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Ecotoxicity Evaluation of Selected Veterinary Pharmaceuticals at Cellular and Molecular Level

Ewa Mulkiewicz, Anna Białk-Bielińska, Marta Wagil, Piotr Stepnowski
University of Gdańsk, Department of Environmental Analysis
Wita Stwosza 63, 80-308 Gdańsk, Poland
ewa.mulkiewicz@ug.edu.pl; a.bialk-bielinska@ug.edu.pl; martawagil@gmail.com;
piotr.stepnowski@ug.edu.pl

Extended Abstract

Veterinary pharmaceuticals (VPCs) are produced and used in very large quantities and their diversity is increasing every year. VPCs are biologically active compounds designed to interact with a target molecule in the animal. In the environment they may affect other organisms having the same target or may exert toxicity via other mechanisms (Gunnarsson et al., 2008). Although concentrations of VPCs in environmental samples are quite low (from ng/L to µg/L level), they are continuously being released into ecosystems (Petrović and Barceló, 2007) and therefore among other pharmaceuticals are classified as emerging environmental contaminants.

Besides primary cell cultures derived from various tissues, fish cell lines are becoming very important *in vitro* tools in aquatic ecotoxicology. The versatility and high potential of fish cell lines in ecotoxicology was illustrated and discussed by Fent (2001). He stressed that toxicological effects might differ from those in mammalian systems; this indicates that risk evaluation for fish can only be meaningfully assessed in fish-specific systems. In aquatic toxicology, cytotoxicity tests using continuous fish cell lines have been suggested as a tool for the screening or toxicity ranking of anthropogenic chemicals, compound mixtures and environmental samples, and replacement or supplementation of *in vivo* animal tests. Permanent fish cell cultures such as hepatoma cells (PLHC-1) and gonadal cells (RTG-2) have been successfully used for acute toxicity assessment of a variety of environmental chemicals such as organotins, substituted phenols and pharmaceuticals. A significant correlation of *in vitro* with *in vivo* acute toxicity in fish (organotins, substituted phenols) and zooplankton *Daphnia magna* (pharmaceuticals) was found (Brüschweile et al., 1995; Fent and Hunn, 1996; Caminada et al., 2006). Although at present there are no EU/OECD guidelines for *in vitro* tests of relevance to aquatic toxicity, the possibility of using fish cell lines in environmental toxicology is being considered. Hence, we have applied a fish cell assay to investigate the influence of VPCs on the aquatic environment by verifying whether they are capable of inducing responses in the rainbow trout (*Oncorhynchus mykiss*) D-11 liver cell line. Selected for the study VPCs (widely used in Poland as feedstuff additives and in aquaculture) belong to different groups: tetracyclines, fluoroquinolones, benzimidazoles, macrocyclic lactones and nitroimidazoles.

The first detectable responses to environmental perturbation are usually changes at the molecular and biochemical levels. The toxic effect of selected VPCs was investigated at the molecular level with acetylcholinesterase and glutathione reductase (GR) inhibition assays, two common biomarker enzymes. The acetylcholinesterase inhibition assay was used to obtain information about possible harmful effects of the chemicals on the nervous system. Many substances can exhibit toxicity related to oxidative stress. Variations in the activities of antioxidant enzymes have been demonstrated in several studies and proposed as biomarkers of pollutant-mediated oxidative stress. Both increases and decreases in the glutathione level have been observed after exposure to different chemicals. GR, a physiologically significant enzyme that plays an important role in maintaining GSH/GSSG homeostasis under oxidative stress conditions (Van der Oost et al., 2003), has been chosen.

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