Effect of atrazine and zearalenone on the number of receptor binding sites for ^{3}H-estradiol in the rat uterus cytosol

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ABSTRACT: The effect of a daily dose of 14 mg atrazine and/or 2.5 mg zearalenone that were administered to rats during 5 days of estrous cycle on the number of receptor binding sites for ^{3}H-estradiol in uterine cytosol was investigated. Comparison with a control group of animals showed no effect of atrazine influence on the number of receptor binding sites for ^{3}H-estradiol in the rat uterine cytosol until day 6 of the experiment, when a significantly lower value was recorded ($P < 0.05$). In the group of animals administered zearalenone, a decline in the number of receptor binding sites for ^{3}H-estradiol in uterine cytosol was observed. On day 4 of the experiment, the number of binding sites was significantly greater ($P < 0.05$) in this group than in the control group of animals, whereafter it gradually decreased to a significantly lower value ($P < 0.05$) as compared to the control group on day 6. In the group of animals administered a mixture of atrazine and zearalenone, the number of receptor binding sites for ^{3}H-estradiol in uterine cytosol did not decrease but showed a similar pattern as in the control group of animals, however, at a statistically significantly ($P < 0.05$) higher level.

Keywords: atrazine; zearalenone; ^{3}H-estradiol receptor; ovary; uterus; rat

Atrazine and zearalenone are toxins that can enter the animal body by feed and water, when reproduction impairments in female animals can occur. Atrazine, an s-triazine herbicide, has been used as herbicide mostly in corn fields. Because of its low reactivity, atrazine is highly stable in nature. After a longterm use its residues have been detected in soil (Goh *et al.*, 1993) and surface water (Hrlec, 1990) as well as in underground water (Graham, 1991) and drinking water (Gojmerac *et al.*, 1996). In addition, atrazine residues have also been found in pretreated forage fodder (Norris and Fong, 1983). Atrazine inhibits biochemical processes that are responsible for normal reproductive function in both male and female animals (Kniewald *et al.*, 1979, 1987; Gojmerac *et al.*, 1995, 1996). In the rat uterus cytosol, atrazine inhibits the development of the estradiol – receptor complex both *in vivo* and *in vitro* (Težak *et al.*, 1992), and delays estrous cycle by protracting the phase of diestrus (Šimić *et al.*, 1994).

Zearalenone is a mycotoxin produced by molds of the genus *Fusarium*. Contamination with *Fusarium* spp. is quite common in this climate zone, ranging from 20% (Munk and Topolko, 1978; Mitak *et al.*, 2001) to even 82% (Kralj *et al.*, 1988), depending on weather conditions. Zearalenone induces characteristic changes, mainly in swine, which are known as the vulvovaginitis syndrome followed by a continuous estrus (Mirocha *et al.*, 1971; Chang *et al.*, 1979; Blaney *et al.*, 1984; Glavits and Vanyi, 1995), and reproduction impairments caused by lower concentrations of zearalenone (Kordic *et al.*, 1992; Vanyi *et al.*, 1994). The physiological activity of zearalenone and its derivatives can be explained by their competition with 17β-estradiol for the specific receptor binding site and interference with steroid enzymes (Kiang *et al.*, 1978; Boyd and Witliff, 1978; Kiessling and Petterson, 1978; Thouvenot and Morfin, 1980; Olsen *et al.*, 1981, 1985).

MATERIAL AND METHODS

Toxins

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine). A pure sample containing 97.5% of atrazine (Herbos Chemical Industry, Sisak, Croatia) was ad-

mixed to liquid paraffin (paraffinum liquidum, Kemika, Zagreb, Croatia) used as a vehicle. The concentration of the active substance was 27.8 mg/ml. Experimental animals received 0.5 ml of the active liquid paraffin suspension or 13.9 mg atrazine/animal/day *p.o.*, or 6.95 mg atrazine/100 g body weight (b.w.) per day.

Zearalenone [6-(10-hydroxy-6-oxo-trans-l-undecenyl) β-resorcyclic acid lactone)] (a desiccant (Sigma, St. Louis, USA) in crystalline form. Total amount of toxic substance was dissolved in 1.0 ml 96% ethyl alcohol and admixed to liquid paraffin used as a vehicle. The working suspension contained 5 mg of zearalenone per milliliter. Animals were administered 0.5 ml of the working suspension *p.o.* by a tube, or 2.5 mg zearalenone per day, that equals 1.25 mg/100 g body weight.

