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ASSESSMENT OF T-2 TOXIN EFFECT AND ITS COMBINATION WITH GROWTH FACTOR AND METABOLIC HORMONES ON 17β-ESTRADIOL SECRETION BY RABBIT OVARIAN FRAGMENTS *IN VITRO*

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ABSTRACT

Assessment of T-2 effect and its combination with growth factor IGF-I, metabolic hormones leptin and ghrelin on 17β-estradiol secretion by rabbit ovarian fragments was studied. Rabbit ovarian fragments were incubated without (control group) or with alone T-2 toxin (0.01; 0.1; 1; 10; and 100 ng.mL^{-1}), or its combination with insulin-like growth factor I - IGF-I (100 ng.mL^{-1}), leptin (1000 ng.mL^{-1}) or ghrelin (500 ng.mL^{-1}) for 24 hours. Secretion of 17β-estradiol was determined by ELISA. T-2 toxin was not shown to be potential regulator of 17β-estradiol secretion in rabbit ovarian fragments. On the other hand T-2 toxin at all used doses combined with IGF-I significantly (P<0.05) inhibited 17β-estradiol secretion by the fragments. Similarly, 17β-estradiol secretion was significantly (P<0.05) inhibited by T-2 toxin at the highest used doses (10 and 100 ng.mL^{-1}) combined with leptin (1000 ng.mL^{-1}). On the other hand T-2 toxin combined with ghrelin was not shown to be potential regulator of 17β-estradiol secretion in rabbit ovarian fragments. Results in this study showed that trichothecene as T-2 toxin combined with IGF-I or leptin was able to modulate 17β-estradiol secretion in rabbit ovarian fragments *in vitro*. We suggest their possible involvement to the process of steroidogenesis.

Keywords: T2-toxin, steroidogenesis, IGF-I, leptin, ghrelin, ovary, rabbit

INTRODUCTION

T-2 is type A trichothecene mycotoxins produced by *Fusarium (F.) poae, F. sporotrichioides, F. kyushuense and F. langsethiae* **(Glenn, 2007)** and contaminates foods, animal foods and agricultural products **(IARC, 1993; WHO, 1990)**. The presence of T-2 toxin was found in grains such as wheat, maize, oats, barley, rice, beans, and soya beans as well as in some cereal-based products (**Patel *et al.*, 1996; Schollenberger *et al.*, 1999**). The toxicity of the T-2 toxin and related mycotoxins can be affected by a variety of factors such as administration route, time of exposure, the number of exposures, dose, animal's age, sex and overall health, and presence of other mycotoxins (**WHO, 1990; JECFA, 2001**). T-2 toxin is readily metabolized by mammalian gut microflora to several metabolites. 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**). There is little data about the effect of T-2 toxin combined with insulin-like growth factor I - IGF-I and the metabolic hormones leptin and ghrelin on animal ovarian cells. A previous study describes the effect of T-2 toxin and its metabolite HT-2 toxin acting alone or combined with growth factor IGF-I and metabolic hormones leptin and ghrelin on progesterone secretion by rabbit ovarian fragments *in vitro* (**Maruniakova *et al.*, 2015**). The aim of this study was to assess the effect of A-trichothecene mycotoxin T-2 and its combination with growth factor IGF-I and the metabolic hormones leptin and ghrelin on 17β-secretion by rabbit ovarian fragments *in vitro*.

MATERIAL AND METHODS

Adult female New Zealand white rabbits (n= 20) from an experimental farm of the Animal Production Research Centre Nitra, Slovak Republic were used. Rabbits (age 150 days, weight 4.00 ± 0.5 kg) were housed in individual flat-deck wire cages under a constant photoperiod of 12h of day-light, the temperature 20–24°C and humidity 55±10%. 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 Ethics committee.

Ovarian fragments

Ovaries were collected and transported to the laboratory at the ambient temperature in a glass container within 30 minutes of slaughter. There after ovaries were washed in 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 T-2 toxin, or their combinations with growth factor IGF-I, leptin and ghrelin (Table 1) at 37 °C and 5 % CO_2 in humidified air at the various doses. Further culture were performed for 24 h, and then the culture media from plate wells were aspirated and kept at –80 ºC for further enzyme-linked immunosorbent assay (ELISA, (Dialab, Wiener Neudorf, Austria). ELISA reader (Thermo Scientific Multiskan FC, Vantaa, Finland). Intra- and inter-assay for 17β-estradiol intra- and inter-assay coefficients were ≤9 % and ≤10 %. Sensitiveness for 17β-estradiol was 8.68 pg.mL^{-1}.

