Bull. Environ. Contam. Toxicol. (1990) 44:623–628

Environmental Contamination and Toxicology

Hepatic Enzyme Activity after Combined Administration of Methylmercury, Lead and Cadmium in the Pekin Duck

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In order to assess adequately the environmental impact of heavy metals it is important to consider that they may occur simultaneously in the environment, where they may interact to alter their individual toxicities on living systems. Metals such as mercury (Hg), lead (Pb) and cadmium (Cd) can be found in all levels of the polluted ecosystem, and in animals inhabiting such areas (Wren 1986). In the polluted aquatic environment waterfowl have been noted to accumulate high levels of these metals in their tissues (Dieter et al. 1976).

A major toxic manifestation of heavy metal exposure is the perturbation of a wide range of enzyme systems in virtually all subcellular compartments (Vallee and Ulmer 1972; Fowler 1978; Prasada Rao and Gardner 1986). These effects are seen in all animals studied, including birds (Scheuhammer 1987). With the exception of lead, little data is available on the effects of metals on avian enzyme systems. The present report describes the effects observed in vivo on acid phosphatase (AP), glutathione S-transferase (GST) and cytochrome c oxidase (cyt c ox) in the liver of pekin ducks exposed to combinations of methylmercury (MeHg), lead and cadmium. Ducks were selected as the experimental animals because of their potential exposure to a variety of heavy metals in contaminated aquatic environments.

MATERIALS AND METHODS

Forty-eight six month old female pekin ducks (Anas platyrynchos) were obtained from King Cole Farms, Aurora, Ontario, Canada, and housed individually in stainless steel cages. The birds were allowed to acclimatize to housing and mash feed for a period of four weeks. The feed was mixed to supply the daily nutritional, mineral and vitamin requirements for laying birds. Birds were allowed food and water ad libitum. At the end of the acclimatization period the birds were randomly placed into eight groups of six birds. Group I (control group) birds were supplied with mash feed containing no added metals and were housed away from treatment groups and

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any known contaminants. Treatment groups were as follows: group II- 8 mg of methylmercuric chloride (MeHgCl)/ kg feed; group III- 80 mg of lead acetate (PbAc)/ kg feed; group IV- 80 mg of cadmium chloride ($CdCl_2$)/ kg feed; group V- 8 mg MeHgCl + 80 mg PbAc/ kg feed; group VI- 8 mg MeHgCl + 80 mg $CdCl_2$/ kg feed; group VII- 80 mg PbAc + 80 mg $CdCl_2$/kg feed; and group VIII- 8 mg MeHgCl + 80 mg PbAc + 80 mg $CdCl_2$/ kg feed. Treatments were administered for 12 weeks in rooms maintained at 20°C with a 14 hour light - 10 hour dark cycle per day. At the end of the 12 week period, the birds were euthanized by decapitation. A portion of the left hepatic lobe was removed, frozen in liquid nitrogen, and stored at -40°C until analysed.

All protein determinations were carried out using the method of Lowry et al. (1951). Acid phosphatase activity was measured spectrophotometrically according to the fixed time method of Moss (1983), after preparation of post mitochondrial supernatant (PMS). The enzyme substrate used was 4-nitrophenylphosphate (Sigma Chemical Co., St. Louis, MO.). Postmitochondrial supernatants were obtained by homogenizing 1 g of liver in a Potter-Elvehjem homogenizer in 3 mL of 0.25 M sucrose and centrifugation at 4°C and 10,000 g for 10 min. Triton X-100 (Sigma) was used to aid in enzyme release from lysosomes. Activity was measured at 405 nm and was recorded as nmol substrate used/min/mg protein. Glutathione S-transferase was assayed using the thioether formation method of Habig and Jakoby (1981), on PMS, using 1-chloro-2,4-dinitrobenzene (CDNB, Sigma) as substrate. Activity was determined by measuring the increase in absorbance at 340 nm and reported as μmol S-2,4-dinitrophenyl glutathione produced/min/mg protein. Cytochrome c oxidase activity was determined according to Cooperstein and Lazarow (1951), with the addition of using Lubrol to aid in the release of the membrane bound enzyme. Mitochondrial suspensions were prepared by homogenizing liver tissue in 3 mL of 0.03 M phosphate buffer, pH 7.4, and centrifugation at 4°C and 9,000 g for 10 min. The pellet was taken and recentrifuged after resuspension in buffer. The resulting mitochondrial pellet was resuspended in 1 mL of the buffer. Purified horse heart type III cytochrome c (Sigma) was used as the enzyme substrate. The reaction between enzyme and substrate at 550 nm was measured and results reported as μmol of cytochrome c oxidized/min/mg mitochondrial protein.

