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Mercury-induced Lipid Peroxidation in the Liver, Kidney, Brain and Gills of a Fresh Water Fish, *Channa punctatus*

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Abstract Simultaneous lipid peroxidation and oxidative stress were examined in the liver, kidney, brain and gills of a fresh-water fish, *Channa punctatus*, after 30 days treatment with inorganic mercury. Although longer exposure caused greater oxidative stress, the degree/rate of injury varied in different organs. Short exposures resulted in increased reduced glutathione (GSH), but longer exposures reduced in all the tissues. The results suggested that the rate of lipid peroxidation did not strictly correspond to oxidative stress. Time dependent effects may represent an early biochemical response, although, the presence of some labile GSH-dependent factors may provide a protective mechanism.

Several reports have described the effects of mercury in fish (Alexander et al., 1973) and other animals (Miller and Clarkson, 1972). Nevertheless, the mechanism(s) of its toxicity have yet to be firmly established. It was initially considered to be an enzyme poison (Rana and Sharma, 1982; Stacey and Kappus, 1982). However, Rana and Kumar (1984) suggested a causative role of lipid peroxidation in mercurial hepatotoxicity. These observations were supported by Yonaha et al. (1982), who reported increased thiobarbituric acid (TBA) chromogen concentrations in renal homogenates of mercury-treated rats. Recent reports including studies of gastropods (Viarengo et al., 1989) and echinoderms (Allemand et al., 1989) have attributed mercurial toxicity to lipid peroxidation. However, increasing evidence that inorganic compounds stimulate lipid peroxidation indirectly, rather than by oxidative stress (Stacey and Kappus, 1982), have now made it essential to study these two phenomena together.

Specific sequestration of metals varies greatly among phyla, even among individual species and individual cell types. A comparative study on lipid peroxidation in four major tissues viz. liver, kidney, brain and gills of a fresh-water fish *Channa punctatus* was therefore, made together with an appraisal of glutathione status in these tissues.

Materials and Methods

Living fish were collected from local river Hindon with the help of a commercial fisherman and maintained in glass aquaria, in which they acclimatized to laboratory conditions for one week prior to mercury treatment. Specimens weighing 60 ± 10 gm were selected and divided into two groups of 25 individuals each. The first group was treated with 5 μg/litre of mercuric chloride (S. Merck, Bombay, M. Wt. 271.50), a sublethal concentration for 30 days. The second (control) group was maintained in dechlorinated water. The water in each aquarium was renewed every second day. Ten fish from each group were killed after 15 and 30 days respectively and pieces of liver, kidney, gills and brain quickly excised and frozen at -4°C. These were subsequently processed for the estimation of malondialdehyde (MDA), using thiobarbituric acid (Jordan and Shenkman, 1982). Protein was estimated by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Reduced glutathione (GSH) was determined following the method suggested by Ellman (1959). Oxidized glutathione (GSSG) was determined following Ohmori et al. (1986). Mercury contents of the liver and kidney were estimated through atomic absorption spectrophotometry (Donaldson and Labella, 1983), the samples being dried and ashed at 600°C and the ash dissolved in concentrated nitric acid and diluted with double distilled water. Analysis were performed using the Instrumentation Laboratory model 551 atomic absorption spectrophotometer at USIC, University of Roorkee. Student's *t* test (Fisher, 1950) was applied for statis-

tical comparisons between the control and treated groups.

Results

The results confirmed that mercury is a potent inducer of lipid peroxidation in *Channa punctatus*, the process being measured on the basis of TBA chromogens that increased sharply in the liver and kidney samples after 15 and 30 days of exposure. However, no significant changes were recorded in the brain and gill samples after 30 days of exposure.

Longer exposures at the same concentration stimulated the formation of malondialdehyde—a lipid peroxidation product. However, the degree of response varied in different tissues, the maximum being in the brain and minimum in the liver after 15 days of exposure. After 30 days of exposure, kidney samples were affected less than the liver (Table 1).

The duration of exposure significantly influenced GSH content, as such increasing in all the tissue samples after 15 days exposure, but decreasing after 30 days (Table 2).

These observations contradicted the results for malondialdehyde formation, suggesting that lipid peroxidation does not depend solely on oxidative injury in this species. Moreover, the response varied with the type of tissue.

A further set of observations on oxidized glutathione (GSSG) in the selected tissues showed a persistent significant increase in GSSG activity. Invariably, the activity was greater after 30 days exposure than 15 days (Table 3).

A duration—dependent cumulative pattern of mercury deposition was observed in both the liver and kidney samples, the greatest accumulation being after 30 days (Table 4).

Table 1. Lipid peroxidation (TBA chromogens and malondialdehyde) in selected tissues of *Channa punctatus*

Sample No.	Treatment	Tissues			
		Liver		Kidney	
		TBA Chromogens	MDA n-moles/mg Protein	TBA Chromogens	MDA n-moles/mg Protein
1.	Control	0.09±0.002	0.409±0.031	0.05±0.003	0.298±0.021
2.	15 days	0.14±0.01**	0.469±0.025^{NS}	0.12±0.012**	0.595±0.098**
3.	30 days	0.11±0.007*	0.882±0.09**	0.30±0.026**	0.671±0.080**

Sample No.	Treatment	Tissues			
		Brain		Gill	
		TBA Chromogens	MDA n-moles/mg Protein	TBA Chromogens	MDA n-moles/mg Protein
1.	Control	0.09±0.002	1.208±0.15	0.07±0.004	0.375±0.04
2.	15 days	0.22±0.015**	1.636±0.95^{NS}	0.12±0.012**	0.654±0.041**
3.	30 days	0.08±0.001**	1.780±0.86^{NS}	0.078±0.003^{NS}	0.689±0.059**

Values are means±SE of 5 observations in each group. p=*<0.02; **<0.01 (control versus experimental groups).

