Bull. Environ. Contam. Toxicol. (1988) 40:178–184

Bulletin of Environmental Contamination and Toxicology

Acute Effects of Mercuric Chloride on Intracellular GSH Levels and Mercury Distribution in the Fish *Oreochromis aureus*

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In recent years there has been much interest in the effects of trace metals on intracellular levels of reduced glutathione (GSH). Most of the research has been performed on rats. As GSH is ubiquitous in living organisms it is of interest to establish a relationship between mercury intoxication and intracellular GSH levels in fish; especially as fish living in rivers and coastal areas are often exposed to mercury as an aquatic pollutant. The role of GSH in fish trace metal toxicity has not been thoroughly investigated. The study of Braddon et al. (1984) established the presence of γ-glutamyl cycle enzymes in various tissues of the black sea bass. Glutathione-S-transferase (a detoxifying enzyme) was also detected in the liver of the black sea bass. These enzymes are important in mammalian glutathione metabolism (Meister and Anderson 1983). The distribution of total glutathione (oxidized + reduced) in selected black sea bass organs seems to follow the established pattern for mammalian organs (Braddon et al. 1984). Thus, it would appear that teleostian and mammalian glutathione metabolism may have many similarities.

There are few reports concerning the effects of mercury during the first few hours of exposure. The aim of this investigation is to establish any changes in organ GSH and mercury levels following just 2 h exposure to mercuric chloride ($HgCl_2$).

MATERIALS AND METHODS

Adult Oreochromis aureus of at least 15 cm length were obtained from a local fish farm. Prior to the experiment they were maintained in glass aquaria containing fresh water. A period of several weeks was allowed for the fish to recover from the stress of transportation and adapt to aquarium conditions. They were fed daily with a commercial pellet food.

Test fish were exposed in pairs to 1 mg/L mercury as $HgCl_2$, made up to a volume of 4 L in fresh water, for a period of 2 h.

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Control fish which were not treated with mercury were maintained in 4 L of fresh water for a period of 2 h. The fish were not allowed access to food during the experimental period. During the experiments the water temperature ranged from 22° to 27°C. The pH varied between 6.5 and 6.9.

After 2 h the fish were killed by decapitation. Gills, liver, brain, intestine, spleen and kidney were dissected out in ice-cold physiological saline. Fat was removed from the visceral organs and the gall bladder was removed from the liver. The gill filaments were trimmed from the gill arches. The intestine was flushed to remove feces and undigested food. Dissected organs were quickly blotted dry and weighed. Organs to be analysed for GSH were stored, frozen at -15°C, until required. This treatment does not influence GSH levels (unpublished data). Organs to be analysed for mercury were oven-dried at 60°C. The method of Agemain and Chau (1976) was used with the following modifications. Organs and tissues which had been pre-weighed and oven-dried in 100 mL conical flasks were digested with 16 mL concentrated HNO_3 (analytical grade, Merck) in a water bath at 60° C. Digestion was allowed to proceed until all traces of the tissues had dissolved. The complete digest of each sample was analysed for mercury content. The method of mercury analysis measures total mercury (organic + inorganic). Authentic standards were prepared from a stock solution of 1000 µg/g $HgCl_2$ in 1% HNO_3. A Perkin-Elmer (model MAS-50A) mercury analyser was used for mercury analysis.

Organs were analysed for GSH after the method of Richardson and Murphy (1975). The organs were homogenized in 5% TCA (trichloroacetic acid) in 1.0 x 10^{-3}M Na_2EDTA (disodium ethylenediaminetetraacetic acid) using 5 mL per 1 g of tissue. An Ultra-Turrax homogenizer (Janke and Kunkel, West Germany) was used at full speed under ice-cold conditions. Complete homogenization required from 10 to 20 sec, depending upon the nature of the tissue. The TCA homogenates were centrifuged at 1000 g for 15 min (0°C) using a Kubota KR/600 centrifuge. A 0.2 mL portion of the supernatant was analysed for GSH spectrophotometrically, using a Shimadzu UV-120-02 spectrophotometer (Richardson and Murphy 1975).

The experiment was repeated four times using separate batches of *O. aureus*. In each experiment there were three or four replicates of each organ for mercury analysis and GSH analysis in test and control groups. Results were calculated as µgHg^{2+}/g wet weight and µgGSH/g wet weight for the organs. The results from the four experiments were subjected to analysis of covariance to determine any differences in effects between experiments. The effects of the covariate were then removed for each set of data and the data were subjected to one way analysis of variance of test and control for each organ (4 experiments combined).

