Environmental Toxicology and Pharmacology 56 (2017) 259–267

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

T-2 toxin induces cytotoxicity and disrupts tight junction barrier in SerW3 cells

Elif Karacaoğlu*, Güldeniz Selmanoğlu

Hacettepe University, Faculty of Science, Department of Biology, 06800, Beytepe, Ankara, Turkey

ARTICLE INFO

Keywords:
Cytotoxicity
N-cadherin
Occludin
Sertoli cell barrier
TEER
T-2 toxin

ABSTRACT

T-2 toxin, which is produced in grain and grain products as a secondary metabolite by Fusarium species, is also potentially dangerous for human health. Up to date, no study was reported the cytotoxicity of T-2 toxin on SerW3 cells in the perspective of junctional barriers. This study focused on revealing the cytotoxic effects of T-2 on Sertoli cells associated with cell junctional barriers. In the present study, SerW3 cells were exposed to T-2 toxin at 12, 120 and 1200 ng/ml doses for 24 and 48 h. Cytotoxicity tests including cell viability (MTT), lactate dehydrogenase (LDH) cytotoxicity test and trypan blue exclusion assay were performed. Occludin, ZO-1, N-cadherin and β-catenin were immunolabelled, expressions of occludin and N-cadherin were determined by western blotting. SerW3 cell barrier integrity was measured by transepithelial electrical resistance (TEER). Cytotoxicity caused by T-2 toxin increased in a dose dependent manner, expressions of proteins and TEER measurement decreased. This study may underlie the early targets of T-2 toxin on SerW3 cells mimicking blood-testis barrier *in vitro*.

1. Introduction

Mycotoxins are toxic fungal metabolites produced by fungi in agricultural products including human food and animal feedstuff worldwide (McKean et al., 2006). Trichotecenes belong to a mycotoxin group, are produced by *Fusarium, Myrothecium and Stachybotrys* spp. and they are the most studied biological natural toxins and threaten human health and economy globally (Cetin and Bullerman, 2005; Krska et al., 2007). Cereals that are mostly contaminated with T-2 toxin can be listed as corn, barley, wheat, oat and rye (Sokolovic et al., 2008). Similar to other trichothecene mycotoxins, T-2 toxin is known to be highly resistant to heat and ultraviolet light and it is not possible to inactivate it in food production process (Sokolovic et al., 2008). It was revealed that T-2 toxin caused decreases in testosterone biosynthesis, induce cytotoxicity, oxidative stress and DNA damage in mouse primary Leydig cells *in vitro* and reproductive abnormalities such as decreases in semen quality, sperm count and testosterone levels in male mice (Yang et al., 2014; Yang et al., 2010; Yang et al., 2016). However it was mentioned that mode of action of T-2 toxin is needed to be elucidated by means of spermatogenesis and on sperm quality (Yang et al., 2010). Recent studies have shown that toxicants may target cell junctions (Bekheet and Stahlmann, 2009; Zhang et al., 2008). To date no study was reported on disclosing the mechanism of perturbing adhesive function *in vitro* exposure of T-2 toxin especially on Sertoli cells in the cell junctional barrier perspective. Herewith, our experimental study was set up to elucidate the early targets of T-2 toxin on Sertoli cells in the perspective of cell junctional complexes by performing several cytotoxicity tests and early toxicity biomarkers.

Spermatogenesis is a highly complicated process with proliferation and differentiation of germ cells (Su et al., 2011). In testis, tight junctions at the surface of Sertoli cells are known to create a physiological barrier and immunologically privilege site for germ cells identified as blood-testis barrier which separates seminiferous epithelium into apical and basal compartments (de Kretser et al., 1998). Toxicity studies indicate that one of the targets of xenobiotics is the blood-testis barrier in testis (Chung and Cheng, 2001; Wan et al., 2013). This knowledge reveals that blood-testis barrier integrity is a crucial parameter for displaying toxicant induced male reproductive disturbances. Dysfunction of blood-testis barrier may directly affect spermatogenesis and sperm counts and may be related to infertility. In the present study, SerW3 cells (17 day old rat Sertoli cells) as a commonly used model for toxicity studies were chosen, because Sertoli cells were reported to be the main target of toxic substances *in vivo* and *in vitro* conditions. Additionally, SerW3 cell line has a characteristic morphology like primary Sertoli cells expressing tight junctions between adjacent cells and exerting functional features (Pognan et al., 1997). SerW3 cells also mimic Sertoli cell barrier *in vitro* because these cells expressed junctional proteins such as occludin, ZO-1, N-cadherin and gap junctional protein

