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BIODEGRADATION OF XENOBIOTICS IN FISH: A SYNTHESIS[1]

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Introduction

Xenobiotics are substances foreign to the body, which have the capacity to interact with it and then exert teratogenic, mutagenic and even carcinogenic effects, such as air pollutants, heavy metals, plastics, pharmaceuticals, pesticides, petroleum products, among others (LANG; PELKONEN, 1999). However, xenobiotics are considered toxic agents, since they break the organic balance, that is, they are substances that cause changes in normal homeostasis of the body that absorbed it (LARINI, 1997). The absorption of xenobiotics in fish occurs through the gastric, gills, dermal, and other routes.

Xenobiotics that enter the body are subsequently excreted through urine, bile, feces, gas exchange, among other secretions, in unaltered or chemically modified form. Unchanged or chemically modified forms of excretion are highly dependent on the kinetic behavior of each substance. Thus, lipophilic substances are easily absorbed, for example by the digestive tract, but their excretion, either by bile or urine is difficult, because given their ease of crossing the cell membranes, they undergo reabsorption. Thus, the tendency of these substances is to accumulate in the organism. However, hydrophilic substances have poorer absorption, but their excretion is mainly via the renal route (LARINI, 1997; OGA, 1996).

To facilitate the excretion of lipophilic xenobiotics, the organism has a biochemical mechanism that transforms the little polar and liposoluble substances into more polar and hidrosoluble substances (OGA,1996). This mechanism is known as biotransformation.

Biotransformation subjects the xenobiotic substance to chemical reactions, usually mediated by enzymes, which convert it into a compound other than the one originally administered. Biotransformation is mainly done in the liver and consists of electrically charging the xenobiotic substance so that, when passing through the renal tubules, it is not reabsorbed.

This process generally inactivates the substance because, in addition to modifying fundamental points of its structure, it decreases the possibility that it reaches the susceptible tissues. Biotransformation is for these substances synonymous with elimination. Sometimes, however, active or even more active metabolites are generated than the administered substance.

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Biotransformation mechanisms involve a series of chemical reactions dependent on hepatic enzymes. A xenobiotic substance may undergo one or more transformations until a derivative with a real possibility of excretion is produced. In this second circumstance, the first reaction is preparatory, producing an intermediate compound (Phase 1) that will still undergo a new reaction, generating in the end, active or inactive metabolites (Phase 2). The processes of phase I and II are independent, i.e., a xenobiotic substance may undergo phase I or phase II reactions, or both sequentially (VAN DER OOST; BAYER; VERMEULEN, 2003).

Biomarkers in fish can indicate both the exposure of organisms to contaminants (exposure biomarkers) and the magnitude of the disturbance caused in response to pollutants (effect biomarkers) (CAJARAVILLE *et al*., 2000). Among the most widely used and recommended biomarkers are enzymes involved in the metabolism of toxic compounds and antioxidant defense systems, since they provide important information regarding the defense capacity of organisms as well as the biotransformation capacity of toxic compounds (BURGEOT *et al*., 1996).

Thus, this work aims to synthesize one of the main biomarkers involved in the biotransformation of xenobiotics, the enzyme ethoxyresorufin O-deethylase (EROD). In addition, it also aims to perform a synthesis on the main expression of proteins in the biotransformation of xenobiotics, being the expression of cytochrome P450 isoform 1A (CYP1A).

Methodology

This work was carried out with the research in periodicals (PUBMED and MEDLINE) with specific descriptors of the enzyme. In addition, information was sought in the book of toxicology and physiology. It is worth mentioning that this work is not a bibliographical review, so the methodology does not cover a temporal cut or even a standard of research.

Results and Discussion

Ethoxyresorufin O-deethylase (EROD)

Measurement of the ethoxyresorufin O-deethylase activity (EROD) in fish is a well-established in vivo biomarker of exposure to xenobiotic compounds (BUCHELI; FENT, 1995; HAHN; STEGEMAN, 1994).

EROD is a highly sensitive indicator of contaminant uptake in fish, providing evidence of induction mediated by cytochrome P450 dependent monooxygenase receptors (specifically the CYP1A subfamily) by xenobiotic chemicals. It is becoming clear that the induction mechanism of CYP1A is closely related, if not directly involved, to harmful effects, such as apoptosis and embryonic mortality, observedin fish exposed to EROD-inducing contaminants (CANTRELL *et al*., 1996).

