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Brain damage and neurological symptoms induced by T-2 toxin in rat brain

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ABSTRACT

T-2 toxin, a trichothecene mycotoxin, is a common contaminant in food and animal feed, and is also present in processed cereal products. The most common route of T-2 toxin exposure in humans is through dietary ingestion. The cytotoxic effects of T-2 toxin include modifications to feeding behavior, nervous disorders, cardiovascular alterations, immunosuppression, and hemostatic derangements. However, to date, effects on the central nervous system (CNS) have rarely been reported. In the present study, female Wistar rat were given a single dose of T-2 toxin at 2 mg/kg b.w. and were sacrificed at one, three, and seven days post-exposure. Histopathological analysis and transmission electron microscope (TEM) observations were used to investigate injury to the brain and pituitary gland. Damage to the brain and pituitary at the molecular level was detected by real time-polymerase chain reaction (RT-PCR), western blot, and immunohistochemical assays. Liquid chromatograph-mass spectrometer/mass spectrometer (LC-MS/MS) was used to investigate T-2 concentration in the brain. The results showed that pathological lesions were obvious in the brain at three days post-exposure; lesions in the pituitary were not observed until seven days post-exposure. Autophagy in the brain and apoptosis in the pituitary suggest that T-2 toxin may induce different acute reactions in different tissues. Importantly, low concentrations of T-2 toxin in the brain were observed in only one rat. Responsible for the above mentioned, we hypothesize that brain damage caused by this toxin may be due to the ability of the toxin to directly cross the blood-brain barrier (BBB). Therefore, given its widespread pollution in food, we should pay more attention to the neurotoxic effects of the T-2 toxin, which may have widespread implications for human health.

1. Introduction

T-2 toxin, a naturally occurring mycotoxin produced by various species of *Fusarium*, widely contaminates barley, maize, oats, and even human food. T-2 toxin contamination of these grains and cereal-based products has frequently been reported. In Israel, from November 2011 to March 2012, T-2 toxin levels were investigated, but were not found to be above their limit of detection (0.6 μg/kg) in any of the investigated samples (15 of wheat and 15 of corn) (Shimshoni et al., 2013). In Germany, the highest amount of T-2 toxin in retail food, which was detected in a sample of oat pastries, was 1.9 μg/kg (Beyer et al., 2009). As barley is the oldest and most economically important crop (Bolechova et al., 2015; Morcia et al., 2016), there are many reports of T-2 toxin contaminants in barley. In Modena and Matera (Italy), a study found that 100% of samples had contamination, with an average level of the T-2 and HT-2 toxin of 443 μg/kg and 165 μg/kg,

Abbrevations: ANOVA, one-way analysis of variance; ATA, alimentary toxic aleukia; atg14, autophagy related 14; atg5, autophagy related 5; bax, Bcl 2 associated X; BBB, Blood brain barrier; BPB, Blood placenta barrier; BSA, bovine serum albumin; Caspase-3, Cysteinyl aspartate specific proteinase-3; Caspase-9, Cysteinyl aspartate specific proteinase-9; CNS, central nervous system; DAB, domain antibody; DMSO, methyl sulfoxide; ESI, electrosprayionization; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM, Gray matter; HE, hematoxylin-eosin; HT-2 toxin, 12,13-epoxytrichothec-9-ene-3-alpha, 4-beta, 8-alpha, 15-tetrol 15-aceta 8-isovalerate; IFN-γ, interferon-gamma; i.g., intragastric administration; IL, interleukin; KBD, Kaschin-beck disease; LC-MS/MS, Liquid chromatograph-mass spectrometer/mass spectrometer; LC3B, microtubule-associated protein 1 light chain 3 beta; LD_{50}, Lethal Dose 50; LSD, least significance difference; MAO, monoamine oxidase; MMP-9, matrix metalloproteinases 9; mRNA, messenger RNA; mTOR, mechanistic target of rapamycin; PBS, Phosphate Buffered Saline; RIPA, radio immunoprecipitation assay; RT-qPCR, Quantitative real time-polymerase chain reaction; TEM, transmission electron microscope; TDI, daily intake; T-2 toxin, 12,13-epoxytrichothec-9-ene-3alpha, 4-beta, 8-alpha, 15-tetrol 4, 15-diacetate 8-isovalerate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresi; WM, white matter; 5-HT, 5-hydroxytryptamine

