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Effect of a Multiherbal Drug on the Distribution and Excretion of Mercury in Mice Treated with Mercuric Chloride

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ABSTRACT

This experiment was designed to study influence of a multiherbal-hepatotonic drug (MHH) on the accumulation and excretion of Hg in mice following administration of mercuric chloride solution as drinking water at 1 mM and 5 mM for 100 and 30 days respectively. Atomic absorption analysis revealed that drug enhanced total Hg- content in the liver and feces while it decreased Hg content in blood, kidney and testis. Drug did not affect Hg content in the brain. In another experiment 250 µg $HgCl_2$ was given i.p./mouse once alone and also followed by DMSA or sodium selenite once or followed by herbal drug for a fortnight. Organ and fecal Hg-contents were measured from all four groups of animals after 1, 5 and 15 days. Atomic absorption analysis suggests distinct action of drug on accumulation and excretion of Hg in mice. Probable effect of drug is discussed to explain its mild chelating action.

INTRODUCTION

Mercuric chloride intoxication is treated with BAL[1], D-penicillamine[2], N, acetyl-DL penicillamine[3], Unithol[4], DMSA[5] and DMPS[6]. These are costly chelators. Effects of penicillamines is not consistent in human being[7-9] in India, heavy metal pollution is becoming evident[10-12], hence there is scope for cheap yet effective drug to cure Hg-intoxication. A multiherbal hepatotonic drug is reported to protect mammalian organs and blood against cadmium[12-15], beryllium[16,17] and mercuric chloride[18,19] hence it appeared worth testing its influence on the uptake and excretion of Hg in mice.

MATERIALS AND METHODS

Five months old male Swiss albino mice were used. First experiment consisted of control group 'C' receiving Hg-free drinking water while in 'P' group mice drunk mercuric chloride solution at 1 mM for 100 days. In 'P+D' group mice drunk mercury solution plus 0.5 ml of MHH 52 drop (A multiherbal hepatotonic drug), per mouse per day for 100 days. In 'PT' (Post-therapy) "100 days $HgCl_2$ drunk mice as in 'P' group" were shifted to Hg-free water and 0.5 ml MHH/mouse/day for a fortnight. In NR group (Natural Recovery) group also '100 days $HgCl_2$ drunk mice were shifted to only Hg-free water for a fortnight. Second experiment was also planned as first one but $HgCl_2$ was administered at 5 mM for 30 days.

Third experiment consisted of four groups (1) 250 µg $HgCl_2$ i.p./mouse once; (2) 250 µg $HgCl_2$ i.p./mouse once; each mouse also received 0.5 ml drug six days before mercuric chloride injection and continued for next fortnight; (3) 250 µg $HgCl_2$ i.p./mouse once plus half an hour later DMSA oral at 30 mg/kg body weight; (4) 250 µg $HgCl_2$ i.p./mouse/once followed by an injection of Na_2SeO_3 at 0.25 mg/kg half an hour later.

Animals of 'C', 'P' and 'P+D' groups were sacrificed on 31st day in first experiment and on day 101 in second experiment; on 46th and 116th day in PT' and 'NR' groups in first and second experiment respectively. In third experiment mice from all four groups were sacrificed after 1, 5 and 15 days. Blood, liver, kidney, brain, testis and fecal matter were processed for atomic absorption spectroscopy (Perkin Elmer – 2380) to measure Hg-content.

STATISTICS

Samples of control group did not show presence of mercury but metal was detected in the samples obtained from all three experimental groups. Data were subjected to 't' test at 5% level of significance.

Table 1: Values obtained in 'P' group were compared with 'P+D', 'PT' and 'NR' groups in the first experiment and significance is shown by superscript sign 'a' (for P vs P+D vs PT and P vs NR) 'b' (for PD vs PT) 'c' (for PD vs NR) and 'd' (for PT vs NR). In second experiment animals died in 'NR' group hence 'd' could not be calculated.

