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Plasma Sorbitol Dehydrogenase, Glutamate Dehydrogenase, and Alkaline Phosphatase as Potential Indicators of Liver Intoxication in Grey Mullet (*Mugil auratus* Risso)

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Monitoring of liver enzymes leakage in the blood has proved to be a very useful tool in hepatotoxic studies (Kaplan and Szabo 1983). As it was ascertained for rainbow trout (Raccicot et al. 1975; Pfeifer et al. 1977; Stathman et al. 1978) and English sole (Casillas et al. 1983), we have also confirmed that alteration of GOT* and GPT activity in plasma could be applied as helpful labels of liver toxicity in grey mullets (Krajnović-Ozretić and Ozretić 1987). Besides, among other enzymes SDH, GLDH and AP were also used to diagnose liver disfunction in mammals (Tietz 1976). SDH is almost exclusively located in liver and its increased activity in plasma can denote hepatic damage. GLDH is also a liver specific enzyme in man and mammals. Owing to its mitochondrial location, GLDH emerges as a highly positive marker of enzyme leakage from the hepatocyte mitochondrial matrix. Similarly AP had attracted considerable attention to indicate specific types of hepatic distress induced by cholestasis. The use of SDH in fish was evaluated only in rainbow trout (Dixon et al. 1987), while Raccicot et al. (1975) and Casillas and Ames (1986) analyzed the potential use of GLDH and AP as possible indicators of liver dysfunction in rainbow trout and English sole, respectively.

The present study concerns the activity of some enzymes and the evidence of selected biochemical indicators and metabolites in blood of grey mullets to assess additional aspects of liver toxicity following exposure to different toxic substances. CCl_4 was used as model hepatotoxic agent; phenol was chosen as general protoplasmatic poison; and cyanide was adopted as extremely poisonous but not hepatotoxic substance. Their effects were studied measuring the activity of SDH, GLDH and AP in mullets

* GOT - Glutamate oxaloacetate transaminase; GPT - Glutamate pyruvate transaminase; SDH - Sorbitol dehydrogenase; GLDH - Glutamate dehydrogenase ; AP - Alkaline phosphatase.

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plasma. Besides, we have examined also the concentration of lipids and triglycerides in plasma as possible indicators of altered fat metabolism in liver tissue. Total proteins, albumin and some metabolites as ammonia and lactate were also measured.

MATERIALS AND METHODS

Grey mullet, *Mugil auratus* Risso average weight 205 g (±25 SD) were used as test animals. Fish were acclimated at least 2 wk in aerated basins (250 L) with a continuous flow of sea water (salinity 37.2 ± 0.4 ‰ and temperature 18 ± 0.5^0 C). Fish were fed daily to satiation and the remaining food was removed. Following previous experience (Krajnović-Ozretić and Ozretić 1987), ten grey mullets per group were injected intraperitoneally (i.p.) with a single dose of the following: 2 mL CCl_4 kg^{-1} or 200 mg of phenol kg^{-1}. Ten fish were exposed for 24 hr to 300 µg NaCN L^{-1} in a sea water continuous flow system with a doser for cyanide. Phenol was first dissolved in distilled water, while CCl_4 was injected without dilution. A separate group of thirty untreated mullet served as control. All parameters were determined 24 hr after the injection, the time when the maximum changes in blood enzyme activities were observed (Krajnović-Ozretić and Ozretić 1987). Blood samples were taken by cardiac puncture. Blood sampling and i.p. injections were performed without the use of anesthetics. Heparin was used as anticoagulant. Blood was kept iced and plasma was separated (10 min at 2000 g) in a refrigerated centrifuge.

GLDH (EC 1.4.1.2) and SDH (EC 1.1.1.14) activities were determined with the kinetic UV method (Schmidt 1974; Gerlach and Wiby 1974) and AP (EC 3.1.3.1) according to the method of Klaus and Schutt (1974). Optimization of SDH and GLDH assay conditions (e.g. pH and substrate concentration) for grey mullet revealed to be very similar to those used in mammalian studies. The activity of the same enzymes was also measured in the extracts of liver, heart, kidneys, gills, white and red muscle. Tissue samples were homogenized with a Polytron grinder in 10 parts of a cold 0.2 M Na-phosphate buffer (pH 7.4) in 20% glycerol with 5 mM mercaptoethanol. Homogenates were centrifuged at 17000 g for 30 min, and the supernatant was immediately used for enzyme assay. For plasma, the enzyme activity was calculated in relation to the unit volume or to the unit weight for body tissues, and it was expressed as International Units (U L^{-1} and U g^{-1}, respectively). Total plasma proteins were determined with Biuret reaction (Weichselbaum 1946). Plasma albumin was estimated by spectrophotometry, using a commercial diagnostic test produced by Sigma (St. Louis, USA). Boehringer (Mannheim, Germany) biochemical test kits were

