

Fish Liver Biomarkers for Heavy Metal Pollution: A Review Article

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Abstract

Heavy metals as dangerous and long lasting pollutants in environment have been widely studied and monitored. Liver is the most important organ in storage and detoxification of these pollutants. Assessment of indicators, including changes in intermediate active components between the liver and pollution effect, provide sensitive liver biomarkers and can be a suitable index for health condition of fish. In addition, fish liver is a favorite model to study interaction between environmental factors and liver structure and performance. This review studies variety of liver biomarkers in molecular- cellular (damage to chromosome, DNA and lysosome; increasing metallothionein and ferritin levels), biochemical-physiological (transaminase enzymes and oxidative enzyme parameters) and morphological-histopathological levels, and investigates advantages and disadvantages of these levels. Moreover, restrictions and landscape in the future is discussed for these biomarkers exposed to heavy metals pollution. There are many liver biomarkers that can be used for identification of initial heavy metal pollution, assessment of health condition of fish and ability of fish to defense against pollution challenges. Some of these biomarkers are special for heavy metal pollution while the others are non-special.

Keywords: biomarkers; heavy metals; fish liver; fish; pollution

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Introduction

Growth in the human population and the development of industries has led to high levels of water pollution that are a serious threat to various aquatic ecosystems. Heavy metal pollution has long-term toxic effects and unlike many of the organic pollutants, they are not degraded over time. Even though some heavy metals (e.g., Cu, Zn and Mn) [1] are needed for the growth of organisms, they are toxic if their concentration exceeds an allowable limit. Other heavy metals do not have any biologic role (e.g., Cd, Ag, Hg, Pb and Cr), and are described as xenobiotic [2]. The heavy metals are easily dissolved in water, and are therefore absorbed by fish and other aquatic organisms. These pollutants are able to be bioconcentrated even at very low concentrations, most specifically in the liver [3], and, therefore, their concentrations in organisms is higher than that in the environment. Fishes have particular sensitivity to environmental changes, and respond more specifically to pollution than mammals [4]. Thus, measurement of biomarkers, either as individual or as population responses to toxic stress resulting from pollution, could be used to easily and rapidly monitor pollution.

Fish liver is the most important organ for detoxification and the main site of many important metabolic reactions involving carbohydrate, lipids and protein [5]. It also is important for the storage of glucose and vitamins A, D, E, K, and B12 [6]. Numerous environmental stressors affect the liver and cause metabolic disturbances and structural damage, possibly leading to death [4]. At all the different cellular levels (i.e., molecular, cellular, biochemical, physiological, morphological, and histopathological), exposure, effective and susceptibility biomarkers can be used to monitor initiative signals of pollution, liver health and ability of this organ to defense against pollution challenges, respectively.

Fish livers have been a favorite model to study the interactions between environmental factors and the functions and structures of the liver [4]. This article reviews liver biomarkers at the different biological levels when exposed to heavy metal pollution, and evaluates their advantages and disadvantages.

Main text

Cellular-molecular biomarkers in the liver

The liver functions to store and detoxify heavy metals. Thus, heavy metals cause changes in the proteins, DNA, and lysosomes of hepatocytes.

Stored metabolites

Fishes are able to absorb heavy metals through the skin mucosa, gill and alimentary tract [7]. They are transferred through ion channels or specialized transporter systems in the plasma membrane into the body [8] and, finally, into the liver and kidney (detoxification organs) where they are absorbed independent of their concentration [9]. They are then stored intracellularly as metallothionein-bound metals, ferritin-bound complex, and bound within vesicle-granules [8]. While considered the most important organ in detoxification, some studies show that liver is the major organ in storage of heavy metals [10]. However, a few heavy metals (e.g. Cr) are preferentially stored in other organs such as the gill [11, 12], and the extent of storage may differ between males and females [13].

Once stored in the fishes, these will become part of the food of other animals and humans higher in the food chain.