Table 2: In the third experiment Hg-content in organs and in feces in all four groups (1 to 4) after each interval (1 day, 5 day and 15 days) were compared. Within the group and significance is shown by mark '*' to show significance when 1 day vs 5 day were and ** when 5 day vs 15 day were compared. A comparison was made among the values given in the last column of Table-2 "Total Hg burden" of first group ('Hg-alone' at 1 day vs 5 days vs 15 days and significance is shown by superscript 'a'. The values of 1 day, 5 days and 15 days in the first group were also compared with corresponding values in Groups 2, 3, 4 and significant difference is shown by 'b' (group 1 vs group 2) 'C' (group 1 vs group 3) and 'd' (group 1 and group 4).

RESULTS (Tables 1 and 2)

1. **Mortality**

   In the first experiment mice drinking 1 mM mercuric chloride solution (P) no mortality was recorded in any group. In the second set of experimental group mice drinking 5 mM mercuric chloride solution there occurred 50% mortality during 30 days exposure hence remaining 50% were sacrificed and used. When drug was also administered to mice drinking mercury mortality was reduced to 20%. Two more identical 'P' groups were also run from which following 50% mortality, surviving 50% were used in either 'PT' group or 'NR' group. In 'PT' group there occurred 40% mortality while all animals died in 'NR' group.

2. **Mercury distribution and excretion pattern**

   (i) Control group – Mice used in the present experiment did not show any trace of mercury in their body. Also, their food, drug and water used were free from mercury.

   (ii) Experimental groups – Measurable mercury did accumulate in the body of mice in all three experiments; details are described under separate headings.

Experiment 1 …. 1 mM (Table 1)

Kidney: 'P+D' group shows less Hg-content than 'P' group. 'P+T' shows low values than 'P' and 'P+D' groups. In 'NR' group value is more than 'P+T' group but lower than 'P' and 'P+D' group.

Table 1: Organwise distribution of Hg content following Mercuric chloride administration to mice (Values are in µg Mean ± SE, n=10)

Organs	Groups in Experiment-I (1 mM for 100 days)					Groups in Experiment-II (5 mM for 30 days)				
	C	P	P+D	PT	NR	C	P (50%)	P+D (20%)	PT (40%)	NR (100%)
Kidney	0	90±2.1	a 66±1.7	ab 42.1+1.3	acd 51.2±1.7	0	101±1.8	a 81±1.4	ab 65.1±1.5	–
Liver	0	244±2.0	a 362±3.1	ab 264±1.9	acd 202±2.0	0	360±3.1	a 460±3.3	ab 480±2.8	–
Blood	0	65±2	a 46.5±2.1	ab 18±1.5	acd 22±1.2	0	80±1.5	a 61±1.7	ab 48±1.5	–
Brain	0	8.8±0.7	8.6±0.5	8.2±0.8	9.1±0.4	0	9.9±0.8	8.6±0.5	8.4±0.3	–
Testis	0	16±1.5	a 10.2±0.6	ab 7.4±1.6	ad 9.2±1.4	0	22±1.2	a 14±1.4	ab 9.1±1.6	–
Feces	0	15.1±0.6	a 27±0.8	ab 11±1.8	ac 7±0.7	0	33±1.1	a 46±2.1	ab 22±1.3	–
Total organ Hg-content	0	428	494	340	294	0	573	624	611	
Its % in feces	0	3.5%	5.46%	3.23%	2.38%	0	5.75%	7.37%	3.60%	

Control mice organs, feces, food, drinking water all were found free from Hg, hence zero is shown. Significance based on 't' test at 5% level of significance are shown as a=P vs PD or PT or NR, b=PD vs PT, c=PD vs NR, d=PT vs NR. % value in bracket shows percentage mortality in that group.