Table 1 Used doses of T-2 toxin or its combination with IGF-I, leptin and ghrelin applied to ovarian fragments of rabbits (24h long treatment)

SUBSTANCES (ng.mL^{-1})	**DOSES** (ng.mL^{-1})					**METHOD**
T-2 toxin	0.01	0.1	1	10	100	
Group	**AI**	**BI**	**CI**	**DI**	**EI**	
T-2 toxin	0.01	0.1	1	10	100	
+	+	+	+	+	+	ELISA
IGF-I	100	100	100	100	100	
Group	**AL**	**BL**	**CL**	**DL**	**EL**	
T-2 toxin	0.01	0.1	1	10	100	
+	+	+	+	+	+	
Leptin	1000	1000	1000	1000	1000	
Group	**AG**	**BG**	**CG**	**DG**	**EG**	
T-2 toxin	0.01	0.1	1	10	100	
+	+	+	+	+	+	
Ghrelin	500	500	500	500	500	

Statistical analysis

Each experimental group was represented by four culture wells of ovarian fragments (each dose = 4 replicates, biological parallels). Assays of hormone levels in the incubation media were performed in duplicate. Significance of differences between the control and experimental groups were evaluated by two-way ANOVA and t-test using the statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA). The data are expressed as means ± SEM. Differences were compared for statistical significance at the *p*-level less than 0.05 ($P<0.05$).

RESULTS AND DISCUSSION

17β-estradiol release by rabbit ovarian fragments after addition of T2 toxin or its combination with growth factor IGF-I, leptin and ghrelin was studied (Figs. 1-4). T-2 toxin treatment at all used doses did not significantly ($P>0.05$) affect 17β-estradiol release by the fragments (Fig. 1). On the other hand 17β-estradiol release was significantly ($P<0.05$) inhibited by T2-toxin combined with IGF-I at all experimental groups (Fig. 2). 17β-estradiol secretion was significantly ($P<0.05$) inhibited by T-2 toxin at the highest used doses combined with leptin (Fig. 3). Combination of T-2 toxin at all doses with ghrelin caused no significant ($P>0.05$) inhibition of 17β-estradiol secretion by the fragments (Fig. 4).

The effect of various *Fusarium* toxins on reproductive functions focused on ovarian steroidogenesis was described in the previous reports (**Medvedova *et al.*, 2011; Kolesarova *et al.*, 2012; Maruniakova *et al.*, 2014; Maruniakova *et al.*, 2015**). In our in *vitro* study the effect of T-2 toxin (at the doses 0.01, 0.1, 1, 10, 100 ng.mL^{-1}) on 17β-estradiol secretion by rabbit ovarian fragments was not found. Similarly, T-2 toxin (at the doses 0.01, 0.1, 1, 10, 100 ng.mL^{-1}) and its metabolite HT-2 toxin (at the doses 0.01, 0.1, 1, 10, 100 ng.mL^{-1}) did not cause significant changes in progeterone release by rabbit ovarian fragments (**Maruniakova *et al.*, 2015**). **Ndossi *et al.* (2012)** detected that estradiol levels were significantly reduced ($P<0.0001$) in H295R cells exposed to 5 ng.ml^{-1} of T-2 toxin. Combination of T-2 toxin at all used doses (0.01, 0.1, 1, 10, 100 ng.mL^{-1}) with IGF-I (100 ng.mL^{-1}) significantly ($P<0.05$) inhibited 17β-estradiol release by rabbit ovarian fragments. Similarly other authors indicate that T-2 toxin at 1, 3, 30 and 300 ng.mL^{-1} completely inhibited FSH plus IGF-I-induced estradiol production (**Caloni *et al.*, 2009**). Another authors **Frizzel *et al.* (2013)** studied the effect of ochratoxin (OTA) on steroid production focused on hormone estradiol. They indicated that treatment of OTA at the dose 1000 ng.mL^{-1} increased the production of estradiol over 3 times. **Kobayashi-Hattori *et al.* (2011)** observed that deoxynivalenol which is B-trichothecene mycotoxin reduced plasma insulin, leptin, insulin-like growth factor I, and insulin-like growth factor acid labile subunit as well as increased hypothalamic mRNA level of the orexigenic agouti-related protein. The release of progesterone was significantly ($P < 0.05$) inhibited by T2-toxin addition (at the doses 0.01, 0.1, 1, 10, 100 ng.mL^{-1}) combined with IGF-I (100 ng.mL^{-1}) at all experimental groups (**Maruniakova *et al.*, 2015**). On the other hand, progesterone release was not affected significantly ($P > 0.05$) by HT-2 toxin (at the doses 0.01, 0.1, 1, 10, 100 ng.mL^{-1}) combined with IGF-I (100 ng.mL^{-1}) **Maruniakova *et al.*, 2015**). Metabolic hormones play an important role in regulation of reproduction. Leptin plays a pivotal role in the development of puberty and in the subsequent regulation of reproductive functions (**Chan *et al.*, 2006**). Our previous report describes the effect of T-2 toxin and its metabolite HT-2 toxin combined with leptin and ghrelin on progesterone secretion by rabbit ovarian fragments (**Maruniakova *et al.*, 2015**). There is no information about effect of T-2 toxin combined with leptin or ghrelin on secretion of 17β-estradiol by rabbit ovarian fragments. T-2 toxin together with leptin was shown to be potential dose-dependent regulator of 17β-estradiol secretion in rabbit ovarian fragments. On the other hand T-2 toxin and HT-2 toxin combined with leptin were not shown to be potential regulators of progesterone secretion in rabbit ovarian fragments (**Maruniakova *et al.*, 2015**). 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 T-2 toxin together with ghrelin was not shown to be potential regulators of 17β-estradiol secretion in rabbit ovarian fragments. Similarly, 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**).

