SUMMARY

Cypermethrin is a synthetic pyrethroid widely used as an agropesticide. The aim of this study was to determine the effect of cypermethrin on reproductive patterns of the planarian Dugesia gonocephala, a bioindicator species from Guacol-lo River, in the Chilean Altiplano, an ecosystem that is free of regular environmental pollutants. Specimens were subjected to doses of 1.455, 0.1455, 0.01455, 0.001455, 0.0001455 and 0.00001455mg·ml⁻¹ cypermethrin (1/10, 1/100, 1/1000, 1/10000 and 1/1000000 LD₃₀ respectively). Planarians incubated in water from the same river were the control. The results showed that cypermethrin modified the morphology of the seminiferous tubules significantly increasing the area and perimeter of the tubules, also causing cellular changes, with a significant increase in (CD133⁻) stem cells. Is concluded that D. gonocephala is an excellent biomarker of environmental pollutants in aquatic ecosystems, and cypermethrin alters the architecture and cell proliferation of germ cells of the seminiferous tubules, possibly as a result of oxidative stress.

Introduction

Pesticides are widely used in the control of agricultural pests. However, they are affecting significantly the quality of soils, water sources and humans who handle these products (Rodríguez and Sanabria, 2005). Cypermethrin is a synthetic pyrethroid derived from pyrethrins extracted from of chrysanthemum (Chrysanthemum ciner-arifolium) bulbs and has a high insecticidal activity (Cantalamessa, 1993; Figueroa et al., 2005; Patel, 2005; Sánchez-Alvarado, 2005). Veteraninarian bulbs and has a high insecticidal activity (Cantalamessa, 1993; Figueroa et al., 2005; Patel, 2005; Sánchez-Alvarado, 2005).

The neurotoxic effect of cypermethrin resides in modifying voltage-dependent sodium channels in neuronal cells, causing a continuous depolarization (Anadon et al., 2009). In parallel, cypermethrin induces oxidative stress, causing disruptions or mutations in DNA, altering not only the architecture of tissues, but also their regeneration capacity. In general, these effects are considered as genotoxic (Hernao et al., 2005; Patel et al., 2006).

The high altitude environment is usually associated with a weather of extremely low temperatures, low relative humidity, high levels of hypoxia, and low atmospheric pressure. These extreme conditions have been crucial in the adaptation of the species that inhabit high altitude regions, including the human species ( Jacobsen et al., 2003; Espinoza-Navarro et al., 2011). The Chilean Altiplano has been considered as an ‘ecological island’, for having both endemic species and a habitat free of common pollutants. Species adapted to high altitude have a large variety of homeostatic mechanisms, such as adaptation to aquatic habitats, reduction of physical activity and development of a dehydration-resistant skin, among others (Raggi, 2000; Rodríguez et al., 2011).

Dugesia gonocephala is a fresh water organism that belongs to the phylum of Platyhelminthes, organisms that are the most geographically widespread in aquatic ecosystems. They are sensitive to environmental contaminants and therefore represent a useful potential biomarker model for toxicological research, due mainly to the fact that they have an exceptional regeneration capacity, so they can be used for the study of differentiation and regeneration of lost or damaged tissues (Newmark and Sánchez-Alvarado, 2002; Salo and Baguna, 2002; Horvat et al., 2005; Sánchez-Alvarado, 2006).

Using immunohistochemistry, an antibody against the membrane protein CD133 (Prominin-1 in human and rodents) and its epitope, has served as a marker to identify various stem cell populations (Gurley and Sánchez-Alvarado, 2008). CD133 protein was isolated from mouse neuroepithelial stem cells and designated initially as promin-1 (Shmelkov et al., 2005). It has been also studied in human cells, where the...
CIPERMETRINA INDUCE ALTERACIONES EN LOS TÚBULOS SEMINÍFEROS DE Dugesia gonocephala, MACROINVERTEBRADO PROVENIENTE DEL ALTIPLANO CHILENO
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RESUMEN