RESULTS AND DISCUSSION

After 2 h exposure to mercury, the gill filaments and kidney had accumulated the highest concentrations of mercury (Table 1). The gill filaments exhibited the highest increase in mercury levels.

This increase is highly significant ($p < 0.001$). The high mercury content of the gill filaments is not surprising since the gills are likely to be the principal route by which mercury enters the body. There is evidence that several heavy metals, including mercury, may be concentrated in the gills of fish exposed to acutely lethal concentrations of metal in the water (Mount and Stephan 1967). Fish maintained in fresh water are unlikely to swallow water for osmoregulatory purposes, in contrast to marine species. Therefore, the intestinal route appears to be an unlikely route for mercury to enter the body of fresh water species. This hypothesis is supported by the present findings; there is no significant

Table 1. Effects of 2 h exposure to 1 mg/L mercury (as $HgCl_2$) on mercury levels of selected organs and tissues.

Organs[b] tissues	Test	Control	F Value[a]	Significance[a] of F
	μg Hg^{2+}/g wet wt.			
Liver	0.60 (14)[c]	0.89 (14)	3.930	0.059
Brain	1.17 (14)	1.57 (14)	1.323	0.261 ns
Gill f	8.03 (15)	0.34 (15)	106.810	0.000
Intest	0.44 (15)	0.41 (15)	0.212	0.649 ns
Spleen	4.36 (13)	9.26 (14)	2.008	0.196 ns
Kidney	10.36 (13)	3.71 (14)	3.572	0.071

[a]Analysis of covariance followed by one way analysis of variance.
[b]Abbreviations: Gill f (gill filaments), Intest (intestine), ns (not significant). Results are expressed as means of test and means of control.
[c]Figures in parentheses indicate the number of organs analysed.

difference between test and control levels of intestinal mercury (Table 1). The absence of feeding during the period of intoxication may have played a crucial role in these findings. If the fish had been allowed access to food during the experimental period it is conceivable that substantial amounts of mercury, adsorbed by the food, would have entered the intestine. In addition, some of the water containing mercury, could have been swallowed with the food.

There is a nearly significant ($p < 0.1$) increase in renal mercury (Table 1) after 2 h exposure to mercury. The kidney is a target organ for mercury. In contrast, the liver has a nearly significant ($p < 0.1$) decrease in mercury content (Table 1). However, there is a highly significant ($p < 0.01$) decrease in hepatic GSH (Table 2).

The liver has higher levels of GSH than any of the other organs (Table 2). As GSH occurs predominantly intracellularly (Meister and Anderson 1983), the liver would appear to be a prime site for the complexion of mercury ions with GSH. Mercury has an exceptionally high affinity for thiol groups (Ballatori and Clarkson 1984). Complexion of mercury ions with GSH in the liver would explain the fall in hepatic GSH levels (Table 2). However, this does not exclude the possibility of mercury ions causing cell membrane damage, leading to a loss of GSH from the hepatocytes; although GSH is supposed to protect the cell membrane (Meister 1983). Other possibilities include the inhibition of enzymes involved in glutathione metabolism. The inhibition of rat liver

Table 2. Effects of 2 h exposure to 1 mg/L mercury (as $HgCl_2$) on GSH levels of selected organs and tissues.

Organs[b] tissues	Test	Control	F value[a]	significance[a] of F
	µg GSH/g wet wt.			
Liver	619.5 (14)[c]	794.9 (15)	10.573	0.003
Brain	260.5 (14)	218.5 (15)	1.539	0.226 ns
Gill f	240.4 (15)	269.0 (16)	0.200	0.658 ns
Intest	325.8 (15)	333.2 (16)	0.031	0.861 ns
Spleen	237.7 (14)	264.5 (14)	0.197	0.661 ns
Kidney	271.3 (14)	263.7 (15)	0.028	0.869 ns

[a]Analysis of covariance followed by one way analysis of variance.
[b]Abbreviations: Gill f (gill filaments), Intest (intestine), ns (not significant). Results are expressed as means of test and means of control.
[c]Figures in parentheses indicate the number of organs analysed.

glutathione reductase by mercury, in vivo, has been reported (Chung et al. 1982). The inhibition of this enzyme could result in increased GSSG (oxidized glutathione) levels at the expense of GSH levels, since this enzyme catalyzes the conversion of GSSG to GSH.