Table 2. GSH (mM/litre) in selected tissues of *Channa punctatus* after mercury treatment for 15 and 30 days

Sample No.	Treatments	Selected tissues			
		Liver	Kidney	Brain	Gill
1.	Control	500±20.21	300±18.01	400±19.34	470±20.13
2.	15 days	1650±35.32***	1000±32.5***	1500±34.01***	1300±30.25***
3.	30 days	350±18.13***	200±15.45***	300±17.92***	300±18.23***

Values are means±SE of 5 observations in each group. p=***<0.001 (control versus experimental groups).

Discussion

The results for *Channa punctatus* showed that inorganic mercury is cumulative in soft tissues, such as the liver and kidney, and is, therefore, capable of manifesting cumulative effects. Observations on the relative tissue distribution of mercury revealed its selective accumulation in the liver and kidneys regardless of the length of the exposure period. Similar observations have been made in rats, regardless of the form of mercury injected (Chan et al., 1992). The binding affinity of metal(s) to carrier proteins may determine subsequent tissue distribution, as suggested by Mehra and Bremner (1983). However, the selective formation of metallothionein (Hg-Mt) in the kidney, where it can be easily reabsorbed due to its low molecular weight, might be the reason for its higher accumulation, especially in the kidneys.

Further-more, it was observed that mercury accelerated the process of lipid peroxidation in the liver, kidney, brain and gills of the fish. However, direct relationship between malondialdehyde and duration of exposure did not exist in all of the tissues examined here. In addition, it has been reported that mercury induces lipid peroxidation in hepatocytes and renal tissue (Fuzimoto et al., 1984; Stacey and Klaassen, 1981). All these findings were further supported by the ability of mercury to affect membrane permeability by altering the membrane structure (Kinter and Pritchard, 1977). Lipids in general are susceptible to mercurial toxicity (Nakada and Imura, 1983). Nevertheless, the increase in malondialdehyde content varied in different organs. Sensitivity of reduced nicotinamide adenine dinucleotide phosphate (NADPH) dependent lipid peroxidation to mercury may vary in different tissues, the simple reason possibly being allied to the mediation of NADPH-dependent lipid peroxidation through NADPH cytochrome reductase in the microsomes (Pederson et al., 1973).

Tissue GSH is known to be involved in the metabolism and detoxication of exogenous and endogenous substances (Meister and Anderson, 1983), particularly mercury ions (Ballatri and Clarkson, 1984). A change in GSH content raises several points, since it reflects the positive influence of deposited mercury on organ function. It should be emphasized, however, that higher accumulation of mercury in *Channa puntatus* was accompanied by a loss of GSH after 30 days exposure. Initially GSH is required for the inhibition of NADPH-dependent lipid peroxidation (Iba and Mannering, 1987). However, during a rapid phase of reaction or prolonged exposure, GSH depletion accompanies increased lipid peroxidation. The time-dependent antioxidant effect of GSH can be explained on the basis of the initial presence of some labile GSH-dependent or microsomal factors i.e. GSH transferases or cytochrome P-450 that can also provide protection as a peroxidase (Tan et al., 1984). The time-dependent effects, however, represent an initial biochemical response.

A subsequent loss of these factors due to con-

Table 3. GSSG (mM/litre) in selected tissues of *Channa punctatus* after mercury treatment for 15 and 30 days

Sample No.	Treatments	Selected tissues			
		Liver	Kidney	Brain	Gill
1.	Control	1700±25.50	2300±30.12	1550±22.31	1850±28.40
2.	15 days	1850±26.20**	2000±28.50***	2200±26.50***	2450±32.90***
3.	30 days	3600±30.50***	3750±35.60***	3000±32.20***	3500±36.20***

Values are means±SE of 5 observations in each group. p=**<0.01; ***<0.001 (control versus experimental groups).

Table 4. Hg content (μg/gm fresh tissues) in liver and kidney of *Channa punctatus* after 15 and 30 days of mercury treatment

Sample No.	Treatments	Selected tissues	
		Liver	Kidney
1.	Control	1.062±0.0450	2.345±0.0736
2.	15 days	3.124±0.1650***	3.854±0.2010***
3.	30 days	4.008±0.3100***	4.348±0.2410***

Values are means±SE of 5 observations in each group. p=***<0.001 (control versus experimental groups).

tinued exposure of the fish to mercury may lead to death. The absence of any protection by GSH can be attributed to the instantaneous destruction of GSH-dependent enzymes. The activity of NADPH cytochrome C reductase may vary in different tissues of the fish. Moreover, traces of mercury, after forming a complex with glutathione, cannot generate hydrogen peroxide, since Hg^{++} cannot catalyze the oxidation of glutathione (Albro et al., 1986).

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淡水魚 *Channa punctatus* における水銀による肝・腎・脳および鰓の脂質過酸化作用

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無機水銀で 30 日間処理した淡水魚 *Channa punctatus* の肝・腎・脳および鰓における脂質過酸化作用と酸化的ストレスを，同時に研究してみた．長時間の曝露は酸化的ストレスを引き起こしたが，障害の程度は各器官で異なっていた．短時間の曝露は，還元型グルタチオンを刺激するが，長時間の曝露は全組織で還元型グルタチオンを阻害した．これらの結果は，脂質過酸化作用が酸化ストレスと厳密には一致しないということを示唆している．時間依存性効果は，初期の生化学的反応を表しているが，若干の不安定還元型グルタチオン依存性因子の存在は，保護機構に帰すことができる．