Biomarkers of damage to chromosomes, DNA and lysosomes

The presence of heavy metals in liver tissue often causes oxidative stress and increases of reactive oxygen species (ROS) through induction of O_2 and transformation into O_2^- (superoxide), H_2O_2 (hydrogen peroxide) and OH^\cdot (hydroxyl radical). The hydroxyl radical is able to react with many compounds. Therefore, these radicals could transfer energy to other molecules including vital molecules. This may cause damage such as DNA single (SSB) and double strand breaks (DSB), micronuclei (MN XX), chromosome aberrations (CA) and sister chromatid exchanges (SCE) in hepatocytes and other cells [14-18].

Among other cell injuries caused by free radicals from heavy metals in hepatocytes is lysosomal damages. Fish hepatocytes often have a small number of lysosomes, and during heavy metal pollution the number and size of the lysosomes increase to store pollutant and lipids [19]. In addition, lysosomal membrane stability decreases due to damage to the lysosomal detoxification system, and enzymes of the lysosomes are released into the cytoplasm and nucleoplasm. This results in more damage to hepatocytes [19, 20]. Although lysosome stability quickly monitors damage and the capacity of hepatocyte in detoxification, observations need to be confirmed with liver histopathologic and other physiologic biomarkers. In addition, the number, size and lysosomal alterations could be affected by sex and season of vitellogenesis in female fish [21] and, therefore, can cause error in pollution monitoring .

Protein biomarkers

After entering heavy metals into the body, some proteins, such as metallothionin, chelate heavy metals via their sulfhydryl groups, and suppress heavy metal activity [22]. These low molecular weight proteins (2-16 kDa) have cysteine amino acids in binding site enriched in thiol ions [23]. Since the liver is the major site accumulating heavy metals, the most metallothionin is found in the liver. Its increase in the liver is a suitable biomarker for heavy metal pollution [24]. P-glycoprotein [25], heat shock proteins [26] and multixenobiotic resistance protein (MXR) [27] are the other known protein biomarkers for heavy metal increases in the liver, although they may also respond to some other xenobiotic compounds.

Biochemical and physiological biomarkers

Reactions in the liver to heavy metal oxidative stress might be enzymatic or non-enzymatic (Figure 1) and seem to be specific for monitoring heavy metals pollution.

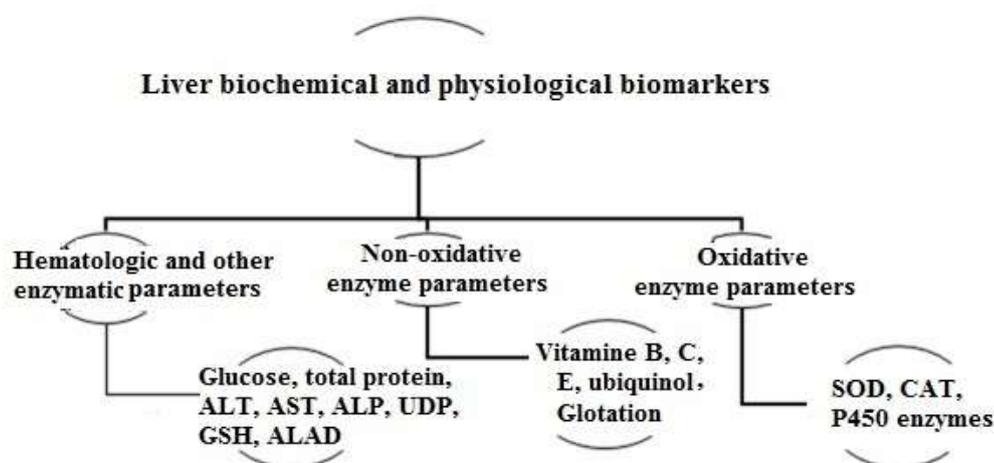


Figure 1 Biochemical and physiological biomarkers in the liver

Hematologic parameters

In fishes, proteins and lipids are used as energy sources [28]. Muscle glycogen obtained from glucose, which in turn is obtained from glycogenesis of exogenous and endogenous amino acids and lactate from anaerobic glycolysis [29]. The liver has the major role in the synthesis of the plasma proteins and the glucose. Some studies showed that pollution, especially heavy metals pollution, disrupted hepatocytes, and, therefore, decreased the removal of plasma glucose and proteins [30]. Other studies showed similar results [31, 32].

