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Effects of diazinon and diazoxon on the lymphoproliferation rate of splenocytes from Nile tilapia (*Oreochromis niloticus*): The immunosuppresive effect could involve an increase in acetylcholine levels

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ABSTRACT

The lymphoproliferation rate of spleen cells from Nile tilapia (*Oreochromis niloticus*) exposed to the organophosphorus pesticide diazinon, to its metabolite diazoxon and to the neurotransmitter acetylcholine, was evaluated in order to explore the immunotoxic mechanism of action of this widely used insecticide.

The lymphoproliferative response of spleen cells to mitogenic stimulus was not affected by either diazinon or diazoxon, indicating that these xenobiotic substances do not have direct immunotoxic properties. Conversely, ex vivo assays showed that spleen from fish exposed to diazinon presented a lower acetylcholinesterase activity and a higher acetylcholine concentration than non-exposed controls. Lymphoproliferation assays also indicated that pre-exposure to acetylcholine depleted the proliferative function of spleen cells.

Thus the combined information from in vitro and ex vivo experiments suggest that the immunotoxic properties of diazinon in Nile tilapia are indirect and could involve the cholinergic system of lymphocytes.

1. Introduction

The fish immune system consists of adaptative and innate components. The adaptive response is characterized by B and T lymphocytes and their highly specific receptors. It first appeared in cartilaginous and bony fishes such as tilapia (*Oreochromis* spp.) [1], a teleost fish with a worldwide representation, especially in warm regions where it has a significant economic value in fishery and aquaculture industries [2]. The immune system of this fish is significantly affected by the presence of pesticides, such as organophosphorus (OP) [3]. The OP pesticides are widely used insecticides which work as inhibitors of cholinesterase enzymes [4], inducing acetylcholine (ACh) accumulation and consequently the activation of ACh receptors (AChR) on cell surfaces, including on lymphocytes [5,6]. Diazinon (0,0-diethyl 0-(6-methyl-2-{1-methylethyl)-4-pirimidinyl phosphorothioate)) represents a broad spectrum OP, frequently used in residential areas, while its most essential use is with regard to agricultural activities. It has been classified as highly toxic and its use has been forbidden in many developed countries, however, it is still commercialized in developing ones [7]. Diazinon is relatively water-soluble, and easily comes into contact with aquatic organisms. Once inside the cells of these organisms, diazinon is metabolized to diazoxon which is the toxic form of this insecticide. Diazoxon, in turn, inactivates the enzyme acetylcholinesterase (AChE) [8]. Most of the data available on the immunotoxic mechanisms of OP comes from rodent experimental models [9], however, OPs are present and contaminate many aquatic environments, especially in extensively cultivated areas where rainfall and irrigation wash them down to rivers and water streams, hence aquatic organisms such as the Nile tilapia (*Oreochromis niloticus*) represent a suitable model to be studied on behalf of the OP toxic effect and mechanisms of action.

The effect of the acute exposure in vivo of Nile tilapia to diazinon ($LC_{50} = 7.830$ and ½ $LC_{50} = 3.915$ ppm) has been evaluated on several parameters of the innate and adaptative immune responses (phagocytic index, percentage of active cells, relative spleen weight, IgM concentration and lymphoproliferation rate). Experimental data showed that diazinon is highly toxic for this species and significantly decreased lymphoproliferation rate, phagocytic index, percentage of active cells and relative spleen weight. However, the IgM concentration was not affected in the experimental conditions described in the paper [3]. Even though this paper provided

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