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REVIEW ARTICLE

DEOXYNIVALENOL: METABOLISM AND REGIONAL DIFFERENCES IN HUMAN EXPOSURE

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Summary

Deoxynivalenol (DON) is one of the most abundant mycotoxins in contaminated food and feed worldwide. It is toxic to humans and inhibits DNA, RNA and protein synthesis. In this review, the metabolism of DON and its exposure in humans from different regions are summarized. Conjugated products DON-3-glucuronide, DON-15-glucuronide, and DON-7-glucuronide are found to be the major metabolites in humans. Human exposure of DON shows some regional differences due to the different DON levels in cereal-based foods and the food intake habits. C12,13-deepoxy metabolite, DOM-1 can be found in most French populations but is rarely detected in UK adults. Spanish exposes lower DON levels than the UK populations. A very high DON exposure is detected in South Africa and Linxian, China. Fetus is shown to expose to DON during pregnancy in human. This review will provide global information of DON metabolism and exposure in humans and facilitate the mycotoxin control strategies.

Key words: Deoxynivalenol; DON; metabolism; human; exposure; DON-3-glucuronide; DON-15-glucuronide

INTRODUCTION

Deoxynivalenol (DON) belongs to the group B trichothecenes and is considered to be one of the most important mycotoxins in cereal commodities (Figure 1). DON can be produced by fungi such as *Fusarium graminearum*, *Fusarium culmorum*, and *Fusarium crookwellense*, which are common pathogens on cereal crops like wheat, barley, and maize in the field (Wu et al. 2011). DON can cause food refusal, vomiting, digestive disorders, weight loss, decrease levels of serum protein and oxidative stress. From molecular level, DON is shown to inhibit DNA, RNA and protein synthesis. Ribosomes in cytosol are reported to be a major target of DON action in different studies (Pestka, 2008). Mitochondrial translation is also targeted by this toxin (Bouaziz et al. 2009). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recently

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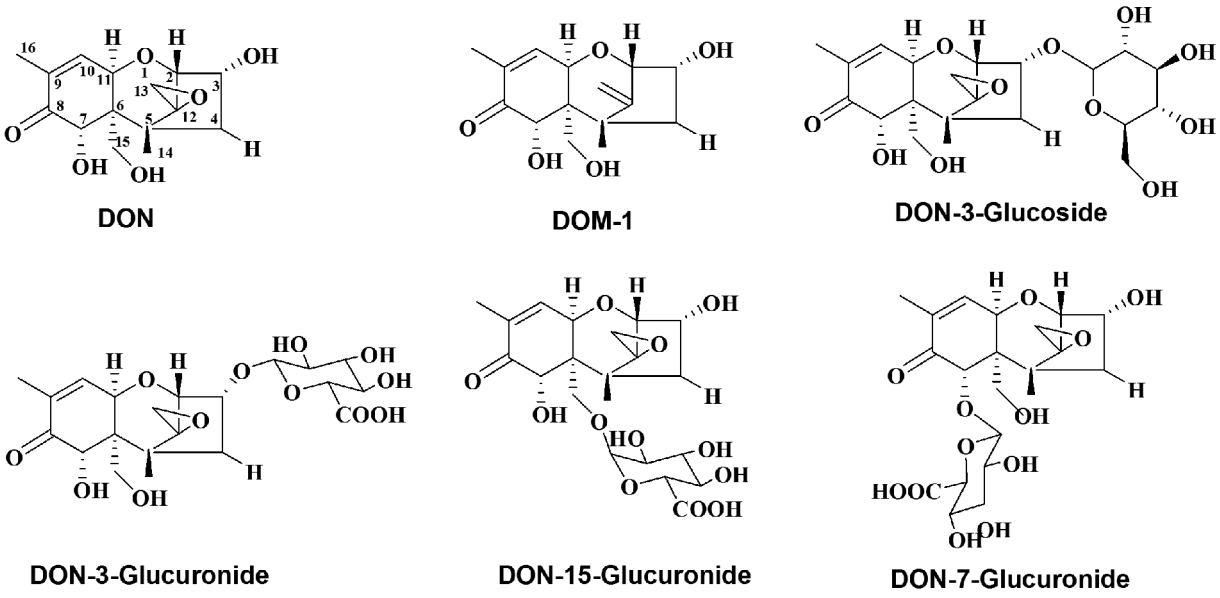

Figure 1. The chemical structure of DON and its related conjugated metabolites

set the limit of group provisional maximum tolerable daily intake (PMTDI) of DON as 1 μg/kg of body weight/day (JECFA, 2010).

