ANTI-XENOBIOTIC DEFENSE MECHANISMS AND ENVIRONMENTAL HEALTH

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Abstract

This work proposes a new approach and methodology to studying and assessing the effect of pollutants on biota. Animals possess several anti-xenobiotic defense mechanisms, particularly a system of active transport of organic anions (SATOA) and a multixenobiotic resistance transporter (MXRtr), whose activity varies within specimens, populations, and species. These defense mechanisms can reduce pollutant damage to cellular functions, structures and metabolism. Comparative studies of these mechanisms, as well as determination of early signs of genotoxicity (one-strand DNA breaks, aneuploidy, micronuclei formation), and pathology, enable assessment of the health of various animals and can be used for reliable early warning monitoring.

Key-words: pathology, monitoring, ecotoxicology

Under contemporary ecological risk assessment there is a necessity for new and innovative scientific ideas and methods to study the biological and ecological effects of anthropogenic pollutants, from early cryptic primary and secondary responses to overt changes in environmental health and community structure. Conventional methods of ecological assessment of environmental stability and alterations in community structure are, in many cases, similar to post-mortem diagnostics in medicine. However, the use of an early warning monitoring system can reflect the more effective diagnostics of determining cryptic signs of disease, as used in prophylactic medicine. Such medicine uses a selected set of physiological, biochemical and morphological parameters which determine human health. An analogous set of parameters can be selected to determine the health of various animal species. For this purpose, it is crucial to monitor the primary and secondary responses of eukaryotic cells to various pollutants (on selected sites), especially the defensive and adaptive reactions and early pathological alterations in cellular metabolism, functions, genetic activity and cytopathology caused by pollutants. We propose a new approach to this type of monitoring, based on the following (1-5) :

- the selection of a set of reliable parameters which characterize the health of all eukaryotic organisms. Several such possible parameters and study methods are presented in Table 1;
- the selection and development of rapid and precise methods for examination of the selected parameters: specific fluorescent probes, markers, analogues, fluorogenic substrates and vital quantitative fluorescent microscopy, especially contact fluorescent microscopy. These enable the *in vivo* and *in vitro* study of metabolism, specific functions and chemical and structural organization at the molecular and subcellular levels, as well as a study of the pathomorphology of cells and organs (Table 1);
- a comparative study of the morphology, physiology, biochemistry, and ecological functions of various anti-xenobiotic defense mechanisms in individuals, populations and species.

In the present work, we offer examples of the interrelations between the activity of anti-xenobiotic defense mechanisms, especially transport systems for xenobiotic elimination, and early signs of genotoxicity and environmental pathology in selected marine and terrestrial species, such as protists, corals, bivalves, gastropods, fishes and turtles (1-7), collected from different sites along the Israeli coast of the Mediterranean Sea, the Red Sea and from the North Sea shore. The examined organisms revealed a number of general anti-xenobiotic defense mechanisms (Table 2). The studied benthic epiphytic community of protozoans also possesses extraorganismal anti-xenobiotic defense mechanisms such as a common mucous matrix, exoenzymes, and other released organic compounds. Two multisubstrate carrier-mediated transport systems for xenobiotic elimination were detected in the studied organisms: a) a system of active transport of organic anions (SATOA) (1-7) and b) a multi-xenobiotic resistance transporter (MXRtr) (5, 9, 10). SATOA eliminates a wide spectrum of organic anionic xenobiotics. Its fluorescent marker transport substrate is fluorescein, whose transport is competitively inhibited by other organic anions such as *p*-aminohippurate and probenecid. SATOA was described in some protozoa (foraminifera), in the Malpighian tubules of insects, in the "kidney" of molluscs and crustaceans, in gills of molluscs and fishes, and in the renal proximal tubules, liver trabecules and choroid plexus of vertebrates (1-7).

Table 1. Main parameters of environmental health and corresponding microfluorometrical methods for their determination.