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respectively, and a highest contamination level of 724 μg/kg and 324 μg/kg, respectively (Morcia et al., 2016). In another study, with regard to T-2 and HT-2 toxins, 30.9% of samples were positive, with a mean content of 11.2 μg/kg, and a highest detected value of 532.5 μg/kg (Bolechova et al., 2015). In Kaschin-beck disease (KBD) endemic villages in China, T-2 toxin contamination is relatively high with an average level of 78.91 μg/kg in wheat and 47.47 μg/kg in flour (Sun et al., 2012). The Panel on Contaminants in the Food Chain set the tolerable T-2/HT-2 daily intake (TDI) level at 100 ng/kg b.w. (EFSA, 2011).

Many studies have reported on the toxicity of this toxin. Symptoms of acute poisoning with T-2 toxin include nausea, vomiting, abdominal pain, diarrhea, and even weight loss (Wan et al., 2015b; Wan et al., 2016; Wu et al., 2017). T-2 toxin exposure induces apoptosis in many cells, including rat ovarian granulosa cells (Wu et al., 2010; Wu et al., 2015), GH3 cells (Liu et al., 2017d), C28/I2, L-02, and HK-2 cells (Lei et al., 2017), primary hepatocytes of broilers (Yang et al., 2017), and even in human neuroblastoma cells (Agrawal et al., 2015). T-2 toxin has the high affinity for the 60S ribosomal subunit, resulting in inhibition of the activity of peptidyl transferase (Doi et al., 2008; Wan et al., 2015a). Therefore, the primary known toxicity mechanism of the T-2 toxin is its inhibitory effect on protein synthesis (Zhang et al., 2017). Additionally, some research has reported that T-2 toxin can affect RNA and DNA synthesis, mitochondrial electron transport systems, mitochondrial function, and cell division and membrane function (Lattanzio et al., 2012; Yang et al., 2015; Wang et al., 2017).

T-2 toxin also targets the immune system. Several studies described that T-2 toxin could decrease the production of interleukin (IL)-2 and the expression of plasma interferon-gamma (IFN-γ)and could upregulate the messenger RNA (mRNA) expression of IL-6, IL-1β, and tumor necrosis factor-α, in a dose-dependent manner in RAW264.7 cells (Wang et al., 2012; Qiu et al., 2016) In animals, the intoxication symptoms also include low milk production, decreased egg production, gastroenteritis, and intestinal bleeding (Marin et al., 2013). In evidence of the above mentioned, we found that the characterization of the T-2 toxin is that widely contaminate food and feed all over the word, have various toxicity and even affect human health. However, the mechanisms by which T-2 toxin affects the CNS have received little attention. Gray matter (GM) and white matter (WM) have long been recognized as the two components of the CNS. WM mediates the essential transmission of electrical signals across the different regions throughout the brain (Filley and Fields, 2016; Liu et al., 2017a). Serious neurobehavioral and cognitive impairments may be induced by WM malfunction (Bennett and Madden, 2014). Pituitary gland, the major neuroendocrine organ, directly or indirectly regulates hormone synthesis and secretion within the body. It is an important part of the hypothalamic-pituitary-adrenal axis (Kessing et al., 2011; Formosa and Vassallo, 2014; Fan et al., 2016). In the 1990s, Sirkka et al. (1992) reported that T-2 toxin has effects on rat behavior, causing an impairment in the passive avoidance test at a dose of 2.0 mg/kg. Another similar study showed that classic feeding behavior, locomotor activity, and body temperature altered after mice had been treated with 0.5–5 mg/kg b.w. of T-2 toxin via gavage (Gaige et al., 2014).