The data was initially tested for normality and homogeneity of variance. Acid phosphatase data was found to be non-normal in distribution, therefore a log transformation was applied. Subsequent testing revealed the transformed data was normal. Data was analyzed by analysis of variance using the general linear model procedure (Steel and Torrie, 1980) and a least square means (adjusted means) analysis was performed to test for differences between the control and treated groups ($P < 0.05$). The presence of interaction effects between administered metals was determined using type III sums of squares.

RESULTS AND DISCUSSION

Acid phosphatase levels were significantly lowered ($P < 0.05$) in all treatment groups receiving Cd, as compared to the control, except for the group administered Cd and Pb concurrently (Table 1). Acid phosphatase is an enzyme capable of hydrolysing phosphate esters at acidic pH, and is primarily

Table 1. Activity of hepatic acid phosphatase in ducks fed metal containing diets

Group	Activity[a] (mean ± SEM)	Interaction Effect[b]	F	Pr > F
I	12.47 ± 1.02			
II	12.60 ± 1.11	Hg	0.02	0.899
III	12.26 ± 1.05	Pb	0.03	0.872
IV	9.80 ± 1.05*	Cd	21.68	0.0001
V	12.48 ± 1.04	HgxPb	0.91	0.346
VI	10.40 ± 1.03*	HgxCd	0.21	0.649
VII	10.96 ± 1.06	PbxCd	0.23	0.638
VIII	9.82 ± 1.04*	HgxPbxCd	1.10	0.300

* Significantly different from Group I (Control, $P < 0.05$) using least means squares analysis

a- nmol 4-nitrophenylphosphate used/hr/mg protein
means represent the antilog of the transformed data

b- Interactions were considered significant if Pr > F was < 0.05 (ie if the interaction would occur < 5% by chance)

located in lysosomes. Lysosomes are able to accumulate not only essential metals, but also non-essential metals such as Hg and Pb (Koenig 1969). Metals may reduce the activity of AP by interfering with fusion of phagosomes with primary lysosomes or by directly affecting the enzyme itself (Li and Traxler 1974). While the present study showed no effects of Pb or organic Hg on duck liver AP, a significant inhibitory effect was seen when Cd was administered. Information regarding the effect of Cd on AP is scant, however Mego and Cain (1975) reported a decrease in activity in mice administered Cd. The inhibition possibly resulted from a decrease in total liver protein or an inhibition of primary lysosome formation. Since Cd avidly binds to sulfhydryls (Vallee and Ulmer 1972), the possibility exists that this metal may complex directly with the enzyme, causing a loss of function.

Levels of GST were significantly depressed only in the Cd alone group (group IV), when compared with controls (Table 2). Glutathione S-transferases are predominantly cytosolic enzymes capable of catalyzing the transfer of glutathione to a variety of potentially toxic electrophilic substances. The alteration in GST activity by metals has been seen to be dependant on the tissue, substrate and system (in vitro or in vivo) used. Published reports have

noted either no change in the activity of GST by Cd during in vivo studies on rat liver (Pak 1988) or a reduction in activity in vitro (Dierickx 1982). Dierickx (1982) also noted that addition of lead chloride to the incubation mixture produced no effect on GST activity, while inorganic Hg caused an inhibition.