* Corresponding author.
E-mail address: elifkush@hacettepe.edu.tr (E. Karacaoğlu).

http://dx.doi.org/10.1016/j.etap.2017.10.005
Received 26 April 2017; Received in revised form 9 October 2017; Accepted 10 October 2017
Available online 12 October 2017

connexin43 (Fiorini et al., 2004).

To date, there was no study addressed on the cytotoxic effects of T-2 toxin related to Sertoli cell junctional barrier and the mechanism of toxicity of T-2 toxin on reproductive system is still remained unclear. In the present study, we focused on revealing whether T-2 toxin causes cytotoxicity and disrupts junctional complexes in SerW3 cell line as an *in vitro* model of blood-testis barrier. Disruption of junctional complexes may be an underlying mechanism in the reproductive toxicity of T-2 toxin. Our hypothesis in this study: T-2 toxin at increasing doses caused cytotoxicity in SerW3 cells and junctional complexes could be early toxicity biomarkers for screening toxicity in Sertoli cells. For this reason, SerW3 cells were exposed to T-2 toxin for 24 and 48 h to investigate the cytotoxicity and several junctional protein analyses were performed as early targets of cytotoxicity were performed. In addition Sertoli cell barrier integrity was tested physiologically for monitoring the barrier function.

2. Material and methods

2.1. Chemicals

T-2 toxin derived from *Fusarium tricinctum* ($C_{24}H_{34}O_9$) with a crystalline solid phase, ≥98% purity was used for the experiments (Cat no:11444, Cayman, USA). T-2 toxin is highly stable and soluble in ~0.5 mg/ml 1:1 DMSO:PBS, ~20 mg/ml in EtOH and ~30 mg/ml in DMF or DMSO (Cat no:11444, Cayman, USA). In the present study, T-2 toxin was dissolved in DSMO as 2.5 mg/ml. Stock solution was diluted to working solutions with DMEM culture media (final concentration of DMSO was < 0.1%). Dulbecco's MEM (3.7 g/l NaHCO3, 4,5 g/l glucose with stable glutamine, Cat no: FG 0445, Biochrom, Germany), fetal bovine serum-gamma irradiated (FBS) (Cat no: S0115GM, Biochrom, Germany), trypsin/EDTA solution-0.05%0.02% (w/v) in PBS w/o Ca^{2+} and w/o Mg^{2+}(Cat no: 02L2143, Biochrom, Germany), trypan blue 0.5% (Cat no: L6323, Biochrom, Germany), penicillin-streptomycin solution 10000 U/ml (100x) (Cat no: PST999, Bioshop, Canada), DMSO cell culture grade (Cat no: A3672, Applichem, Germany), MTT (≥97.5% (HPLC), Cat no: M5655, Sigma, USA), and LDH cytotoxicity assay kit (Cat no: K313-500, Biovision, USA).

2.2. Cell culture conditions and T-2 toxin exposure

SerW3 cells were purchased from German Collection of Microorganisms and Cell Cultures (DSMZ, Germany). SerW3 cells were grown in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin solution at 37 °C in a humidified atmosphere with 5% CO_2.

Cytotoxicity was graded as follows:
non-toxic: viability was > 90%,
slight toxic: viability 60–90%,
moderately toxic: viability 30–59%
toxic: viability < 30% (Dahl et al., 2006; Kılıç Süloğlu et al., 2015).