PARAMETER	METHOD
Cell and tissue respiration: Metabolic state of mitochondria in living cells and tissues *in situ*	Microfluorometry of inherent blue and green fluorescence of NADH and FAD
DNA, RNA, proteins and lipids content and dynamics	Quantitative fluorescent cytochemistry
Enzyme activity in living cells *in situ* a. non-specific esterases, b. detoxifying enzymes, c. marker enzymes	Fluorogenic substrates, specific inhibitors and microfluorometry. Determination of main enzyme kinetic parameters: K_M, K, and V_{max}
Permeability of plasma membranes, epithelial layers and histohaematic barriers	Fluorescent markers of permeability and microfluorometry
Carrier-mediated transport systems for xenobiotic elimination: System of active transport of organic acids (SATOA) and multixenobiotic resistance (MXR) transporter	Fluorescent substrates, specific inhibitors and microfluorometry. Determination of main transport kinetic parameters: K_M, K, and V_{max}
Xenobiotic-binding proteins	Fluorescent analogs of ligands and microfluorometry
Intra- and extracellular depot for xenobiotic accumulation and storage	Fluorescent analogs of xenobiotics and microfluorometry
State of lysosomes and cell viability	Vital test with acridine orange or neutral red and mictofluorometry
Functional state of nuclear chromatin and cell cycle phases	Staining with acridine orange and microfluorometry at 530 and > 590 nm
Complete pathological and histopathological examination	Section and macroscopic examination. Organo-somatic indexes. Staining of tissue blocks and contact fluorescent and epi-microscopy
Cytogenetic examination	Functional activity of nuclear chromatin, one-stranded DNA break, aneuploidy, apoptosis, micronucleus test, anaphase chromosome aberrations

Table 2. Main general anti-xenobiotic defense mechanisms of eukaryotic organisms.

Mechanism	Functions
External diffusion barriers: membrane lipid bilayer, epithelial layers, additional structures (mucin, chitin, keratin, shells)	Impermeable for watersoluble compounds, decreased permeability for lipidsoluble compounds
Internal (histo-haematic) barriers	Protect brain, gonads and endocrine organs from all xenobiotics
Multisubstrate detoxifying enzymes mediated by cytochrome P450	Detoxify some polycyclic aromatic xenobiotics in all species
Enzymes conjugating xenobiotics	Make xenobiotics less toxic and more useful for transport systems
Peroxidases and nonspecific esterases	Protect from excess of oxygen, iodine and bromine, bind some xenobiotics
Multisubstrate carrier-mediated pumps for xenobiotics' elimination: MXRtr and SATOA	MXRtr eliminate lipophilic xenobiotics, SATOA eliminate watersoluble anionic xenobiotics
Extracellular xenobiotic-binding proteins: mucins, serum albumins etc	Bind xenobiotics and decrease their reactivity or eliminate them
Cellular metal-binding proteins	Bind and eliminate heavy metals
Intracellular compartments: lysosomes	Accumulate, store and eliminate some cationic xenobiotics
Extracellular structures/compartments: concretions, fat tissue etc	Accumulate and store xenobiotics

MXRtr eliminates various lipophilic cationic xenobiotics (Rhodamin B, ethidium bromide and Acridine Orange are its fluorescent marker substrates) and is inhibited by the specific blocker, Verapamil, or by its transport substrates. MXRtr was primarily discovered in drug-resistant cancer cells but has recently been described in some parasitic protozoa, sponges, worms and molluscs (5, 9, 10). Recently, we discovered and studied MXRts in marine foraminifera and ciliates, in the pseudogills and mantle of the gastropods *Patella coerulea* and *Cellana rotha*, in the gills and mantle of various bivalves, as well as in the gills of fish, and in the proximal tubules and liver of fish, amphibia (tadpoles) and turtles. Our observations show that the activity of these transport systems varies among different taxons and also varies among populations from different sites. For example, MXRtr and SATOA in the gills of *Patella coerulea* and its cotaxon *Cellana rotha* from polluted sites along the Mediterranean Sea and Red Sea in both cases showed higher activity than those in populations of the same species from clean sites. Increased activity of the MXR transporter was also detected in the gills and mantle of various bivalve molluscs from the polluted sites along the Mediterranean and Red Sea coasts, as