Cipermetrina es un piretroide sintético utilizado como agropesticida. El objetivo de este estudio fue determinar el efecto de la cipermetrina en los patrones reproductivos de Dugesia gonocephala, una especie bioindicadora del río Guacollo, en el altiplano chileno, ecosistema libre de contaminantes ambientales. Los individuos fueron sometidos a dosis de 1,455, 0,1455, 0,01455, 0,001455, 0,0001455 y 0,00001455mg·ml-1 de cipermetrina (1/10, 1/100, 1/1000, 1/10000 y 1/1000000 LD50, respectivamente). Las planarias del grupo control fueron incubadas en agua obtenida del mismo río. Los resultados muestran que cipermetrina modifica la morfología de los túbulos seminíferos, aumentando significativamente el área y el perímetro de los mismos, y además se observa un aumento significativo en el recuento de células madre (CD133). Se concluye que D. gonocephala es un excelente biomarcador de contaminantes ambientales en los ecosistemas acuáticos. Cipermetrina es un agropesticida que altera la arquitectura celular y la proliferación de las células germinales de los túbulos seminíferos, posiblemente como resultado de estrés oxidativo.

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RESUMO

Cipermetrina é um piretroide sintético utilizado como agropesticida. O objetivo de este estudo foi determinar o efeito da cipermetrina na reprodução de Dugesia gonocephala, uma espécie bioindicadora do rio Guacollo, no altiplano chileno, ecossistema livre de contaminantes ambientais. Os indivíduos foram submetidos a doses de 1,455, 0,1455, 0,01455, 0,001455, 0,0001455 e 0,00001455mg·ml-1 de cipermetrina (1/10, 1/100, 1/1000, 1/10000 e 1/1000000 LD50, respectivamente). As planarias do grupo controle foram incubadas em água obtida do mesmo rio. Os resultados mostram que cipermetrina modifica a morfologia dos túbulos seminíferos, aumentando significativamente o área e o perímetro dos mesmos e, adicionalmente, verifica-se um aumento significativo no recuo de células mãe (CD133). Conclui-se que D. gonocephala é um excelente biomarcador de contaminantes ambientais nos ecossistemas acuáticos. Cipermetrina é um agropesticida que altera a arquitetura celular e a proliferação de células germinais dos túbulos seminíferos, possivelmente como resultado de estresse oxidativo.
and 0.00001455mg·ml\(^{-1}\), respectively). The dilution was performed with water from the Guacollo River and a control group was kept in river water. Incubation in a volume of 10ml of each solution was at 18-24°C for 48h, considering that the renal bioelimination of cypermethrin in mammals is from 12 to 24h (Iannacone and Tejada, 2007; Crawford et al., 1981). Then the specimens were fixed in formalin for histological techniques and paraffin embedding. Sections of 5μm were mounted on previously xylanized glass slides.

**Morphometric analysis**

Mayer’s hematoxylin-eosin stain was used. Analyses were performed under an Olympus CX31 microscope with integrated digital camera and 100× objectives and previous calibration made using a millimeter ruler. Micrographs were examined with the program Micrometrics SE Premium 2.0. Twenty seminiferous tubules of each individual were analyzed to determine the area and perimeter, based on a standard measurement for a longitudinal section of the animal, with evidence of the gut in the second third of the planarian.

**Immunohistochemistry**

The presence of CD133 (prominin in human and rodents) positive cells was analyzed with Antibody-Stem Cell Marker (ab19898, USA, as positive control human colon and in calibration dilution; negative control without antibody-stem cell marker). Twenty-five seminiferous tubules were observed and analyzed in each sample, with the same instrumental and program used for the morphometric analysis (HRP/DAB).

**Statistical analysis**

The area, tubular perimeter and number of stem cells positive for CD133 monoclonal antibody were tabulated in Microsoft Excel 2010 and the mean and standard deviation were calculated. The differences between the control group and those exposed to cypermethrin were analyzed statistically with Nonparametric ANOVA (Kruskal-Wallis test) and post test (Dunn’s multiple comparison test) using GraphPad InStat 3 for Windows. Statistical significance was considered at p<0.05.

**Results**

At the time of histologic processing and microscope observation all animals had developed seminiferous tubules and were in sexually mature state conditions. Figure 1 shows a significant increase in the area of seminiferous tubules of Dugesia gonocephala in all groups incubated with cypermethrin with respect to the control in river water (p<0.05). The experimental group in the highest dilution (1/1000000) shows the largest increase in the tubular area compared to the control group (80000 vs 200000μm\(^2\), respectively).

