Physiol. Res. 66: 705-708, 2017

RAPID COMMUNICATION

The Effect of HT-2 Toxin on Ovarian Steroidogenesis and Its Response to IGF-I, Leptin and Ghrelin in Rabbits

A. KOLESAROVA[1], N. MARUNIAKOVA[1], A. KADASI[1], M. HALENAR[1], M. MARAK[1], A. V. SIROTKIN[2]

[1]Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic, [2]Department of Zoology and Anthropology, Constantine the Philosopher University, Nitra, Slovak Republic

Received February 5, 2017
Accepted May 2, 2017

Summary

T-2 toxin and its metabolite HT-2 toxin are one of the most toxic mycotoxins of type A-trichothecenes, which are produced mainly by *Fusarium* species. Therefore, study of *Fusarium* toxins T-2 toxin and HT-2 toxin is an essential issue because they could also play role in failures of reproductive functions as well as endocrine system of domestic animals. Assessment of the effect of A-trichothecene mycotoxin HT-2 toxin alone or combined with insulin-like growth factor (IGF-I), leptin and ghrelin on estradiol secretion by rabbit ovarian fragments *in vitro* was done. Rabbit ovarian fragments were incubated without (control group) or with HT-2 toxin, or its combinations with IGF-I, leptin and ghrelin at various concentrations for 24 h. Secretion of 17β-estradiol was determined by ELISA. Firstly, HT-2 toxin at the doses 10 and 100 $ng.ml^{-1}$, but not at 1 $ng.ml^{-1}$ decreased 17β-estradiol secretion by ovarian fragments. Secondly, 17β-estradiol secretion was not affected by HT-2 toxin exposure combined with growth factor IGF-I, metabolic hormones leptin and ghrelin. In conclusion, HT-2 toxin has potent direct dose-dependent effects on ovarian steroidogenesis in rabbits. These direct effects of HT-2 mycotoxin on ovarian steroidogenesis could impact negatively on the reproductive performance of rabbits.

Key words

Trichothecenes • IGF-I • Leptin • Ghrelin • Steroidogenesis • Ovary • Rabbit

Corresponding author

A. Kolesarova, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, 949 76 Nitra, Slovak Republic. E-mail: Adriana.Kolesarova@uniag.sk

Fusarium mycotoxins such as trichothecenes occur on a worldwide basis in cereal grains, animal feeds and forages (McNamee *et al.* 2017). *Fusarium* mycotoxins have a great impact on ovarian functions of pigs (Ranzenigo *et al.* 2008, Caloni *et al.* 2009, Medvedova *et al.* 2011, Kolesarova *et al.* 2012, Maruniakova *et al.* 2014, Halenar *et al.* 2015) and rabbits (Maruniakova *et al.* 2015). The influence of trichothecene mycotoxin, such as T2 toxin and its metabolite HT-2 toxin, on porcine ovarian granulosa cells (Maruniakova *et al.* 2014) and rabbit fragments (Maruniakova *et al.* 2015) focused on the processes of steroidogenesis was described in the previous studies. HT-2 toxin is a primary metabolite in the gut and is absorbed into the blood after ingestion of T-2 toxin. Metabolism continues in the liver (with biliary excretion), resulting in a substantial, combined first-pass effect in the gut and liver (Canady *et al.* 2010). Not only these toxins but also their plant metabolites may contribute to toxicological effects (Meng-Reiterer *et al.* 2016). The toxic effects of T-2 toxin and its metabolite HT-2 toxin could not be differentiated, and the toxicity of T-2 toxin *in vivo* might be due at least partly to HT-2 toxin (Creppy *et al.* 2002). The occurrence of *Fusarium* mycotoxins in cereals causes the greatest financial loss to the animal industry, because their ingestion induces a reduction in feed intake and growth, as well as immunosuppression

© 2017 Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
Fax +420 241 062 164, e-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

and reproductive disorders (Charmley *et al.* 1994). T-2 toxin induced apoptosis in ovarian granulosa cells of rats through reactive oxygen species-mediated mitochondrial pathway (Wu *et al.* 2011). Decreased porcine granulosa cell proliferation was reported by Caloni *et al.* (2009). Disruption of the process of folliculogenesis and corpus luteum formation can lead to adverse reproductive outcomes such as anovulation, infertility, decreased fecundity, estrogen deficiency, and premature ovarian failure (Craig *et al.* 2011). The panel on contaminants in the food chain of the European Food Safety Authority has set the tolerable daily intake for the sum of both, T-2 and HT-2 toxin, at 100 ng.kg^{-1} body weight (EFSA 2011). For a 2 kg rabbit, it is estimated that the lower-bound and upper-bound exposure to the sum of T-2 and HT-2 toxins would be 2.0 and 3.4 μg.day^{-1}, respectively. Expressed on a body weight basis, this is equivalent to 0.98 and 1.7 μg.kg^{-1} b.w. per day, respectively (EFSA 2011).

