trichothecene mycotoxin, relatively few DON metabolites have been identified, and whether other DON metabolites exist needs to be revealed. Integrating transcriptomics, proteomics, and metabolomics technologies to establish physiology-based clinical diagnostic tools for livestock health risk assessment, and analyzing the correlations between DON exposure concentrations, residue concentrations in physiological specimens, and corresponding clinical symptoms, remains a significant challenge.

Note: Figure translations are in progress. See original paper for figures.