Table 2: Effect of DMSA, Na_2SeO_3 and a multiherbal drug on the accumulation and the excretion of mercuric chloride in mice. Values are µg/wt. wt. for organs and µg/dry wt. for feces (Means ± SE, n=90)

Treatments	Total mercury measured after	Total mercury content in the following samples						Total of organ Hg burden
		Liver	Kidney	Brain	Blood	Testis	Feces	
$HgCl_2$ alone	1 day	68±2.0	24.1±0.6	6.8±0.6	26±0.5	10.2±0.8	12.5±1.3	135.1
	5 day	52±2.2*	19.5±1.7*	4.8±0.2*	5.5±0.1*	6.2±0.7*	7±0.8*	94.6[a]
	15 day	32±1.7**	10.5±1.1**	4.3±0.3	3.1±0.2**	5.8±0.6	4.2±0.2**	55.7[a]
$HgCl_2$ + Herbal drug	1 day	86±1.7	6.3±0.7	7±0.7	15.2±0.4	9±1.1	17±0.9	123.5[b]
	5 day	63.2±1.1*	4.4±0.2*	4.6±0.2*	3.5±0.4*	5.1±0.5*	6.5±0.3*	80.8[b]
	15 day	35.1±1**	2.1±0.1**	4.1±0.1	1.1±0.1	3.7±0.1**	5.6±0.6	46.2[b]
$HgCl_2$ + DMSA	1 day	32±0.2	38.4±0.9	4.2±0.2	7.5±0.1	5.5±0.7	14.0±0.3	87.6[c]
	5 day	24.6±0.6*	12.9±0.2*	3.68±0.1*	3.5±0.4*	4.5±0.8*	5.5±.0.4*	49.1[c]
	15 day	15.2±0.4**	1.8±0.3**	3.4±0.1	1.1±0.3**	4.3±0.2	1.9±0.3**	25.8[c]
$HgCl_2$+Na_2SeO_3	1 day	62±1.1	27±0.4	5.6±0.1	20.5±0.2	7.7±0.2	12.44±0.1	122.8[d]
	5 day	29.1±1.8*	17.6±0.6*	4.4±0.6*	10.7±1*	4.5±0.1*	5.5±0.4*	66.3[d]
	15 day	22±0.6**	3.1±0.3**	4.2±0.1	3.2±0.4**	4.4±0.2	2.3±0.1**	39.4[d]

Note:
250 mg $HgCl_2$ contains 197 µg "Hg".
For total organ value (Table 2 last column); µg/mg value was multiplied by total weight of that organ
Statistics: All values are subjective to "t" test at 5% level of significance
For Table 2: *=Significant difference when 1 day value vs 5 day compared, and ** when 5 day vs 15 day compared except for last column where:
a=1 day vs 5 day vs 15 day i.e. comparison within Group 1 b=Group 1 vs Group 2 comparison of corresponding interval.
c=Group 1 vs Group 3 comparison of corresponding interval. d=Group 1 vs Group 4 comparison of corresponding interval.

Liver: 'P+D' group shows higher Hg-content than 'P' group. In 'PT' group value is more than 'P' group but less than 'P+D' group. In 'NR' group value is lower than 'P', 'P+D' and 'PT' group.

Blood: In 'P+D' group less Hg contents is recorded than 'P' group. In 'PT' value is quite less than 'P' and 'P+D' groups. In 'NR' group value is more than 'PT' but less than 'P' and 'P+D' groups.

Brain: Hg did accumulate in the brain but content remained unaffected by any treatment.

Testis: In 'P+D' group less Hg is recorded than 'P' group. In 'PT' group low value is noted than 'P' and 'P+D' groups. In 'NR' group low value is recorded than 'P' but value is more than what is recorded in PT (but equal to P+D group).

Feces: In P+D group, Hg content is higher than 'P' group (highest among all groups). In 'PT' group value is less than 'P' and 'P+D' groups. In 'NR' group value is lowest among all groups.

Experiment II … 5 mM (Table 1)

Kidney: Hg content is less in 'P+D' group than 'P' group. In 'PT' group value is lowest i.e. lower than both groups 'P' and 'P+D' groups.