The aims of this *in vitro* study were to examine the effect of HT-2 toxin alone or combined with growth factor IGF-I, and the metabolic hormones leptin and ghrelin on the release of steroid hormone 17β-estradiol by rabbit ovarian fragments.

Adult female New Zealand white rabbits (n=20, age 150 days, weight 4.00±0.5 kg) from an experimental farm of the Animal Production Research Centre Nitra, Slovak Republic were used. Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic (no. 3398/11-221/3) and Institutional Ethics Committee. Ovaries were collected and transported to the laboratory at the ambient temperature in a glass container within 30 min of slaughter. Thereafter ovaries were washed in sterile physiological solution and dissected using a blade knife to 8 approximately equal parts (weight 4.8-5.6 mg). These ovarian fragments were washed again 2 times in sterile physiological solution and cultured in 1 ml of medium supplemented with 10 % fetal calf serum and 1 % antibiotic-antimycotic solution and without (control group) or with HT-2 toxin (1, 10 and 100 ng.ml^{-1}), or its combinations with IGF-I (100 ng.ml^{-1}), leptin (1000 ng.ml^{-1}) or ghrelin (500 ng.ml^{-1}) at 37 °C and 5 % CO_2 in humidified air. Further culture was performed for 24 h, and then the culture media from plate wells were aspirated and kept at -80 °C for ELISA. Intra- and inter-assay coefficients for 17β-estradiol were ≤4 % and ≤9.3 %, respectively. Each experimental group was represented by four culture wells of ovarian fragments (each dose = 4 replicates, biological parallels). Assays of hormone level in the incubation media was performed in duplicate. The data presented are means of values obtained from three separate experiments performed on separate days using separate pools of ovaries from 20 animals. Significance of differences between the control and experimental groups were evaluated by two-way ANOVA and t-test. The data are expressed as means ± SEM. Differences were compared for statistical significance at the level P<0.05.

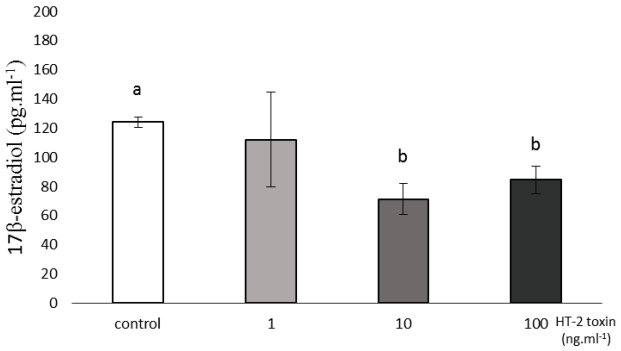

Fig. 1. 17β-estradiol release by rabbit ovaries after addition of HT-2 toxin. Differences between the groups were evaluated by two-way ANOVA, t-test. a, b – significant differences (P<0.05) between control and experimental groups. The data are expressed as means ± SEM. ELISA.

17β-estradiol release by rabbit ovarian fragments after HT-2 toxin addition is shown in Figure 1. Firstly, 17β-estradiol release by rabbit ovarian fragments was significantly (P<0.05) inhibited by HT-2 toxin treatment at the doses 10 and 100 ng.ml^{-1}, but not at 1 ng.ml^{-1}. Our results are in accordance with results of Ndossi *et al.* (2012) who detected that estradiol levels were significantly reduced (P<0.0001) in cells exposed to 5 ng.ml^{-1} of T-2 toxin by about 2.5 folds less than in control cells, and after addition of same concentration of HT-2 toxin estradiol concentrations decreased significantly (P<0.05). T-2 toxin may be able to alter growth of the granulosa layer within ovarian follicles in addition to their effect on steroidogenesis (Caloni *et al.* 2009). Medvedova *et al.* (2011) observed significant (P<0.05) stimulation of progesterone release by porcine ovarian granulosa cells after addition of 1000 ng.ml^{-1} of deoxynivalenol (DON) which is B-trichothecene. On the other hand, T-2 toxin and HT-2 toxin treatments to ovarian fragments of rabbits did not cause significant (P>0.05) changes in progesterone release at the doses 0.01, 0.1, 1, 10 and 100 ng.ml^{-1} (Maruniakova *et al.* 2015).

