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The food born mycotoxin deoxynivalenol induces low-grade inflammation in mice in the absence of observed-adverse effects

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HIGHLIGHTS

- The present work shows that sub-chronic low doses DON exposure induces an increase in cytokine mRNAs expression in both peripheral organs and brain.
- The present work demonstrates that sub-chronic low doses DON exposure provokes histological liver alterations.
- The present work provides the first demonstration that sub-chronic administration of low DON doses in mice results in a low-grade inflammation state.
- The present work questions about the harmlessness of chronic human dietary DON exposure and challenges the provisional maximum tolerable daily intake (PMTDI) of 1 μg/kg bw.

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ABSTRACT

Scope: Deoxynivalenol (DON) is the most common fungi toxin contaminating cereals and cereal-derived products. High consumption of DON is implicated in mycotoxicoses and causes a set of symptoms including diarrhea, vomiting, reduced weight gain or immunologic effects. However, such clinical intoxications are rare in humans, who are most frequently, exposed to low DON doses without developing acute symptoms. The adverse effect of chronically consumed low DON doses can not be totally excluded. Using a mouse model, we evaluated the impact on inflammatory status of subchronic administration of DON given at doses comparable to the daily human consumption.

Methods and results: The inflammatory status was evaluated in mice receiving 1, 2.5 or 25 μg/kg bw/day DON during a 10 or 30 days period. The systemic interleukin-1 beta (IL-1β) concentrations were evaluated by Elisa and inflammatory biomarker mRNA expressions were quantified by qPCR within brain structures and peripheral organs. While DON intake failed to modify physiological markers, we observed a systemic IL-1β increase and a modulation of pro-inflammatory gene expression in brain structures, liver, duodenum and adipose tissue.

Conclusion: We bring here the first evidence that subchronic DON intake, at doses that match daily human intake, induces, in a murine model, a central and peripheral low grade inflammation.

1. Introduction

Produced by various *Fusarium* fungi species, deoxynivalenol (DON), also called vomitoxin, is the most abundant type B trichothecene found in cereals (Binder, 2007). The extent of cereal and grain contamination is strongly influenced by rainfall and moisture at the time of flowering. The 72nd joint Food and Agriculture Organization of the United Nations and World Health Organization FAO/WHO expert committee on Food Additives

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(JECFA, 2011) collected and gathered surveys originating from 42 countries dealing with the levels and patterns of DON contamination in cereals. From a total of 23, 980 samples analyzed for DON contamination, mean levels for raw cereals were reported as follow: wheat, 9900 μg/kg; maize, 4772 μg/kg; rice, 183 μg/kg; barley 6349 μg/kg; oats, 537 μg/kg and rye, 190 μg/kg. Unsurprisingly given the reported contamination levels, DON has been implicated in mycotoxicoses in both humans and farm animals (Pestka, 2010). Acute DON toxicity is characterized by a set of symptoms including diarrhea, vomiting, reduced weight gain and immunologic effects. In some case, leukocytosis, hemorrhage, circulatory shock and death have been reported in farm animals following consumption of cereals highly contaminated with DON (Pestka, 2010). In humans, epidemiological studies have reported acute illnesses including nausea, vomiting, abdominal pain, diarrhea, headache, and dizziness in populations who have consumed *Fusarium*-contaminated grains or DON-containing cereals (3–93 mg/kg) (Bryden, 2007). Trichothecenes are characterized by a high stability under different environmental conditions and resistance to high temperature and to industrial processing of food/grains (Bretz et al., 2006; Wolf-Hall et al., 1999). This high stability explains its widespread presence in human food commodities. In this context, DON constitutes a very common contaminant of food-stuff such as bread, breakfast cereals, baby food, pasta, pastries, pizza or beers (Rodrigues and Naehrer, 2012). Based on cereal and food commodity DON concentrations and diet consumption data, the total DON human intake was estimated in 2011 to range from 0.2 in the African diet to 14.5 μg/kg bw/day in the Middle Eastern diet (JECFA, 2011). The average dietary DON exposures were recalculated by multiplying the mean DON concentration of each commodity by the amount of each commodity consumed in the different regional diets. Wheat was considered as the main source of DON intake in three regional diets *i.e.*, European and Middle Eastern (64–88% of total intake), whereas the sources were more varied in African diet (wheat, rice and maize) and Far Eastern diet (wheat and rice). Accordingly, chronic human exposure to low DON doses is highly predictable in many worldwide regions and the question of the potential risk of such chronic exposure remains unanswered. Modulation of the immune system is a classical hallmark described during acute or chronic DON intoxication (Pestka, 2010). Acute DON intoxication (0.5–25 mg/kg) was shown to up-regulate the expression of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6) or tumor necrosis factor alpha (TNF-α) (Amuzie et al., 2008; Girardet et al., 2011a).