In addition to xenobiotic induction, EROD activity may be influenced by a large number of abiotic and biotic factors, such as age, reproductive phase and water temperature (ANDERSSON; FÖRLIN, 1992).

In practice EROD activity describes the rate of the CYP1A mediated deethylation of the substrate 7-ethoxyresorufin (7-ER) to form the product resorufin. The catalytic activity towards this substrate is an indication of the amount of enzyme present and is measured as the concentration of resorufin produced per mg protein per minute (mol/mg/min) (KENNEDY; JONES, 1994). Because metabolism is generally highest in hepatic tissue, the assay is typically conducted using fish liver (WHYTE *et al.*, 2000).

Expression of cytochrome P450 1A isoform (CYP1A)

Cytochromes P450 are a diverse multigene family of heme-containing proteins that oxidize, hydrolyze, or reduce compounds through the insertion of an atom of atmospheric oxygen to the substrate during the reaction cycle (NEBERT *et al.*, 1993; NELSON *et al.*, 1996). In fish, these enzymes are concentrated mainly in the liver, but have been detected in the kidney, gastrointestinal tract and gill tissue (VARANASI, 1989). Embedded in the smooth endoplasmic reticulum, they metabolize both endogenous and exogenous compounds (phase I reactions), generally increasing the water solubility of substrates, thereby enhancing their elimination (ANDERSSON; FÖRLIN, 1992). In this way, cytochromes P450, such as CYP1A, tend to detoxify xenobiotic chemicals; however, phase I metabolites of some contaminants may be more toxic than the parent compound (GUENGERICH; LIEBLER, 1985).

The most useful aspect of CYP1A for biomonitoring purposes is the enzyme's tendency to increase in concentration upon chemical exposure. Induction of CYP1A is mediated through the binding of xenobiotics to a cytosolic aryl hydrocarbon receptor (AhR) (WHYTE *et al.*, 2000). AhR ligands generally have isoteric configurations and are similar in structure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), a model CYP1A inducer (WHYTE *et al.*, 2000).

Receptor binding is followed by a series of molecular events leading to the expression of several genes (including CYP1A) known as the "Ah-gene battery" (NEBERT *et al.*, 1993). The toxic effects of xenobiotic compounds are thought to be mediated through the AhR, with induced proteins causing alterations in cellular homeostasis (DEVITO; BIRNBAUM, 1994). In fish, early life stages appear to be particularly sensitive to AhR ligands (MEHRLE *et al.*, 1988; WALKER; PETERSON, 1991), and recent evidence indicates the involvement of CYP1A enzymes specifically in this toxic response (CANTRELL *et al.*, 1996).

Conclusions

Different strategies can be adopted to assess the level of contamination of the aquatic environment, such as the identification and quantification of pollutants present in water, sediments and in organisms. Currently, environmental analyzes constitute ecotoxicological tests. At the level of biological organization, the tools of analysis are called biomarkers, which make it possible to correlate the intensity of exposure with the biological effect of the substance.

There are a number of advantages of using biomarkers in environmental monitoring studies, such

as detecting early changes in biological responses, preventing the effects of organism intoxication from reaching an irreversible point. Among some biomarkers of xenobiotic metabolism, stands out the CYP1A-induced phase I 7-ethoxirresorufin O-deethylase EROD enzyme. That is, the activity of the cytochrome P450 isoform 1A in the conversion of the non-fluorescent 7-ethoxyresorufin substrate into a fluorescent product, resorufin.

The use of CYP1A induction as an assessment technique has increased in recent years. This is due mainly to the optimization of protocols for the rapid and relatively inexpensive measurement of its catalytic activity as EROD.

Induction of EROD as a biomarker in fish has several advantages. The EROD activity provides a fingerprint of the presence of AhR active compounds. EROD is an extremely sensitive indicator of environmental changes and is usually one of the first measurable detectable responses to exposure. In addition, EROD represents the cumulative impact of all inducing chemicals, whether or not they are detected analytically.

Keywords: ethoxyresorufin-O-deethylase (EROD), cytochrome P450, polycyclic aromatic hydrocarbons.

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