used to estimate total plasma lipids (colorimetric method of Zollner and Kirsch, 1962) and triglycerides (enzymatic colorimetric GPO-PAP test, Wahlefeld 1974).

Student's t test analysis was applied to check the difference between treated groups and control using the STATGRAPHICS computer program (STSC, Rockville, MD, USA).

RESULTS AND DISCUSSION

The activity of GLDH, SDH and AP in several body tissues of the control group of grey mullet are displayed in Table 1. The values provide a helpful estimation of the potential sources of the enzymes from where, in particular circumstances, they could be delivered into fish plasma. As in other vertebrates, the liver of mullet was distinguished by the highest content of SDH and GLDH.

Table 1. ***Mugil auratus.*** **Distribution of GLDH, SDH and AP in mullet plasma (U L^{-1}) and in tissue (U g^{-1} wet wt.). Each value represents the mean (N=30) and ± SD.**

	GLDH	SDH	AP
Plasma	18.3 ± 9.4	1.16 ± 0.20	20.0 ± 5.98
Liver	66.0 ± 35.5	4.20 ± 0.39	4.50 ± 1.48
White muscle	0.22 ± 0.13	0.05 ± 0.01	0.21 ± 0.02
Red muscle	4.10 ± 2.4	0.19 ± 0.02	0.23 ± 0.05
Kidney	9.90 ± 5.3	1.70 ± 0.3	13.7 ± 3.0
Heart	19.5 ± 7.3	0.39 ± 0.02	0.40 ± 0.01
Gill filaments	3.90 ± 1.0	0.13 ± 0.01	2.40 ± 0.27

The activity ratio of the liver extracts, compared to the other tissues was in the range of 2.4 - 77 for SDH and 3.4 - 300 for GLDH. The activity of AP was the highest in kidney, but it was only three times higher of the liver extracts. The activity of all three enzymes was the lowest in plasma: for SDH very near to the detection limits.

The activity of SDH, GLDH and AP in mullet plasma significantly increased after exposure (24 hr) to CCl_4 and phenol, while cyanide did not generate any change (Table 2). SDH compared with the control group increased 16 and 8.8 times, respectively. Pertinent to the highest activi-

Table 2. *Mugil auratus*. Effect of CCl_4, phenol and cyanide measured on the basis of changed plasma parameters.

	Control	Phenol	CCl_4	Cyanide
GLDH ($U\ L^{-1}$)	18.3 (9.4)**	495.6* (324.0)	102.4* (71.3)	16.1 (6.2)
SDH ($U\ L^{-1}$)	1.16 (0.20)	10.2* (1.7)	19.1* (2.7)	1.09 (0.2)
AP ($U\ L^{-1}$)	20.0 (5.98)	75.9* (38.8)	150.9* (51.7)	21.8 (5.9)
Triglycerides (mg 100 mL^{-1})	91.1 (50.1)	98.3 (55.4)	196.6* (59.2)	90.8 (48.3)
Total lipids (mg 100 mL^{-1})	1404.0 (297.6)	913.5* (186.8)	1296.0 (268.5)	1358.0 (263.0)
Total Proteins (g 100 mL^{-1})	4.1 (0.6)	2.9* (0.7)	3.5 (0.9)	4.4 (0.4)
Albumin (g 100 mL^{-1})	1.2 (0.1)	1.4 (0.2)	1.1 (0.5)	1.4 (0.2)
Alb./Prot.	0.29	0.48*	0.31	0.34
Ammonia (µg 100 mL^{-1})	194.0 (83.7)	371.8* (83.8)	322.4* (37.0)	-
Lactate (mg 100 mL^{-1})	13.9 (3.1)	14.9 (3.6)	17.6 (4.4)	15.1 (3.5)
Hemoglobin (g 100 mL^{-1})	9.1 (1.0)	6.4* (1.3)	10.6 (1.2)	9.8 (1.0)
Hematocrit (%)	31.0 (3.5)	22.0* (5.0)	35.3 (8.7)	34.5 (4.3)
MCHC*** (%)	30.8 (2.9)	29.0 (2.9)	30.8 (4.7)	28.4 (3.1)