The metabolic profile of DON in animals is relatively clear. DON can be transformed to C12,13-deepoxy metabolite, DOM-1 in rodents, pigs, chickens and cattle (Wu et al. 2010) (Figure 1). DON is mainly distributed in bile, liver, kidney, spleen, and is mainly excreted from feces and urine (Prelusky et al. 1986; Prelusky and Trenholm, 1991). Currently, more researches are focusing on the metabolism of DON in humans. In addition to DOM-1, the conjugated products seem to be the major metabolites. DON-3-glucuronide (DON-3-GlcA), DON-15-glucuronide (DON-15-GlcA) and the tentatively identified DON-7-glucuronide (DON-7-GlcA) are the metabolites of DON in humans (Maul et al. 2012; Sarkanj et al. 2013; Warth et al. 2013) (Figure 1). Generally, *in vitro* models, including the liver microsomes and the feces incubations, are usually used for the metabolic study in humans. However, one *in vivo* study in human is recently reported and provides very direct evidence (Warth et al. 2013).

Recently, human exposures of DON in different countries are increasingly reported. In China, DON from the high exposure region-Linxian was detected to be 37 ng/mL. In French populations, DOM-1 is commonly detectable, but this metabolite cannot be detected in most UK adults (Turner et al. 2010a, 2011a,b). The populations in China usually expose higher levels of DON, but because of the food habit, lower DON levels are reported from Shanghai, China (Turner et al. 2011a). In Spain, DON was confirmed in 33.3% of the human urine samples (Rubert et al. 2011b). In Austrian adults, DON and its glucuronide conjugates (DON-GlcA) are determined as biomarkers of exposure in the urine (Warth et al. 2012). Although there are increasing references on the metabolism and the exposure of DON in humans from different countries, the reports are scattered and it is difficult for us to understand the global profiles.

In this review, we aim at discussing the metabolism of DON in humans and the human exposure of DON in different countries and regions. Moreover, some regional differences of DON exposure in humans are suggested. This review will provide further information in the metabolism and exposure of DON in humans and facilitate mycotoxin control strategies.

GLOBAL INSIGHT IN DON METABOLISM

DON is able to be transformed to 12,13-deepoxy-DON (DOM-1), in rodents, swine, chickens, and ruminants (Wu et al. 2010). This metabolite is common in animals and can largely reduce the toxicity. In a BrdU bioassay, when the C-12-C-13-epoxide ring was lost, the LD_{50} of DON was increased by 54 times

(Eriksen et al. 2004). However, DOM-1 is rarely detectable in humans. In several studies, human feces were incubated under anaerobic conditions for 48 hours with 3-acetyl-DON (Eriksen et al. 2003; Eriksen and Pettersson, 2003) but no deepoxidated metabolites were detected in the fecal incubates. This observation suggests that humans may lack the relative microflora for key detoxification of DON. Different with the findings in animals, more conjugated DON are detected as the metabolites in humans. DON-15-GlcA and DON-3-GlcA are the major phase II metabolites in humans. Other conjugates such as DON-7-GlcA are also monitored in several studies (Maul et al. 2012; Sarkanj et al. 2013; Warth et al. 2013).

To date, it is still unclear which enzymes are catalyzing the C12,13-deepoxy pathway. UDP-glucuronosyltransferases (UGTs) possibly play an important role in the formation of DON-GlcA, but the exact subfamily of the UGTs has yet to be elucidated (Tukey and Strassburg, 2000; Obol'skiĭ et al. 1998).

METABOLISM OF DEOXYNIVALENOL IN HUMANS

The metabolic fate of DON in animals is clear and currently more studies are focusing on the metabolism of DON in humans. Generally, researchers are using the *in vitro* models, including the liver microsomes and feces incubations, to investigate the metabolism of DON in humans. In order to uncover the real face of DON metabolism in humans, *in vivo* metabolism of DON in human is also studied (Warth et al. 2013). Conjugated products are the major metabolites of DON in humans.