Liver: 'P+D' group has more Hg-content than 'P' group. In 'PT' group highest value is recorded i.e. more than 'P' and 'P+D' groups.

Brain: Hg accumulated in the brain but its content did not change following any treatment in any group.

Blood: Less Hg is recorded in 'P+D' group than 'P' group. In 'PT' lowest value is recorded i.e. lower than 'P' and 'P+D' groups.

Testis: In 'P+D' group less Hg is recorded than 'P' group. In 'PT' group lowest value is found i.e. lower than 'P' and 'P+D' groups.

Feces: Higher Hg-content is recorded in 'P+D' group than 'P' group. 'PT' group revealed lowest value i.e. lower than 'P' & 'P+D' group.

Experiment III (Table 2 last column):
Analysis of data reveals that in the presence of herbal drug (Group-2 = $HgCl_2$ + MHH) after 1 day following Hg administration, total Hg-burden of body is 14.54% less than corresponding value in first group (grp-1 = $HgCl_2$ alone). In the presence of DMSA this value is 48.31% lower and in the presence of selanite this is 29.95% low.

When values obtained after 15 days following Hg-exposure in Group I are compared with corresponding values in Groups 2, 3 and 4 it becomes clear that 17.09% Hg is retained in the body of mice in herbal drug treated group i.e. group 2 and is 53.69% less in DMSA treated group (gr-3) and is 29.28% less in Selenite treated group (gr-4). This way results indicate that in the presence of herbal drug MHH statistically significant less Hg is retained in the body of mice and more is excreted in feces.

DISCUSSION

There exists no report on the chelating action of any herbal compound in animal system in the literature hence probable role of this drug in relation to present findings is discussed.

Only 1-2% of an orally administered dose of mercuric chloride given to mice is absorbed[20]. In the present case 1 mM and 5 mM (i.e. 270 µg/ml and 1035 µg/ml) were used as drinking water. If each mouse consumed 1 to 2 ml of either solution per day for 100 or 30 days respectively than observed Hg-content values do not seem unexpected. High mortality in 'P' group at 5 mM is not unexpected as LD_{50} for mercuric chloride in mice is 10 mg/kg body weight (EHC-1991).

- DMSA and Na_2SeO_3 reduced Hg-burden in mice, these being known facts[5,21] do not require discussion.
- Results show high Hg-content in the liver, aquatic mammals like Porpoise and pilot-whale had been shown to have 18.3 mg/kg and 157 mg/kg Hg-content in the liver[22,23]. In another report[24], common seal had been found to have 765 mg/kg Hg in the liver. Here also occurred high selenium along with Hg. Authors suggested that as selenium is a protective substance and it caused Hg to bind with – SH containing proteins. In the present case drug might have induced metallothionein (as plants can synthesize phytochelatins)[30], which in turn have bounded large quantity of Hg. Such action of this drug was suspected in relation to the protection afforded towards mice liver and kidney following cadmium chloride intoxication[14,15].
- This multiherbal drug (like DMSA), did not cause rise in the Hg concentration in the blood hence in brain. A redistribution caused by BAL leading to high Hg in the brain is a major contraindication in the use of BAL[26,27]. Moreover, BAL is toxic, DMSA is 35 times less toxic and herbal drug used is not at all toxic[25]. In the presence of drug, less Hg is recorded in the testis. In a report beryllium-induced reproductive impairment in rats was restored with the use of this drug[28]. Action of drug was not explained. Drug might have reduced beryllium from the reproductive organs as it did with Hg.

In animals, initial excretion is predominantly via feces, when more is absorbed it is lost through kidney via urine. Drug possesses mild laxative property hence more Hg appeared in feces and less in the kidneys.

In plant kingdom phytochelatins of the general formula [Glu (-Csy)n-Gly (n=2…. 11) act as principal heavy metal detoxifying components. Possibility of occurrence of precursors of phytochelatins in the drug and their chelating action cannot be ruled out.

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