Secondly, 17β-estradiol release by rabbit ovarian fragments was not significantly (P>0.05) influenced by HT-2 toxin addition combined with IGF-I, leptin or ghrelin. There are less data about the effect of T-2 toxin and HT-2 toxin in combination with IGF-I, leptin or ghrelin on secretory activity of ovarian cells to produce estradiol hormone. However, steroid hormone progesterone was examined after T2 and HT-2 toxins combined with the growth factors IGF-I and the metabolic hormones leptin and ghrelin (Maruniakova *et al.* 2015). T-2 toxin, but not HT-2 toxin combined with IGF-I has been shown to be potential regulator of progesterone secretion in rabbit ovarian fragments. T-2 toxin combined with IGF-I decreased progesterone release by rabbit ovarian fragments (Maruniakova *et al.* 2015). Our previous study showed that T-2 and HT-2 toxins combined with IGF-I could modify progesterone secretion by porcine ovarian granulosa cells and potentially regulate process of steroidogenesis in porcine ovaries (Maruniakova *et al.* 2014). Previous authors indicated that T-2 toxin at 1, 3, 30 and 300 ng.ml^{-1} completely inhibited FSH plus IGF-I-induced estradiol production by porcine granulosa cells. At 30 and 300 ng.ml^{-1}, T-2 toxin completely inhibited FSH plus IGF-I-induced progesterone production (Caloni *et al.* 2009). Leptin is a hormone that mediates the effect of the metabolic state on several biological functions, including reproduction. Leptin affects reproductive functions *via* alterations in the release of hormonal regulators (Harrath *et al.* 2016). Its pivotal role is in the development of puberty and in the subsequent regulation of reproductive functions (Chan *et al.* 2006). In our study HT-2 toxin did not affect changes in leptin-induced estradiol secretion by rabbit ovaries. Our data confirmed findings of Maruniakova *et al.* (2015) on the progesterone release by rabbit ovarian fragments after T-2/HT-2 toxins combined with leptin. Similarly, T-2 and HT-2 toxins with leptin did not induce changes in progesterone secretion by rabbit ovaries. Ghrelin is expressed in very small amounts in the pancreas, lung, kidney, lymphocytes, placenta, testis, and ovaries where it may act as an autocrine/ paracrine factor (Castaneda *et al.* 2010). In our study, HT-2 toxin did not affect changes in ghrelin-induced 17β-estradiol secretion by rabbit ovaries. These results are in accordance with previous *in vitro* studies. T-2 toxin and HT-2 toxin combined with ghrelin were not shown to be potential regulators of progesterone secretion in rabbit ovarian fragments (Maruniakova *et al.* 2015).

There are limited data about the effect of trichothecene mycotoxins on process of steroidogenesis. In conclusion, HT-2 toxin has potent direct dose-dependent effects on ovarian steroidogenesis of rabbits. These direct effects of HT-2 mycotoxin on ovarian steroidogenesis could negatively impact the reproductive performance in rabbits.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects no. VEGA, 1/0039/16, KEGA 011SPU-4/2016, Slovak Research and Development Agency APVV-0304-12, APVV-15-0543, SK-FR-2015-0009 European Community under project no 26220220180: Building Research Centre „AgroBioTech" and BMBS COST Action BM1308 Sharing Advances on Large Animal Models (SALAAM).

References

CALONI F, RANZENIGO G, CREMONESI F, SPICER LJ: Effects of a trichothecene, T-2 toxin, on proliferation and steroid production by porcine granulosa cells. *Toxicon* **54**: 337-344, 2009.

CANADY AR, COKER RD, EGAN SK, KRSKA R, OLSEN M, RESNIK S, SCHLATTER J: T-2 and HT-2 toxins. IPCS Inchem home, 2010. http://www.inchem.org/documents/jecfa/jecmono/v47je06.htm.

CASTANEDA TR, TONG J, DATTA R, CULLER M, TSCHOP MH: Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol* **31**: 44-60, 2010.