In vitro metabolism of DON in human proximal tubule cells and lung fibroblasts in primary culture was carried out but no metabolites, including the phase II metabolites, were detected in the two cell cultures (Königs et al. 2007). However, the toxic effect of DON on the cells should not be ignored, since in one report, DON up-regulated the genes which are closely related to ribosomal structure and function, mitochondrial function, oxidative stress and apoptosis in human peripheral blood mononuclear cells (Katika et al. 2012).

In order to mimic different stages of DON digestion in human gut, an *in vitro* model system was used to investigate the stability of DON-3-glucoside (D3G) to acidic conditions, hydrolytic enzymes, and intestinal bacteria. D3G was resistant to hydrochloric acid and human cytosolic glucosidase had no effect on its degradation. However, several lactic acid bacteria showed a high capacity to hydrolyze D3G (Berthiller et al. 2011). This study suggests that DON detoxified by the plant into D3G may become partly bioavailable due to D3G hydrolysis by bacterial β-glucosidases in the colon.

The metabolism of DON in human liver microsomes was investigated by Maul et al. (2012). In addition to DON-3-GlcA, human liver microsomes could also form DON-15-GlcA. The third metabolite was tentatively identified as DON-7-GlcA, but the confirmation of identity of this metabolite requires further investigation. In addition to the metabolism in liver, the biotransformation of DON-3-GlcA by human microbiota was assessed as well (Gratz et al. 2013). The fecal microbiota released DON from D-3-GlcA very efficiently and the fecal microbiota from one volunteer transformed DON to DOM-1. Urine from the same volunteer also contained DOM-1, whereas DOM-1 was not detectable in urine from other volunteers. Their results suggest that the metabolite DOM-1 may be from the biotransformation of DON or DON-3-GlcA in intestine.

For the first time, Warth et al. (2013) investigated the *in vivo* metabolism of DON in human after the volunteer consumed the contaminated diet containing 138 μg DON over 4 days. DON-15-GlcA was identified as the major metabolite besides DON-3-GlcA. The third metabolite was determined but its structure was not identified. Moreover, the masked form of DON (DON-3-glucoside and 3-acetyl-DON) in urine was preliminary analyzed but none of the masked forms were detectable in this study.

REGIONAL DIFFERENCES IN HUMAN EXPOSURE

Currently, there are a series of studies focusing on human exposure of DON in different countries. Interestingly, the exposure of DON in humans shows some potential regional differences. The differences are possibly due to the DON levels in cereal-based foods and are correlated to cereal intake, particularly bread consumption in different countries and regions.

For the fist time, DON was detected in human urine by Meky et al. (2003). In their study, urine samples were collected from female inhabitants of Linxian, China, an area of potentially high DON exposure, and Gejiu, a low risk region of China. DON was detected in all the samples following β-glucuronidase treatment. DON from the high and low exposure regions of China was 37 ng/mL and 12 ng/mL, respectively. Conjugated-DON was suggested to exist in human urine.

Turner et al. (2010b) first reported the DOM-1 residue in the urine from French population. DOM-1 was found in urine from 80.7% of indi-viduals who worked on farms. However, in the UK adults, DOM-1 was not detectable in most urine samples (Turner et al. 2011b). The potential reasons for this difference could be the accidental transmission of animal microbiota to farm workers, which has the capacity for DON conversion to DOM-1.

The urinary levels of DON in UK adults are positively correlated to cereal intake, particularly bread consumption (Turner et al. 2008). Moreover, a strong correlation between DON intake and the urinary biomarker was observed in models adjusting to age, sex and body mass index in the UK adults. Their data demonstrate a quan-titative correlation between DON exposure and urinary DON, and serve to validate the use of urinary DON as an exposure biomarker (Turner et al. 2010a, b). After the cereals consumption of 300 g/day , DON in urine was detected in the level of 13.24 μg/day (11.7 ng/mg creatinine) in UK adults. However, in a study of Shanghai women's health (Turner et al. 2011a), only 4.8 ng DON/mL urine (5.9 ng DON/mg creatinine) were detected, which was much lower than that found in UK adults. In Shanghai, maize and barley are rarely consumed, but rice consumption is by far the predominant cereal con-sumed, and rice is not a major source of DON (CAST, 2003).