SerW3 cells were incubated with T-2 toxin at 0–5 μg/ml doses to calculate LC_{50} [LC_{50} was calculated as 2.5 μg/ml (2500 ng/ml) by probit analysis]. Doses were chosen based on the calculated LC_{50} dose of T-2 toxin in SerW3 cells, (as folds of LC_{50}). Additionally, previous study conducted with granulosa cells (Maruniakova et al., 2014) were based for the dose selection considering the granulosa cells belonging to reproductive system because there were no study reported on Sertoli cells previously. SerW3 cells were incubated with 12, 120 and 1200 ng/ml of T-2 toxin (respectively, high dose group: half fold of LC_{50} ~ 1,2 μg/ml = 1200 ng/ml, middle dose group: 10 fold less than high dose group: 120 ng/ml, low dose group: 100 fold less than high dose group: 12 ng/ml) and culture media as a control group and with DMSO as a vehicle control group for 24 and 48 h for further analyses.

2.3. Cell viability assay

Cell viability assay was performed with MTT with minor modifications (Mosmann, 1983). Briefly, cells were cultured in 96-well culture plates at a density of 3×10^4 cells/well. Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h. Culture media were discarded and cells were exposed to T-2 toxin doses for 24 and 48 h. Afterwards, 0.5 mg/ml MTT was added into wells and incubated for 3 h at 37 °C, then MTT solution was discarded and formazan crystals were dissolved in isopropanol. Finally, absorbance of the samples were read in microplate spectrophotometer at 570 nm (BIO-TEK μQuant, BIO-TEK Instruments, Inc, USA). Experiments were performed triplicate. Absorbance belonging to control group was assigned as 100%. Cell viability percent was calculated as:

$$viability\% = \frac{absorbance\ of\ sample}{absorbance\ ofcontrol\ sample} \times 100$$

2.4. Lactate dehydrogenase (LDH) cytotoxicity assay

Lactate dehydrogenase (LDH) cytotoxicity test was performed according to instructions of the commercial kit. Briefly, SerW3 cells were cultured in 96-well cell culture plates at a density of 2×10^4 cells/well and incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h. Culture media was discarded and cells were exposed to T-2 toxin doses for 24 and 48 h. In order to calculate cytotoxicity percent in this assay, a high control group (non-treated cells were exposed to lysis solution) was needed for max LDH levels. All culture media were collected and centrifuged at 600*g* for 10 min. Then 100 μl of LDH reaction mix were added onto 10 μl supernatant of sample and incubated for 30 min at room temperature. Absorbance of samples were read at 450 nm and 650 nm reference wavelength in microplate spectrophotometer (BIO-TEK μQuant, BIO-TEK Instruments, Inc, USA). Experiments were performed triplicate. Cytotoxicity percent was calculated as:

$$\text{cytotoxicity}\% = \frac{absorbance\ of\ sample - absorbance\ of\ control\ sample}{(absorbance\ of\ high\ control\ sample - absorbance\ of\ control\ sample)} \times 100$$

2.5. Trypan blue exclusion assay

SerW3 cells were cultured in 6-well cell culture plates at a density of 3×10^5 cells/ml and incubated for 24 h. Culture media was replaced with T-2 toxin doses for 24 and 48 h. Thereafter, culture media was collected to count floating cells. Adherent cells were treated with trypsin/EDTA solution. Cell samples were dyed with 0.5% trypan blue solution and counted by using Bürker counting chamber for live/death cells under light microscope (Olympus CX21, USA). Experiments were performed triplicate. Cell viability percent was calculated as:

$$viability\% = \frac{number\ of\ viable\ cells}{(number\ of\ viable\ cells + number\ of\ death\ cells)} \times 100$$

2.6. Immunolabelling of junctional proteins

SerW3 cells were cultured in 8-well chamber slides at a density of 5×10^4 cells/well. After incubation with T-2 toxin doses for 24 and 48 h, cells were fixed in −20 °C methanol for 15 min and were washed with PBS. Cells were incubated with avidin/biotin blocking kit (Vector Laboratuaries, USA). Additionally, cells were incubated with 5% goat serum for 20 min at room temperature to prevent non-specific binding. Primary antibodies were diluted in 5% goat serum. Cells were incubated with 20 μg/ml occludin antibody (PA5-20755, Thermo Scientific, USA), 5 μg/ml N-cadherin antibody (PA5-19486, Thermo Scientific, USA), 1 μg/ml β-catenin antibody (PA5-19469, Thermo Scientific, USA) or 3 μg/ml ZO-1 antibody (40–2200, Invitrogen, USA)