CHAN JL, MATARESE G, SHETTY GK, RACITI P, KELESIDIS I, AUFIERO D, DE ROSA V, PERNA F, FONTANA S, MANTZOROS CS: Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. *Proc Natl Acad Sci* **103**: 8481-8486, 2006.

CHARMLEY LL, ROSENBERG A, TRENHOLM HL: Factors responsible for economic losses due to Fusarium mycotoxin contamination of grains, food and feedstuffs. In: *Mycotoxins in Grain: Compounds Other than Aflatoxin*. MILLER JD, TRENHOL HL (eds), St. Paul, Eagan Press, USA, 1994, pp 471-486.

CRAIG ZR, WANG W, FLAWS JA: Endocrine-disrupting chemicals in ovarian function: effects on steroidogenesis, metabolism and nuclear receptor signaling. *Reproduction* **142**: 633-646, 2011.

CREPPY EE: Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Lett* **127**: 19-28, 2002.

EFSA (European Food Safety Authority): Scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. *EFSA J* **9**: 2481, 2011. http://www.efsa.europa.eu/en/efsajournal/doc/2481.pdf

HALENAR M, MEDVEDOVA M, MARUNIAKOVA N, KOLESAROVA A: Assessment of a potential preventive ability of amygdalin in mycotoxin-induced ovarian toxicity. *J Environ Sci Health B* **50**: 411-416, 2015.

HARRATH AH, ØSTRUP O, RAFAY J, KONIČKOVÁ FLORKOVIČOVÁ I, LAURINCIK J, SIROTKIN AV: Metabolic state defines the response of rabbit ovarian cells to leptin. *Reprod Biol* **17**: 19-24, 2017.

KOLESAROVA A, CAPCAROVA M, MARUNIAKOVA N, LUKAC N, CIERESZKO RE, SIROTKIN AV: Resveratrol inhibits reproductive toxicity induced by deoxynivalenol. *J Environ Sci Health A* **47**: 1329-1334, 2012.

MARUNIAKOVA N, KADASI A, SIROTKIN AV, BULLA J, KOLESAROVA A: T-2 toxin and its metabolite HT-2 toxin combined with insulin-like growth factor-I modify progesterone secretion by porcine ovarian granulosa cells. *J Environ Sci Health A* **49**: 1-6, 2014.

MARUNIAKOVA N, KADASI A, SIROTKIN AV, LEŚNIAK A, FERREIRA AM, BULLA J, KOLESAROVA A: Assessment of T-2 toxin effect and its metabolite HT-2 toxin combined with insulin-like growth factor I, leptin and ghrelin on progesterone secretion by rabbit ovarian fragments. *J Environ Sci Health B* **50**: 128-134, 2015.

MCNAMEE SE, BRAVIN F, ROSAR G, ELLIOTT CT, CAMPBELL K: Development of a nanoarray capable of the rapid and simultaneous detection of zearalenone, T2-toxin and fumonisin. *Talanta* **164**: 368-376, 2017.

MEDVEDOVA M, KOLESAROVA A, CAPCAROVA M, LABUDA R, SIROTKIN AV, KOVACIK J, BULLA J: The effect of deoxynivalenol on the secretion activity, proliferation and apoptosis of porcine ovarian granulosa cells *in vitro*. *J Environ Sci Health B* **46**: 213-219, 2011.

MENG-REITERER J, BUESCHL C, RECHTHALER J, BERTHILLER F, LEMMENS M, SCHUHMACHER R: Metabolism of HT-2 toxin and T-2 toxin in oats. *Toxins* **8**: 364, 2016.

NDOSSI DG, FRIZZELL C, TREMOEN NH, FÆSTE CK, VERHAEGEN S, DAHL E, ERIKSEN GS, SØRLIE M, CONNOLLY L, ROPSTAD E: An *in vitro* investigation of endocrine disrupting effects of trichothecenes deoxynivalenol (DON), T-2 and HT-2 toxins. *Toxicol Lett* **214**: 268-278, 2012.

RANZENIGO G, CALONI F, CREMONESI F, AAD PY, SPICER LJ: Effects of Fusarium mycotoxins on steroid production by porcine granulosa cells. *Anim Reprod Sci* **107**: 115-130, 2008.

WU J, JING L, YUAN H, PENG SQ: T-2 toxin induces apoptosis in ovarian granulosa cells of rats through reactive oxygen species-mediated mitochondrial pathway. *Toxicol Lett* **202**: 168-177, 2011.