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Short Communication

Changes in bovine *GST* expression after exposure to thiacloprid-based insecticide

Martina Galdíková*, Katarína Šiviková, Beáta Holečková, Ján Dianovský

University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic

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The effect of thiacloprid-based insecticide was investigated for the changes of GST expression in our experiment. The bovine cultures were exposed to the insecticide at the concentrations ranged from 30 to 480 μg mL^{-1} for 24 h. For evaluation of expression of bovine GSTM3 real-time PCR was applied. A decrease in expression of bovine *GSTM3* was observed at the lower doses. The higher concentrations of thiacloprid formulation caused an increase in mRNA expression.

Keywords: thiacloprid, bovine peripheral lymphocytes, glutathione S-transferases, real-time PCR

1 Introduction

Thiacloprid belongs to the new and commercially very successful family of the neonicotinoids which are now widely used for control piercing and sucking insect pests around the world (Pandey et al. 2009). They possess unique biological and chemical properties, such as broad-spectrum insecticidal activity, low application rates, excellent uptake and translocation in plants, new mode of action and favourable safety profile (Maienfisch et al., 2001). Mechanism of action of the neonicotinoids class involves disruption of the insect's nervous system by stimulating nicotinic acetylcholine receptors. This class of insecticides constitute little or no risk to mammals (Tomizawa and Casida, 2003). However, a high degree of acute toxicity to fish was found in a number of neonicotinoids (Tomizawa and Casida, 2005) and this could cause delayed lethal and sublethal effects on freshwater arthropods at relatively low concentrations (Beketov and Liess, 2008).

The adverse effects usually induced by pesticides are limited by the action of a large set of metabolic enzymes. Our study focused on expression of bovine glutathione S-transferase (GST) gene in bovine lymphocyte cultures after exposure to different concentrations of commercial preparation Calypso 480SC with the active ingredient thiacloprid. Glutathione S-transferase family is considered as one of the most important detoxification enzymes groups (Isgor et al., 2010).

2 Material and methods

The thiacloprid-based insecticide, (trade name Calypso 480 SC, with active agent N-{3-[(6-Chloro-3-pyridinyl)methyl]-1,3-thiazolan-2-yliden}cyanamide (Bayer AG, Germany), was solved in water and used in the experiments.

Experiments were carried out with healthy bull donors (Slovak spotted cattle, 6-8 months old). Whole blood cultures (0.5 ml) were cultivated for 72 h at 37 °C in 5.0 ml of RPMI 1640 medium supplemented with L-glutamine, 15 μM HEPES (Sigma, St. Louis, MO, USA), 15% foetal calf serum (BOFES, Sigma, Chemical Co. St. Louis, MO, USA), antibiotics (penicillin 250 U mL^{-1} and streptomycin 250 μg ml^{-1} and phytohaemagglutinin (PHA, 180 μg ml^{-1}, Welcome, Dartford, England).

RNA was extracted from bovine lymphocyte cultures treated with different thiacloprid concentrations (30, 60, 120, 240, 480 μg.ml^{-1}) and from negative control cultures. Before RNA extraction, cultures were stored in RNA later® Solution (Ambion®, Inc., Austin, Texas) at −20 °C. Total RNAs were extracted using the Aurum™ Total RNA Mini Kit (BioRad, USA). The concentrations of RNA samples

* **Corresponding Author:** Martina Galdíková. University of Veterinary Medicine and Pharmacy in Košice, Department of Genetics, 041 81 Košice, Slovak Republic. E-mail: martina.galdikova@uvlf.sk

were determined using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA). cDNA was prepared with an iScript™ cDNA Synthesis Kit (BioRad, USA). Real time PCR was performed using the CFX96 Touch™ Real-Time PCR Detection System (BioRad). Relative expression values were calculated in accordance with the comparative threshold cycle method using the formula: $RQ=2^{-(\Delta\Delta Ct)}$ (Livak and Schmittgen, 2001). Student's t test was performed for statistical analysis of real-time PCR data

3 Results and discussion

The changes in expression of GST genes in cultured bovine lymphocyte cultures are seen in Table 1. In our experiments, the decrease in the expression in bovine GSTM3 was observed with the lowest dose (30 μg ml^{-1}) of the insecticide, whereas at concentrations ranging from 60 to 480 μg ml^{-1} an increase in mRNA expression was seen.

Decreased activity of antioxidant enzymes has been defined as indirect inhibition of the enzymes resulting from the binding of oxidative molecules produced during pesticide metabolism (Peulkonen, et al., 1998; López et al., 2007). Increased activity might be result from activation of the compensatory mechanism leading to the induction of free radical scavenging enzymes to counteract the oxidative stress generated by pesticides (López et al., 2007).

Table 1 The results of real-time PCR- thiacloprid testing in relation to the expression of GSTM3 gene

	Cell culture	GOI – REF	ΔCt – ΔCt **E C**	RQ $2^{(-\Delta\Delta Ct)}$
		ΔCt	ΔΔCt	$GSTM3_N$
Control **C** (basal expression)	Without pesticide	10.82 ±0.65	0.00	1.00
Experiment **E** (influenced expression)	Thiacloprid (μg ml^{-1}) 30	11.74 ±0.95	0.92	0.528
	60	10.51 ±1.87	-0.31	1.240
	120	9.17 ±2.47	-1.65	3.138
	240	8.15 ±0.96	-2.67	6.635
	480	9.98 ±0.69	-0.84	1.790

RQ – relative quantification; POLR2 – reference gene (REF); GSTM3 – glutathion-S-transferase gene (gene of interest-GOI) ; $GSTM3_N$ – normalized values of gene expression compared with basal value 1.00

4 Conclusions

There is no doubt that GSTs play an important role in the detoxification of xenobiotics and in the protection of organisms against their toxic effects**.** Further studies on GST expression should contribute to animal health protection and preventing oxidative stress.

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