Exposure to mercury for 2 h failed to change renal GSH levels significantly (Table 2), despite an increase in renal mercury levels (Table 1). The failure of mercury to change GSH levels may be due to several factors. The turnover of glutathione is much more rapid in the kidney than in the liver of mice and the kidney has much higher levels of γ-glutamyl cycle enzyme activities (Sekura and Meister 1974). If a similar situation exists in fish

it would support the following hypothesis. Some of the free mercury ions entering the fish kidney would presumably complex with intracellular GSH, resulting in lower GSH levels. However, the rapid turnover of glutathione may allow the kidney to maintain GSH levels within the normal range. Since γ-glutamyl-cysteine synthetase activity is controlled by feedback inhibition by GSH (Richman and Meister 1975), the loss of GSH to form complexes would increase the activity of this enzyme.

Another possibility is that much of the free mercury entering the kidney may bind to intracellular thiol groups which are not associated with GSH. Metallothionein is a low molecular weight protein with many thiol groups. It is responsible for binding most of the mercury in the rat kidney when mercuric chloride is administered (Wisnienska et al. 1970). If metallothionein is present in the teleostian kidney in substantial quantities, it may spare GSH during metal intoxication.

In mammals there is evidence that mercury-glutathione complexes leave the liver and, following modification, are taken up by the kidney tubule cells. Methyl mercury is rapidly excreted in rat bile, bound to glutathione (Alexander and Aaseth 1982). There is evidence that more than 70% of this bound mercury is then reabsorbed from the intestine (Alexander and Aaseth 1982). Methyl mercuric glutathione complexes are degraded to cysteine-methyl mercury complexes in the rat duodenum (Hirata and Takahashi 1981). After glomerular filtration mercury enters the kidney tubule cells as a mercury-cysteine complex. The biliary route would appear to make an important contribution to renal mercury levels in rats as ligation of the bile duct leads to decreased renal mercury levels (Alexander and Aaseth 1982). There is no reason to doubt the existence of a similar pathway in teleosts.

If much of the mercury entering the fish kidney had already formed mercury-glutathione complexes in the liver, an increase in renal mercury levels could occur without an associated change in renal GSH levels; thus accounting for the present findings. It must be pointed out that mercury-glutathione complexes are not detected by the present GSH assay.

The highly significant increase in gill mercury levels (Table 1) is not accompanied by a change in gill GSH levels (Table 2). Heavy metals, including mercury, have been implicated in the precipitation of mucus secreted by the gills (Mount and Stephan 1967). Since mucus chelates heavy metals, much of the mercury measured in the gill filaments may have been associated with mucus. Consequently, a large proportion of the mercury measured in the gill filaments would be extracellular and would not be expected to influence intracellular GSH directly. However a superficial examination revealed no increased mucus in the gills of the experimental fish with only 2 h exposure.

The brain, spleen and intestinal mercury levels in the fish did not change significantly after 2 h exposure to mercury (Table 1).

Accordingly, there was no significant change in the GSH levels of these organs (Table 2). However, chronic exposure of Tilapia mossambica to 0.5 mg/L mercury (as mercuric nitrate) resulted in a significant increase in brain mercury levels after 7 d of exposure (Panigrahi and Misra 1980). These findings suggest that the amount of mercury entering the fish in just 2 h may be too low to affect the brain, intestine or spleen. Initially the liver may be successful in detoxifying the blood and preventing reactive mercury ions from reaching these organs in significant quantities. A higher mercury dosage, or a longer period of exposure, might surpass the detoxifying capacity of the liver.

In conclusion, it appears that in fish the liver is the primary site for interactions between mercury and GSH, particularly during the initial period of exposure. It is proposed that the liver plays a role in detoxifying reactive mercury ions and, in partnership with the kidney, limits the transport of these ions to other organs. An interorgan cycle of GSH, from the liver to the kidney, has been proposed in mammals (Meister 1981). It seems probable that GSH complexes follow a similar pathway in fish.

Although a relationship between mercury and GSH has been established, there is uncertainty that this relationship is beneficial. GSH is generally thought to be important in the metabolism of endogenous and exogenous toxins (Meister and Anderson 1983; Dudley and Klaassen 1984). However, depletion of GSH in rats prior to the adminstration of methyl mercuric chloride resulted in a decreased renal deposition of mercury (Richardson and Murphy 1975). A high degree of correlation was found between renal mercury accumulation and renal GSH concentration. It has also been reported that glutathione depletion inhibits the acute toxicity of methyl chloride in mice (Chellman et al. 1986). This is contrary to the role usually proposed for GSH in detoxifying xenobiotics. Further research is required to provide a better understanding of the role of GSH in detoxifying trace metals.

Acknowledgments. This work was supported by a research grant from the National University of Singapore. We wish to thank Dr J J Counsilman for his help and advice with the statistical analysis. We also wish to thank Mrs Y N Chan for typing the manuscript. Mr P Allen is the recipient of a National University of Singapore research scholarship.

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Received February 27, 1987; accepted July 27, 1987.