* Values significantly different from controls: $P < 0.05$
** (N) - ±SD
*** MCHC - mean corpuscular hemoglobin concentration

ty of SDH in liver and to its considerable size we presumed that impaired liver could release comparably more SDH into plasma than any of the other analyzed tissues. That was substantiated by the high positive correlation computed in comparison with the increased activity of GOT and GPT (Krajnović-Ozretić and Ozretić 1987) as exclusive

liver enzymes (r = 0.818 and 0.945, respectively). Since the actual SDH activity in plasma of control mullet is extremely low (1.16 U L^{-1}) even any moderate activity increase can be easily detected. Comparing our results with the records obtained with rainbow trout with the same toxicant, but after 48 hr (Dixon et al. 1987) the increase of SDH in grey mullets was four times higher.

The activity of GLDH increased 5.5 and 27 times in relation to CCL_4 and phenol. GLDH, an allosteric enzyme, in connection with transaminases (GOT and GPT), plays an important role in ammonia detoxification in fish (Hochachka and Somero 1973), and it may determine the direction of amino acids metabolism (catabolism versus anabolism). Concerning the concentration of ammonia and lactate as metabolites, the level of plasma lactate was not changed while ammonia was significantly increased. Thus the increased activity of GLDH jointly to the increased concentration of ammonia, suggested that protein catabolic processes prevailed as a result of toxic stress. On the other side, the unchanged concentration of lactate in plasma denoted that anaerobic processes were not activated.

The activity of plasma AP also increased: about 7.5 in CCl_4 and 4.9 times in phenol treated mullets. In fish toxicology studies the interpretations about the function of this enzyme system are rather contradictory. While Raccicott et al. (1975) in rainbow trout plasma evidenced the inconsistence between liver intoxication with CCl_4 and AP activity, Casillas and Ames (1986) proposed it as useful to assess the hepatotoxic effects of CCl_4 in English sole. In mullets, we actually found that kidneys contained the highest activity of AP, but since we were not able to prove its liver origin, we cannot speculate about the use of this enzyme as indicator of intrahepatic cholestasis.

In grey mullets plasma, after the exposure to CCl_4 we found increased level of triglycerides. In clinical medicine, serum triglycerides are used to evaluate lipid metabolism (Tietz 1974). As in mammals, also in fish triglycerides are central metabolites in lipid metabolism (Love 1980). Since homeostasis of lipids is one of the principal functions of liver, any change of triglyceride concentration in serum can be used as indicator of liver dysfunction. Increased plasma triglycerides associated with cytological changes and fat accumulation in liver may be indicative of altered or impaired synthesis and transport of lipids in liver.

In mullets treated with phenol, following the increased enzyme activity, plasma proteins and lipids were significantly decreased. Hemoglobin and hematocrit, consistent

with our previous findings, also decreased (Krajnović-Ozretić and Ozretić 1988). Theoretically we can suppose that plasma protein decrease can result from hemodilution, loss of proteins with urine following kidney damage, or by increased protein utilization without replenishment. Since the concentration of proteins in mullets plasma decreased at the same time as total lipids, hemoglobin and hematocrit, the dilution of plasma with water could perhaps be supposed.

In laboratory animals treated with CCl_4, Berryman and Bollman (1943) and in rainbow trout Gingerich and Weber (1979), Pfeifer and Weber (1979) and Dixon et al. (1987) observed that total plasma protein decreased. On the contrary, in mullet the plasma protein level did not significantly decrease neither was the albumin/protein ratio altered.

Exposure to cyanide did not cause any change either in enzyme activity or in other measured parameters. That was consistent with the mode of action of HCN as a metabolic inhibitor instead of a hepatotoxic agent.

Searching for confident methods to assess the impaired metabolism and biochemistry in fish exposed to toxic substances, we verified the significance of aminotransferase activity in mullet plasma (GOT and GPT) as indicators of liver intoxication (Krajnović-Ozretić and Ozretić 1987). We have currently confirmed that the increased activity of SDH and GLDH and the enhanced concentration of triglycerides in plasma can be also used as indicators of hepatoxicity induced by CCl_4 and phenol.

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