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Response of blastocyst–endometrium interactions in albino rats to sublethal doses of biological and synthetic insecticides

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ABSTRACT

The present study compared morphological, histochemical and histomorphometric characteristics of the sublethal effects of XenTari® and deltamethrin in blastocyst–endometrium interactions in female rats. Pregnant rats received 185, 1850 and 3700 mg of XenTari® or 1.0, 2.0 and 4.0 mg of deltamethrin (all doses per kg of body weight) and were sacrificed on the seventh day of pregnancy. The rats treated with the higher doses of insecticides exhibited a significant reduction in the number of implantation sites, vacuolated trophoblast cells, rare cytotrophoblasts, accentuated leukocyte infiltration, increase in vascularization of sites and blood in the uterine lumen. The decidua was more fibrous, particularly in the rats treated with the highest dose of XenTari®. In conclusion, sublethal doses of both XenTari® and deltamethrin produced qualitative/quantitative alterations in the blastocyst–endometrium interaction in female rats, thereby compromising the implantation process. Further studies are needed particularly at verifying the effects of these insecticides in the pregnancy to term in rats, order to investigate possible correlated effects on women working or living near agricultural fields.

1. Introduction

Deltamethrin is a type-II pyrethroid class insecticide that is still widely used in the control of agricultural pests and is of interest to medical-veterinary science and public health. Acute poisoning with this insecticide results in symptoms such as contortion, salivation and convulsion (Burr and Ray, 2004; Anadón et al., 2006, 2009). Moreover, some unforeseen short-term sublethal effects in humans may occur as a result of the use of these products particularly affecting women who work in or live near agricultural areas.

Due to the increasing concern regarding environmental contamination and the disposal of harmful substances to humans, such as pyrethroids, a number of studies have stressed the use of pesticides from biological origin including those made from bacteria such as *Bacillus thuringiensis* (*Bt*) (Berliner, 1909) which is obtained through large scale fermentation processes. Several commercial formulations are currently available (Oliveira Filho et al., 2009) and one of them, XenTari®WG, contains *B. thuringiensis* subsp. *aizawai*. Despite of being considered safe this product is registered as a toxic pesticide in Brazil due to its formulation (Sivasupramaniam et al., 2000; Moino, 2003).

A considerable number of rural workers are potentially exposed to pesticide sprays in Brazil (Garcia and Almeida, 1991; Waichman et al., 2002, 2007). Both Decis®25EC and XenTari® are still in use in many areas of the country, especially in horticulture which is usually carried out in a family farming structure. The most important contamination route in this situation is through direct contact with the product during the dilution or spraying, however some other pathways include the contact with the agricultural product during harvest, ingestion of food containing insecticide residues above the established limits and the use of pesticide bottles as recipients for water or other potable liquids.

Women constitute approximately 60% of the agricultural workforce in Brazil and are especially exposed to increased amounts of pesticides in the production of crops such as grapes, Barbados cherry and tomatoes (Branco and Vainsencher, 2001). The communication between mother and fetus via placenta during pregnancy may allow pesticide residues to reach fetal tissues. The exposure to pesticides at concentrations that do not cause clinical signs of systemic poisoning in the mother may still harm fetal development (Cantarutti, 2005; Myllynen et al., 2005; Lopez-Espinosa et al., 2007). Therefore this outcome may likely become intensified among female agricultural workers. Early on in these circumstances, when the worker might not be aware of the pregnancy, the constant exposure to pesticides may affect the implantation of blastocysts in the uterus, which may lead to an erroneous interpretation of infertility.

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Studies have demonstrated a relationship between the changes in sex hormone levels and the quantity of pyrethroid substances eliminated through urine in men with some types of infertility (Xia et al., 2008; Meeker et al., 2009). Andrade et al. (2002) observed that 4.0 mg/kg of deltamethrin in adult male rats caused a reduction in testicular and epididymal absolute weights and sperm production. Vázquez-Padrón et al. (1999) and Calderón et al. (2007) report changes in the antigenic levels of the immune system in rats following intragastric administration of the Cry1Ac *Bt* toxin at a dose of 100 μg/kg and of the p130 *Bt* toxin at doses of 0.1–2.5 mg/kg administered intraperitoneally. However, few studies have assessed the effects of these products on the female reproductive system.

The aim of the present study was to analyze and compare the sublethal effects of biological and chemical insecticides on the blastocyst–endometrium interaction in female rats. Outcomes from tests using rats could then be used for making inferences regarding the potential effects on women and other female mammals. XenTari®WG (*B. thuringiensis* subsp. *aizawai*) and Decis®25EC (deltamethrin) were used at doses that did not cause clinical signs of poisoning in the female rats and the blastocyst–endometrium interaction was evaluated using morphological, histochemical and histomorphometric parameters.

2. Material and methods

The experiments were carried out at the Histology Laboratory in the Department of Animal Morphology and Physiology of the Federal Rural University of Pernambuco (Brazil).