In order to evaluate the human exposure of mycotoxins and its risk in the Valencian population in Spain, Rubert et al. (2011) analyzed the levels of DON in the urines from 27 volunteers (age 21-77 years old) during September and November, 2010 in Valencia (Spain). T-2 and HT-2 toxin were not detected in any of these samples analyzed but DON was confirmed in 33.3% of the urine samples, which was much lower than the occurrence in UK populations (98.7%) (Turner et al. 2008).

In Croatia, the DON exposure in pregnant women urine was analyzed (Sarkanj et al. 2013). DON (18.3 μg/L) and its metabolites DON-15-GlcA (120 μg/L) and DON-3-GlcA were detected in 97.5% of the studied samples. Several highly contaminated urine samples contained a third DON conjugate, which was tentatively identified as DON-7-GlcA by MS/MS scans. Forty-eight percent of subjects were estimated to exceed the provisional maximum tolerable daily intake (1 μg/kg BW.). In South Africa, multiple mycotoxin exposure was determined by urinary biomarkers in rural subsistence farmers in the former Transkei (Shephard et al. 2013). After the hydrolysis with β-glucuronidase, DON was detected in 100% of the samples with the concentration of 20.4 ± 49.4 ng/mg creatinine, suggesting a very high DON exposure in this area.

Warth et al. (2012) have conducted a pilot survey to investigate the level of DON exposure in Austrian adults by measurements of DON and its glucuronide conjugates (DON-GlcA), as biomarkers of exposure, in the urine. The average concentration of total DON was estimated to be 20.4 ± 2.4 μg/L. For the first time, in vivo metabolisms of DON in humans were performed by this group, and DON-15-glucuronide was found to be a major DON metabolite in human urine. About 75% of total glucuronides were derived from this metabolite while DON-3-GlcA accounted for 25%. In these earlier studies, DON exposure is usually estimated from dietary average intakes or by measurement of the native toxin in urine after enzymatic hydrolysis with β-glucuronidase. The DON-3-GlcA standard was synthesized and was successfully used for directly quantification of this product (Warth et al. 2011).

Fetus is shown to expose to DON during pregnancy in human (Nielsen et al. 2011). Placentas were used to study DON transfer with the *ex vivo* dual perfusion model and about 21% of the initially incubated toxin was transported to the foetus. Since DON has effects on foetal growth and is immunosuppressive, the risk assessment of DON in fetus should be considered (Pestka, 2010).

Several studies also focused on DON exposure on the intestine. In order to evaluate the oral subchronic exposure of DON on the composition of human gut microbiota, Saint-Cyr et al. (2013) have established a human microbiota-associated rat model. During oral DON exposure, a significant increase of 0.5 log 10 was observed for the *Bacteroides/Prevotella* group during the first 3 weeks of administration. Concentration levels for *Escherichia coli* decreased at day 27. Compared with DON, 15-acetyl-DON

shows higher permeability in the intestinal transportation and causes higher potential risk for humans (Kadota et al. 2013). Until now, the toxicities of acetylated DONs are considered to be equivalent to DON. However, higher toxicity of 15-actyl-DON should be considered to evaluate toxicity of DON and acetylated DONs (Kadota et al. 2013).

In summary, the exposure of DON in humans shows regional dependent profiles. The deepoxy metabolite DOM-1 is commonly found in French populations but rarely detectable in UK adults. The urinary levels of DON in human are positively correlated to cereal intake. Spanish expose lower DON levels than the UK populations. A very high DON exposure is detected in South Africa. Fetus is shown to expose to DON during pregnancy in humans.

CONCLUSIONS

Conjugated products are the major metabolites of DON in humans. DON-3-GlcA, DON-15-GlcA, and DON-7-GlcA are the conjugated metabolites; however, DON-15-GlcA seems to be the major one. The exposure of DON in humans shows some potential regional differences. DOM-1 is commonly found in French populations but rarely detectable in UK adults. Spanish exposed lower DON levels that the UK populations. A very high DON exposure is detected in South Africa. These differences are very possibly due to the food intake habits and the occurrence of DON in cereals from different regions. However, human exposure of DON from more countries is still needed to facilitate mycotoxin control strategies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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