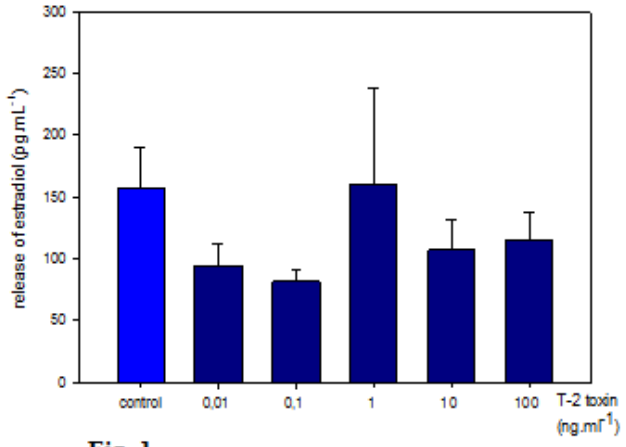

Fig. 1

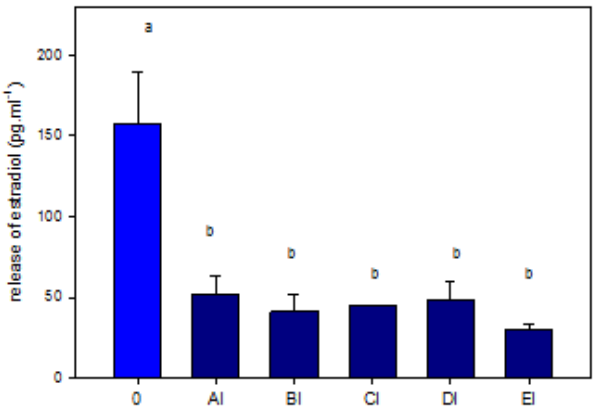

Fig. 2

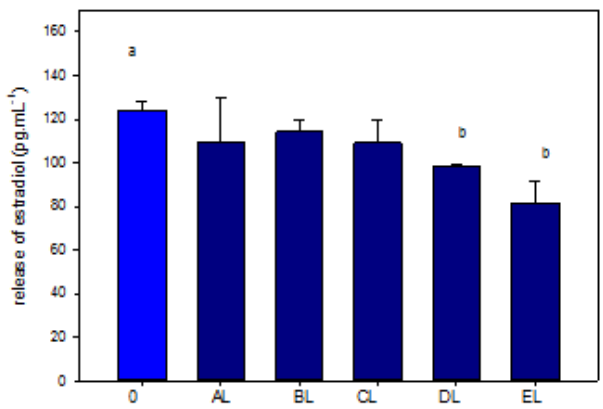

Fig. 3

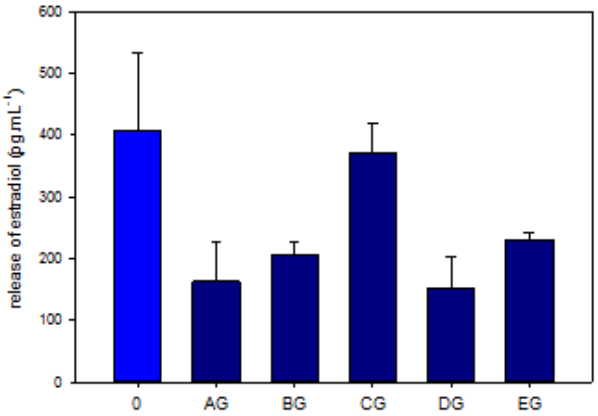

Fig. 4

Figures 1-4 17β-estradiol release by rabbit ovarian fragments after addition of T-2 toxin (Fig. 1), T-2 toxin combined with IGF-I (Fig. 2), T-2 toxin combined with leptin (Fig. 3) and T-2 toxin combined with ghrelin (Fig. 4). Signs *a*,*b* denote value significantly ($P<0.05$) different from control group. 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. ELISA.

CONCLUSION

Toxic substances can influence the process of steroidogenesis depending on used doses, time exposure as well as different cell types. In our study the effect of the T-2 toxin on secretion of 17β-estradiol secretion in rabbit ovarian fragments was not found. On the other hand its combination with growth factor IGF-I modified the secretion of 17β-estradiol. Similarly, T-2 toxin combined with leptin modified of 17β-estradiol secretion by rabbit ovarian fragments. Results in this study showed that trichothecene as T-2 toxin combined with IGF-I or leptin was able to modulate 17β-estradiol secretion in rabbit ovarian fragments *in vitro*. We suggest their possible involvement to the process of steroidogenesis.

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