The aim of the present study was to evaluate the impact of subchronic intoxication with DON given at doses below the non observed-adverse effect level (NOAEL), *i.e.*, 100 μg/kg bw (Iverson et al., 1995–1996) on mice inflammatory status. In accordance, mice received 1, 2.5 and 25 μg/kg bw/day by oral gavage during a 10 or 30 days period. We first checked daily the absence of DON-induced body weight and food intake modification in our treatment paradigms. The systemic IL-1β concentration was quantified in all tested conditions and inflammatory biomarker mRNA expressions were next evaluated by qPCR both in peripheral organs and within brain structures. Given the low levels of DON administered, oral gavage was chosen to provide consistent and controlled doses throughout the treatment period.

2. Materials and methods

2.1. Animal housing

Experiments were performed on adult male C57BL/6 mice (26 ± 3 g; Charles River, France). Animals were individually housed at 22 °C in a facility at controlled temperature on a 12/12-h light/dark cycle with standard powder diet (AO4 P2.5, SAFE UAR, France) and water available *ad libitum*. Individual cages were designed in order to limit spillage (Pecchi et al., 2008). Experiments were performed in strict accordance with European Economic Community guidelines (86/609/EEC) and the local committees' recommendations (C-13-055-6, Aix-Marseilles University) for the care and use of laboratory animals.

2.2. Per os *administration of DON*

Prior to DON treatment, mice received a volume of distilled water *via* oral administration procedure for a habituation period of seven consecutive days. Then, mice were orally administered 1–25 μg/kg bw DON (D0156, Sigma–Aldrich, France) *via* gavage, using a 22 gauge intubation needle (Popper and Sons). Each mouse received 5 μL/g bw of the appropriate solution (~100 μL/mouse). The same volume of distilled water was given to control animals.

2.3. Food and water intake measurements

2.3.1. Powdered food consumption

Immediately after intra-gastric DON administration, a fresh supply of pre-weighed food was given to mice. The measurement of powdered food remains was the same as in previous studies (Girardet et al., 2011b). Food intake was calculated as the difference between the pre-weighed and the remaining powder measured with a precision balance (0.01 g; Denver Instrument from Bioblock).

2.3.2. Water intake

Mice had a free access to water. The measurement of water intake was the same method as for food consumption.

2.4. Hematoxylin labeling

Fresh liver pieces (8 mm^3) were frozen in liquid nitrogen. Sections (10 μm thick) were cut on a cryostat (Leica CM3050, France) and collected on gelatin-treated glass slides. Slides were then incubated during 40 s in hematoxylin solution (#GHS316, Sigma–Aldrich) and rinsed in water bath. The sections were dehydrated in increasing alcohol solutions (50°, 75°, 96° and 100°), then in xylenes, and coverslipped with mounting medium for microscope preparation (Eukitt®). The labeling was evaluated using a Nikon Eclipse E600 light microscope and photomicrographs were acquired using a 10 and 20 fold lens with a DXM 1200 Camera (Nikon) coupled to ACT-1 software. The microscope was set at a specific illumination level, as was the camera exposure time.

2.5. IL-1β assay

Retro-orbital blood collection was performed prior to treatment and at the end of the intoxication period using haematocrit tubes Na-Heparinized (Milian). Blood samples (0.5 mL/animal) were collected in eppendorf tubes containing 0.6 U aprotinine (Sigma–Aldrich, A1153), EDTA 7.5% (Sigma–Aldrich, E5513) and 5 U heparin (Choey) and tubes were centrifuged (1500 × *g*) at 4 °C during 15 min. Plasma IL-1β levels were quantified using IL-1β mouse ELISA Kit (Ab 100704, Abcam) in accordance with manufacturer's procedures.

2.6. Quantitative PCR analysis

Animals used for RT-PCR analysis were sacrificed 24 h after the last DON administration. mRNA extraction and RT were performed as previously described (Gaigé et al., 2014). Briefly, total RNA was extracted from frozen organ using TRI Reagent® (Sigma–Aldrich).