Figure 2 shows significant increases in the perimeter of the seminiferous tubules analyzed in all treated groups, with a greater circumference than that of the control group. The group kept at the lowest cypermethrin concentration (1/1000000 dilution) showed values of 1200μm, while 600μm were recorded for the control group.

Figure 3 shows the quantification of CD133 positive stem cells in the periphery of the seminiferous tubules of D. gonocephala incubated in different dilutions of cypermethrin. All treatment groups showed significant increases in CD133 positive stem cells. It is emphasized that the group of lowest dilution has a higher average than the control group kept in river water (18 and 2, respectively).

Figure 4 shows a seminiferous tubule from the cypermethrin-treated group (1/1000000 dilution), with presence of CD133-positive stem cells inside the tubule. The epithelium is disordered, with most gonial cells (or neoblasts) and scarce sperm cells in the tubule lumen.

**Discussion**

Most pesticides or environmental pollutants enter aquatic ecosystems, affecting the physiology and survival of species living in them. These genotoxic chemicals also generate teratogenic effects by their action on germ cells. An appropriate assessment of these damages requires suitable bioindicator agents. The
freshwater planarian *Dugesia gonocephala* is an important component of aquatic ecosystems in the Chilean Altiplano, it is sensitive to pollutants, has a high capacity of regeneration and is easily cultivated in the laboratory. In general, planarians are very useful experimental models (Chen et al., 1997; Salo and Baguna, 2002; Horvat et al., 2005).

The seminiferous tubules of the planarian *D. gonocephala* are composed of Sertoli cells, intertubular Leydig cells and germ cells that are arranged in a stratified manner (Menéndez-Valderrey, 2005; Alonso and Camargo, 2011).

In mice, the seminiferous tubules incubated with the pyrethroid cypermethrin exhibit alterations in morphology and in cell proliferation causing changes on DNA and the mitotic process (Patel et al., 2006). In other studies in mice it has been shown that this pesticide induces severe histopathological alterations and interferes with spermatogenic activity. In *Drosophila melanogaster* it leads to extensive damage in the composition of DNA in a dose-dependent manner (Elbetieha et al., 2001; Mukhopadhyay, et al., 2004; Caneguim, et al., 2009; Salo et al., 2009; Wang et al., 2009; Al Hamdani and Yajurvedi, 2011). In general, these effects were attributed to a phenomenon of oxidative stress. Similar morphological changes were observed in seminiferous tubules of *D. gonocephala* treated with cypermethrin; an increase in the tubular area with an irregular, disorganized epithelium, and detachment of the tubular wall.

Planarians have the ability to regenerate any organ, as determined by the presence of totipotent stem cells in the stroma. Also, they have germ cells in seminiferous tubules and ovaries. Gashaw et al. (2007) have identified in human fetal seminiferous tubules the presence of CD133+ cells during the second trimester of pregnancy.

The expression of CD133+ stem cells in *D. gonocephala* was consistently higher in all groups treated with cypermethrin, the effects being dose dependents. Control animals show presence of CD133+ cells in the periphery of the seminiferous tubule that probably correspond to neoblasts; in treated group the presence of CD133+ cells is seen inside the seminiferous tubule, which might correspond to a proliferative response against oxidative stress caused by cypermethrin.

Neoblasts or undifferentiated cells may be multipotent stem cells and express the CD133 protein, the potential to differentiate into somatic or germ cells depending on whether microenvironmental conditions are favorable or not (Onal et al., 2012). Given the impact that pesticides have on the ecology and public health, the use of these products demand a better and sustainable management (Chirinos and Geraud-Pouey, 2011).

**Conclusions**

The results obtained indicate that the planarian *Dugesia gonocephala* from the Chilean Altiplano is an excellent biosensor (biomarker) to determine toxic effects on reproductive ecotoxicology.

The pesticide cypermethrin alters the cell structure of the seminiferous tubules, significantly increasing both the area and the tubular perimeter. It also increases the count of totipotent cells positive for the CD133 antigen. Overall, cypermethrin shows a dose dependent effect.

The high sensitivity of planaria to environmental conditions will allow a better assessment of the quality of waters where this species is found.

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**REFERENCES**


Henao B, Palacio J, Camargo M (2005) Evaluación genotóxica de los plaguicidas cipermetrina y...
diazinón en Tilapia roja (Oreochromis sp.). Actual Biol. 27(2): 43-55.