Animals

Sixty female Sprague-Dawley rats aged 90 days (sexually mature), body weight about 200 g per animal, were divided into 4 groups of 15 animals. The animals were properly caged at a temperature of 21°C, relative humidity of 56%, and 12-hour light – dark cycles. The animals were fed by a commercial chow for laboratory rats (4 RF 21, Muscedola s.r.l., Settimo Milanese, Italy), and had access to food and water *ad libitum*. During the 5 days of estrous cycle, the animals received test substances *p.o.* by a tube: group 1 atrazine, group 2 zearalenone, group 3 a mixture of atrazine and zearalenone, and group 4 liquid paraffin (control group). The administration of the experimental substances was started on the day of estrus (determination by microscopic examination of vaginal swab; Weihe, 1987), in order to ensure that each group consisted of animals in the same estrous phase. The experiment started on the day of estrus, with the next estrus expected on experiment day 5. On experiment days 4, 5 and 6 (between 9.00 and 10.00 a.m.), 5 animals from each group were sacrificed per day.

Determination of specific estrogen receptors

The animals were sacrificed by decapitation after receiving ether narcosis. Uteri were collected on days –1, 1 and +1 of expected estrus. The samples (a portion of the horn of uterus) were stored in liquid nitrogen until hrey were analysed. Then the samples were minced and transferred to a microdismembrator S (Braun, Melsungen, Germany) and stirred with a tungsten carbide ball for 45 seconds. The fine powder thus obtained was mixed with 4 parts of TED buffer (TRIS, ethyldiaminoacetic acid, dithiotretiol) and homogenized with cooling on a vortex. The homogenate was centrifuged at 3 000 × g and 4ºC for 10 minutes, then the supernatant was centrifuged again at 13 000 × g for 60 minutes in an MSE high speed centrifuge. Upon centrifugation, the fatty layer was carefully removed by a vacuum capillary, and the supernatant was used for protein and receptor determination.

Saturation analysis

Aliquot parts of the uteri (200 µl) in duplicates were incubated with increasing amounts of appropriate ligand. Total concentrations of ^{3}H-F_2 and ^{3}H-ORG2058 were 0.06–0.8 and 0.22–2.56 nM, respectively. The samples were incubated overnight (16–18 h) at 4ºC, then 500 µl of 0.5% adsorbent suspension (active charcoal and dextran) were added to each sample, and incubation was continued with stirring. Upon centrifugation, the aliquot portions of the supernatant (500 µl) were mixed with scintillation solution and measured on a sample counter emitting beta radiation (LKB Spectral 1219). Results were corrected for the value of nonspecific binding. Nonspecific binding was determined in the presence of 200-fold amount of an appropriate competitor (stable ligand). The data obtained, was analysed by a specific software, followed by printing of the results.

Statistical analysis

Statistical analysis was done by use of the STATISTICA for Windows Release 4.3", StatSoft Inc., 1993.

RESULTS AND DISCUSSION

The results of testing for estrogen receptors represent the number of binding sites in the five uterine portions from animals of the same group sacrificed on the same day of the experiment (Table 1 and Figure 1).

Atrazine administered in a concentration of 6 or 12 mg/100 g b.w. reduces the number of receptor binding sites for ^{3}H-estradiol in uterine cytosol by 35.1–29.4% after 7 days (Težak *et al.*, 1992). In the present study, however, atrazine had no effect on the number of receptor binding sites for ^{3}H-estradiol in uterine cytosol during the first 5 days of the experiment