Transaminase enzymes, including alanine transferase (ALT), aspartate transferase (AST), and alkaline phosphatase (ALP) are important for amino acid and protein metabolism in fish tissues, and are key metabolic enzymes released into the blood from damaged hepatocytes [33] and also have increased synthesis due to induction [34]. Thus, alterations in plasma levels of transaminase enzymes could be a sign of hepatocytes damages.

With heavy metal pollution, decreases or increases of some other easily measured liver detoxification enzymes, such as glucuronosyltransferase (UDP-GT) [35] and δ -aminolevulinic acid dehydratase (ALAD) [36] can also be used as suitable biomarkers, although they may not have been heavy metal specific.

Oxidative enzyme parameters

Production of antioxidant compounds is part of an organism's response to free radicals, by trying to neutralize them to slow or prevent oxidative damage. Antioxidants generally stop oxidative chain reactions by giving a hydrogen atom to free radicals. The yield and performance of an antioxidant is dependent on how its hydrogen is released [37].

In the liver, antioxidant defenses occur both enzymatically and non-enzymatically. Antioxidant enzymes of the mitochondria and cytoplasm are able to decompose hydrogen peroxide (H_2O_2) [37, 38]. Thus, measurements of these enzymes are a valid biomarker for monitoring free radicals. Among the most important antioxidant enzymes whose activity changes with pollution are catalase (CAT), glutathione peroxidase (GPx) [39], and superoxide dismutase (SOD) [40]. Although some P450 enzymes also respond to heavy metals [41], their alterations with other organic pollutions make these enzymes more non-specific biomarkers.

Histopathological-morphological biomarkers

Histopathological alterations have special advantages [42]. Differences in biochemical processes in different organelles lead to different responses that can be determined histocytologically. Long-term exposure to heavy metals causes more changes in detoxification organs such as the kidney [43] and the liver. Studies of the livers of fish and birds exposed to heavy metals show degenerative, destructive and inflammatory alterations [43-46]. Again, however, they are non-specific biomarkers.

Bile as a biomarker in fish

The bile secreted by the liver contains salts and bile acids, which have role in emulsification and absorption of fatty acids from the gastrointestinal tract. It also has an important role in the excretion of many xeno- and endobiotics that the kidney is not able to remove them from the liver and blood [47]. Several studies have shown that many heavy metals enter into the gall bladder, and then are excreted from the fish [47-50]. The bile liquid can be more easily analyzed and is not age or sex dependent [50]. With cannulation, bile can be obtained from the bile duct without killing the fish [47]. Thus, monitoring bile and establishing correlations of heavy metals in bile and its storage in the liver can improve the value of

data obtained using the bile.

Problems and future of study on liver biomarkers in response to heavy metals

Some of the limitations mentioned require further research to allow fish to be more successfully used as biomarkers for heavy metal pollution:

Fishes are the most diverse group among vertebrates. Species diversity and factors such as sex and reproduction season may affect some liver biomarkers. So, these need to be better documented.

To better understand the changes in liver biomarkers with heavy metal pollution, it is necessary to understand which non-biological factors (e.g., temperature, salinity, and pH) and biological factors (e.g., fish age and size, liver structure, and fish genotype) affect these biomarkers so that measurement conditions can both be standardized and optimized.

Using cell culture, one can more easily study the effects of heavy metals on hepatocytes. These can be used to determine dose effects and the impact of other pollutants in the same water sample. These then need to be correlated with results in the field.

So are liver biomarkers appropriate and valid as compared to other biomarkers for use in real ecosystems? Additional questions that need to be answered include understanding the time course of liver changes. It may also be important to have a better understanding of the molecular mechanisms by which heavy metals change liver transporters, genes and proteins under different pollution scenarios.

Conclusion

This study showed that there are many liver biomarkers at all different cellular levels that can be used for identification of initial heavy metal pollution (exposure biomarkers), assessment of health condition of fish (effect biomarkers) and ability of fish to defend against pollution challenges (susceptibility biomarkers). Moreover, these biomarkers are useful for environmental monitoring and assessment; some of these biomarkers, such as metallothionein and ferritin, are special for heavy metal pollution while the others are non-special.

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