Recently, an increasing number of researchers have begun to pay close attention to cerebral lesions induced by T-2 toxin. For example, a study exposed mice to 5.94 mg/kg b.w. or 1.54 mg/kg b.w. of T-2 toxin, by dermal or subcutaneous route, respectively, and sacrificed them at one, three and seven days post-exposure; they found that reactive oxygen species generation, lipid peroxidation, glutathione depletion, and protein carbonyl content were increased in a time dependent manner in the brains of the mice (Chaudhary and Rao, 2010). Further, percutaneous exposure of 1 Lethal Dose 50 (LD_{50}) T-2 toxin was shown to cause a reversible alteration in BBB permeability, as observed by extravasation of Evans blue dye (Ravindran et al., 2011). Many studies have suggested that cerebral lesions induced by T-2 toxin may occur through oxidative stress in the brain or through destruction of the BBB (Weidner et al., 2013b; Gaige et al., 2014; Zhang et al., 2014). One study simulated the BBB *in vitro* using porcine brain capillary endothelial cells, and investigated whether T-2 toxin could cross the BBB. The study found a fast permeation of T-2 toxin across the BBB from the mimicked blood to the brain side, with an accumulation of T-2 toxin on the basolateral side (Weidner et al., 2013a). This was the first paper to reveal that T-2 toxin can penetrate the BBB and accumulate in the brain in *vitro*. Our recently study presented that the pituitary might be a novel target organ of T-2 toxin (Liu et al., 2017c).

These studies suggest that the ability of T-2 toxin to penetrate the BBB may be a cause of cerebral lesions. However, whether the T-2 toxin can penetrate into the brain *in vivo* has not been directly examined. Further, the pathologic lesions and infrastructure changes in the brain induced by T-2 toxin have not been reported. Finally, there is also no study in the literature reporting the effect of T-2 toxin on the pituitary. In this study, we try to clarify these research questions using molecular biology experimental techniques including histopathological analysis, TEM observation, and LC-MS/MS.

2. Materials and methods

2.1. Reagents and chemicals

T-2 toxin (CAS NO. 21259-20-1) was purchased from TRC (Canada) and methyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (France).

2.2. Animals

Specific pathogen-free female Wistar rats, weighting between 150 and 200 g b.w., from the Centre of Laboratory Animals of Hubei Province, Wuhan, P.R. China, were used for this study. The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine (Huazhong Agricultural University, Wuhan, P.R. China). Animals were kept in well-maintained animal rooms at a temperature of 20–26 °C, with a relative humidity of 40–70% and a 12 h light/dark cycle. During the first week acclimatization period, all animals received basic feed and fresh water. The rats were housed four per cage with hardwood shavings as bedding.

2.3. Experimental design

T-2 toxin was dissolved in DMSO, then was diluted in Phosphate Buffered Saline (PBS). For the time course study, animals were divided into four groups of five animals each, for each time point (0 h, 1, 3 and 7 days). The single-dose 2 mg/kg b.w. (intragastric administration (i.g.)) of T-2 toxin was determined by Gad and Weil's method. The oral LD_{50} of T-2 toxin in female rats is 7.0 mg/kg b.w. Several studies have shown that after treatment with 2 mg/kg b.w. T-2 toxin, obvious neurologic symptoms are observed in rats (MacDonald et al., 1988; Sirkka et al., 1992). A day before i.g. exposure to T-2 toxin, all animals received fresh water only, without basic feed. Control rats received only PBS-DMSO. All animal work was in compliance with the NIH publication “The Development of Science Based Guidelines for Laboratory Animal Care” (NRC 2004).

2.4. RT-PCR examination

The mRNA expression of genes related to autophagy and apoptosis (eg. LC3B, atg14, atg5, mTOR, caspase-3, caspase-9, bax, and Bcl-x) in rat cerebral cortex and pituitary were measured. The levels of mRNA expression of the genes were determined by real-time quantitative reverse RT-PCR. Total RNA from the cerebral cortex and pituitary was isolated using the TRIzol extraction method according to the manufacturer's instructions (Invitrogen Inc., Carlsbad, CA). The quality of RNA was verified by evaluating the optical density at 260 nm and