2.1. Animals

Thirty-five virgin albino female rats, *Rattus norvegicus albinus*, from the Wistar lineage, at 90 days of age and weighing nearly 200 g were obtained from the animal housing facility of the Federal Rural University of Pernambuco and used in the study. The animals were kept in cages maintained at 22 °C and artificial light with a 12:12 photoperiod (light from 6 am to 6 pm) and received food and water "*ad libitum*". Vaginal smears were collected for determining the estrous cycle after the animals were allowed an adaptation period. Animals with three regular estrous cycles were mated and divided into the following groups: Group I – pregnant rats that received placebo (water); Groups II, III and IV – pregnant rats that received 185, 1850 and 3700 mg of XenTari®/kg, respectively; Groups V, VI and VII – pregnant rats that received 1.0, 2.0 and 4.0 mg of deltamethrin/kg, respectively. Female rats from all groups were sacrificed on the seventh day of pregnancy which is the period when the blastocyst is totally inserted in the endometrium (Lee and De Mayo, 2004; Claire et al., 2006). The experimental protocol received approval from the ethics committee of the Federal Rural University of Pernambuco (process number: 23082.019868/2009).

2.2. Insecticide administration

The insecticides were administered daily immediately following the confirmation of copulation by oral gavage with doses using a method modified from Shaban et al. (2003) for the biological insecticide and Andrade et al. (2002) for the synthetic insecticide. The rats were weighed daily until the seventh day of gestation to monitor their weights in order to correct the insecticide doses to be used accordingly. The LD50 for the oral administration of the studied pesticides are: >5000 mg/kg of body weight for XenTari®WG (*B. thuringiensis* ser. *aizawai*) and >1190 mg/kg of body weight for Decis®25CE (deltamethrin).

2.3. Histopathological and histochemical analyses of implantation sites

The females in all groups were anesthetized with an intramuscular injection of ketamine hydrochloride (80 mg/kg) and xylazine (6 mg/kg) on the seventh day of gestation (end of the bioassay) and euthanized using T-61® (Hoechst Roussel Vet GmbH – Rheingaustraße 190 – D 65203 Wiesbaden/Germany) administered at 0.2 mL/kg by intravenous injection. The uterine horns containing the implantation sites were collected and immediately transferred to Boüin's fluid for a period of 48 h. The uterine horns were examined afterward with a magnifying glass for the count of implantation sites using the dilated areas as reference. The implantation sites were immediately embedded in paraffin and the cuts stained with hematoxilin–eosin (H.E.) and Mallory's trichromic stains.

2.4. Morphometric analysis

For the vascularization quantification in the region of the implantation sites, a reticulum with 121 intersection points (Olympus, model U-OCMSQ10/10) was attached to a 10X ocular lens and analyzed with a 40X objective. Ten randomly chosen fields were examined on each of five slides (one from each animal of the group) totaling 1210 points per implantation site and taking into account the points directly over the lumen and wall of the blood vessels. The mean number of points was obtained for each group representing the area occupied by blood vessels.

2.5. Statistical analysis

Due to the number of samples ($n = 5$) the test of normality cannot be improved and therefore the distribution of samples can not be assumed as normal. Thus, the nonparametric test was used. Data from the mother's weight, number of implantation sites and vascularization morphometry were submitted to the Kruskal–Wallis test. Mean values were compared using the Wilcoxon-Mann–Whitney test ($P > 0.05$).

3. Results

3.1. Number of implantation sites

The statistical analysis of the number of implantation sites among the experimental groups revealed that both the XenTari®WG and Decis®25EC treatments at the highest administered doses significantly differed from the control group. There was also a significant difference between the lowest (Group V – 1.0 mg/kg) and highest (Group VII – 4.0 mg/kg) doses of the insecticide Decis®25EC (Table 1).

3.2. Histopathology and histochemistry

The implantation sites in the control group were well developed and composed of trophoblasts (some with mitotic activity), polyploid cytotrophoblasts and rich vascularization (Fig. 1A–C). The luminal epithelium was characteristically of simple columnar type and several endometrial glands were evident in the decidua (Fig. 1D and E). The 185 and 1850 mg/kg doses of XenTari®WG and the 1.0 mg/kg dose of Decis®25EC did not cause histological changes in the sites or decidua (Figs. 1F, and 2A and B). However, at the 2.0 mg/kg dose of Decis®25EC leukocyte infiltration occurred in the region of the implantation site (Fig. 2C). Significant histological changes occurred after the use of the 3700 mg/kg dose of XenTari®WG (Fig. 2D–F) and the 4.0 mg/kg dose of Decis®25EC (Fig. 3A–C). These changes were characterized by the presence of vacuolated trophoblastic cells, rare cytotrophoblasts, large amount of blood vessels (some with accentuated leukocyte infiltration), degeneration in the decidua region and the presence of blood in the uterine lumen. The Mallory's trichrome staining produced reactions with different intensities in the decidua between the control group and the groups treated with the higher dose of both

Table 1
Means ± standard deviation of the number of implantation sites of the experimental groups.

Groups	*N*	Means*
Rats that received placebo	5	14.0 ± 1.58a
185 mg/kg XenTari®	5	11.2 ± 0.83abc
1850 mg/kg XenTari®	5	11.2 ± 2.28abc
3700 mg/kg XenTari®	5	9.4 ± 1.14bc
1.0 mg/kg deltamethrin	5	13.0 ± 1.23ab
2.0 mg/kg deltamethrin	5	11.6 ± 1.14abc
4.0 mg/kg deltamethrin	5	8.20 ± 4.71c

C.V. = 19.22%.
N = number of repetitions per treatment; degrees of freedom (DF) = 6; statistics (F^P) = $4.004^{0.0051}$.
* Means followed by the same letter do not differ.