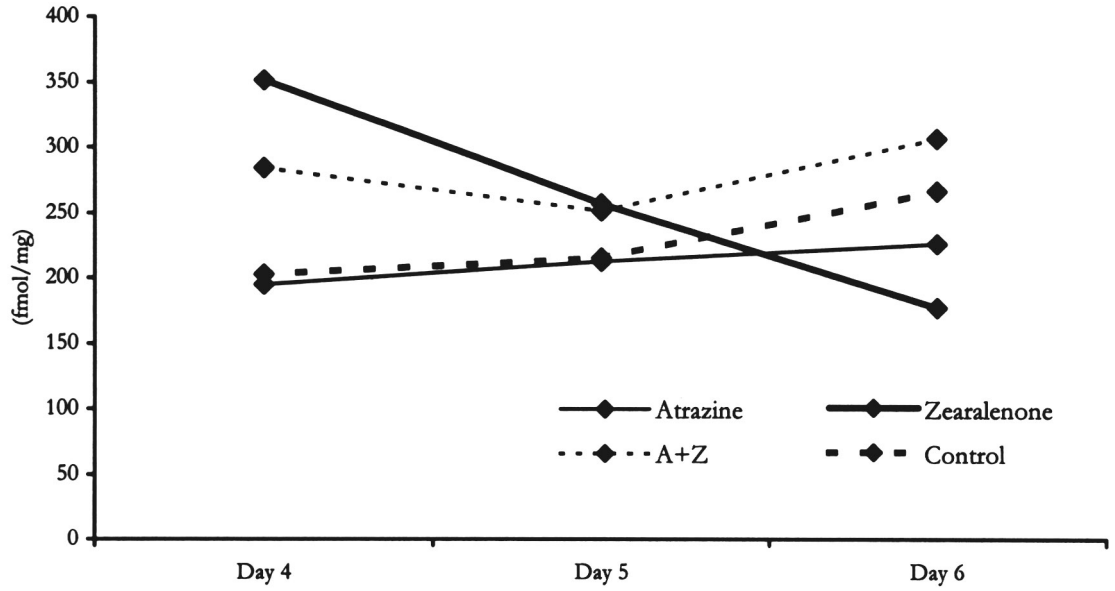

Figure 1. Number of binding sites for ^{3}H-estradiol on uterine cytosol receptors

(Table 1). A reduction in the number of binding sites ($P < 0.05$) in comparison with the control group was only recorded on the last day of the experiment.

The estrogen activity of zearalenone manifests through the specific binding of zearalenone and its metabolites to uterine receptors, as demonstrated by the test of competitive binding with 17β-estradiol (Katzenellenbogen *et al.*, 1979; Greenman *et al.*, 1979; Sheehan *et al.*, 1984). In our study, a decrease in the number of receptor binding sites for ^{3}H-estradiol in uterine cytosol was also observed with the passage of the experiment in the group of rats administered zearalenone (Figure 1) as compared to the control group. At the beginning of the experiment, a significantly higher ($P < 0.05$) number of binding sites on uterus receptors were recorded in the group that received zearalenone, which may have been due to individual differences among the experimental animals. With progressing of the experiment, the number of binding sites for ^{3}H-estradiol on uterine receptors unavoidably decreased, being significantly lower ($P < 0.05$) than the values measured in the control group on the last day of the experiment. Our results are consistent with the data reported by Kiang *et al.* (1978), who have shown that zearalenone competes with 17b-estradiol for binding to uterine estrogen receptors.

Table 1. Number of binding sites for ^{3}H-estradiol on receptors in rat uterine cytosol after treatment with atrazine and/or zearalenone

Toxins (mg/kg b.w.)	Day of experiment	Number of binding sites for ^{3}H-estradiol (fmol/mg protein ± SE)
Atrazine (69.5)	4	194.8 ± 3.30
	5	212.5 ± 5.39
	6	226.0 ± 7.05
Zearalenone (10.8)	4	351.4 ± 8.04
	5	257.0 ± 12.51
	6	177.1 ± 9.47
Atrazine + Zearalenone	4	284.1 ± 9.99
	5	251.4 ± 8.60
	6	306.3 ± 7.35
Control (0)	4	202.5 ± 1.34
	5	215.3 ± 2.49
	6	266.5 ± 2.95

The group of animals given a mixture of atrazine and zearalenone had a significantly higher number of binding sites ($P < 0.05$) for ^{3}H-estradiol on uterine receptors than the control group throughout the 3-day experiment (Table 1). Even though the curve of the number of binding sites for ^{3}H-estradiol on uterine receptors was identical to that obtained for the control group (Figure 1), a higher level of the curve appeared.

However, zearalenone in a mixture with atrazine had no major effect on the reduction of binding sites for ^{3}H-estradiol on receptors in the rat uterine cytosol

(Figure 1). This might point to an interaction of atrazine and zearalenone, because of which the compound formed had no affinity for estrogen receptors in the ovarian cytosol.

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