Our results show an inhibition of GST in duck liver after chronic treatment with Cd and thus are similar to the acute in vitro study of Dierickx (1982). Little information exists on the effects of chronic administration of metals on GST activities in other species. It is interesting to note that in the present study only the group administered Cd alone (group IV) showed a significant decrease in GST activity. It appeared that combined administration of MeHg and/or Pb with Cd may have affected Cd's action on GST since the activity of GST was not significantly different from control levels in these combined groups. However, no significant interaction effects were observed. Since GST is an important enzyme in the phase II biotransformation system any interference in its activity by heavy metals such as Cd may predispose the tissue to toxic effects (eg. lipid peroxidation) of other environmental contaminants.

Table 2. Activity of hepatic glutathione S-transferase in ducks fed metal containing diets

Group	Activity[a] (mean ± SEM)	Interaction Effect[b]	F	Pr > F
I	0.179 ± 0.019			
II	0.172 ± 0.009	Hg	1.17	0.287
III	0.158 ± 0.010	Pb	0.01	0.908
IV	0.140 ± 0.015*	Cd	1.09	0.303
V	0.168 ± 0.006	HgxPb	0.10	0.755
VI	0.163 ± 0.012	HgxCd	0.90	0.350
VII	0.159 ± 0.010	PbxCd	2.21	0.145
VIII	0.175 ± 0.020	HgxPbxCd	0.39	0.534

* Significantly different from Group I (Control, $P < 0.05$) using least means squares analysis

a- μmol S-2,4-dinitrophenyl glutathione produced/min/mg protein

b- Interactions were considered significant if $Pr > F$ was < 0.05

No significant differences in the activity of cyt c ox in the liver of the ducks were observed between the control group and individual treatment groups. However, the Hg interaction effect was significant, indicating that, overall, groups administered Hg had significantly lower activity than groups not given this metal (Table 3). Although Cd and Pb have been observed to cause a reduction in cyt c ox activity (Iannaccone et al. 1976; Prasada Rao and Gardner 1986), only organic Hg inhibited the activity of this enzyme in the present study.

Various authors have observed reduced activities of cyt c ox in the kidneys of rodents caused by acute inorganic Hg (Verity and Brown 1970), while Fowler and Woods (1977) observed a reduction in the activity of hepatic cyt c ox in fetal rats whose mothers were exposed to methylmercury hydroxide. Mann and Auer (1980) showed that the purified enzyme was inhibited by low amounts of both inorganic and organic Hg. It appeared that only one equivalent of the organic mercurial used (ethylmercuric chloride) was able to penetrate into the interior of the enzyme and inhibit it. The inhibition occurred by the binding of the mercurial to a sulfhydryl group in the heme *a* portion of beef heart cyt c ox (Mann and Auer 1980). Our in vivo study has also shown an inhibition of this enzyme by an organic mercurial, methylmercuric chloride.

Table 3. Activity of hepatic cytochrome c oxidase in ducks fed metal containing diets

Group	Activity[a] (mean ± SEM)	Interaction Effect[b]	F	Pr > F
I	2.21 ± 0.44			
II	2.03 ± 0.28	Hg	4.47	0.041
III	2.70 ± 0.33	Pb	3.27	0.079
IV	2.22 ± 0.33	Cd	1.70	0.200
V	1.35 ± 0.17	HgxPb	1.79	0.189
VI	2.05 ± 0.24	HgxCd	1.78	0.191
VII	1.53 ± 0.30	PbxCd	1.84	0.183
VIII	1.35 ± 0.30	HgxPbxCd	1.68	0.202

a- μmol cytochrome c oxidized/min/mg mitochondrial protein
b- Interactions were considered significant if Pr > F was < 0.05

The inhibitory effects of low levels of MeHg and Cd on the enzyme systems studied here were relatively mild, with Cd producing the most pronounced effects. No significant interactions affecting the activity of the studied enzymes were noted between MeHg, Pb and Cd.

Acknowledgements: The authors wish to thank Dr. PVV Prasada Rao for assistance in enzyme determinations. The financial assistance of the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Agriculture and Food is gratefully acknowledged.

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Received July 26, 1989; accepted November 6, 1989.