Kawasaki Med. J. Vol. 4: No. 2, 113-119, 1978

EFFECT OF MERCURIC CHLORIDE ON THE THYROID FUNCTION IN RATS

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Accepted for Publication on June 27, 1978

Abstract

Mercuric chloride ($HgCl_2$) intraperitoneally administered to male Sprague-Dawley rats was associated with decrease in 24-hr thyroid ^{131}I-uptake, thyroid weight, serum protein bound^{131}I, conversion ratio and thyroid-to-serum^{131}I ratio. Chromatographic analyses of pronase-thyroid hydrolysates of the $HgCl_2$-treated rats revealed a depression in synthesis of thyroid hormones. The thyroid function in male Sprague-Dawley rats seemed to be more susceptible to $HgCl_2$ than in male Wistar rats, while no significant difference was observed between both sexes of Wistar rats.

INTRODUCTION

Mercury is one of the wide spread, hazardous environmental contaminants both in the forms of organic and inorganic compounds[1,2], and is known to principally associated with central neuropathy and renal failure[3], but little is known about its toxic effects on the endocrine organs, including the thyroid gland[4].

The present study was undertaken to assess the influence of mercuric chloride ($HgCl_2$) on the thyroid function in rats by examining some of the kinetics of iodine metabolism following single intraperitoneal injection of the compound.

MATERIALS AND METHODS

Male Sprague-Dawley rats or male and female Wistar rats, 10-11 weeks old, were used in this study. The animals were fed Oriental MF pellet (iodine content 1.06 mg/100 g) and given tap water *ad libitum*.

The rats were given an intraperitonal injection of a fresh solution of $HgCl_2$ so that they received 2.6 mg (1/4 mM) or 1.3 mg (1/8 mM) of mercury for every 1 kg of body weight. A group of rats in each strain and sex were injected intraperitoneally with 0.5 ml of deionized water and served as a

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control. Twenty-four hours after the injection of the compound, 10 μCi of carrier free $Na^{131}I$ was injected intraperitoneally. Twenty-four hours later, the rats were sacrificed and blood was drawn from the abdominal aorta of each rat under ether anesthesia and the serum was separated by centrifugation.

Radioiodine(^{131}I) in 1 ml of serum from each rat was counted in a Packard Autogamma Scintillation Spectrometer Type 53203 (serum^{131}I), then 2 ml of 10% trichloroacetic acid (TCA) was added to the serum, and the resulting precipitate was washed twice with 3 ml of 5% TCA. The precipitate was counted in the scintillation counter to determine the level of protein bound radioiodine ($PB^{131}I$) in the serum, as the percentage of the dose of injection. The ratio of $PB^{131}I$ to serum^{131}I in each rat was also calculated as the conversion ratio.

The thyroids were dissected out, cleaned, weighted and ^{131}I was assayed to determine the thyroid^{131}I-uptake as the percentage of the injected dose.

The determination of the thyroid-to-serum^{131}I-ratio (T/S ratio) was performed as the ratio of ^{131}I per mg of thyroid to ^{131}I per ml of serum.

Each thyroid was hydrolyzed in 1.0 ml of physiological saline with 10 mg of Pronase-P (Kaken Kagaku Co.) for the 24 hours at 37°C. The iodinated aminoacids were fractionated by ascending paper-chromatography in a solvent system of n-butanol : ethanol : 2N-NH_4OH (5 : 1 : 2, v/v/v) for approximately 16 hours. The radioactive papers were dried, and the positions of the radioactive zones were located by a Packard Radiochromatogram Scanner Type 7201, then cut them into 5 mm strips. Each strip was counted in the counter and the radioactivity in each band was divided by the total paper count to derive the percentage of each constituent[5].

The measurements of serum triiodothyronine (T_3) and thyroxine (T_4) levels, hematocrit (Ht) value, hemoglobin (Hb) content and blood urea nitrogen (BUN) level were performed in a separate series of experiment, in which male Wistar rats were similarly treated with $HgCl_2$, without ^{131}I-administration. Serum T_3[6] and T_4[7] levels were assayed with radioimmunoassay (T_3RIAKIT and T_4RIAKIT, Dainabott Co.). The Hb content was measured as cyan-methemoglobin, and the Ht value was determined by the micro-capillary method. The BUN level was determined by the urease-indophenol method (Urea NB-Test, Wako).

RESULTS

1) Effect of $HgCl_2$ on the thyroid function in male Sprague-Dawley rats:

The results are summarized in Table 1. It can be seen in Table 1 that the administration of $HgCl_2$ in Sprague-Dawley rats was associated with a decrease in thyroid weight accompanied by a significant decrcase in 24-hr

thyroid^{131}I-uptake, PB^{131}I, conversion ratio and T/S ratio. The weight of kindneys significantly increased in the $HgCl_2$-injected rats.

2) Radioiodine distribution in the components of pronase-hydrolyzed thyroid of male Sprague-Dawley rats treated with $HgCl_2$:

Chromatographic analyses of thyroid hydrolysates are shown in Table 2. The data in the table show that a significant increase in percentage of ^{131}I in MIT was observable, while ^{131}I in DIT decreased in the $HgCl_2$ treated groups. A significant reduction in percentage of ^{131}I in T_4 was observable, while the percentage of T_3 remained unchange in the groups treated with $HgCl_2$. The depression of DIT labeling in the $HgCl_2$-treated rats was also indicated by elevation of the MIT/DIT ratio, as compared with the control group. These changes are considered as a classic illustration of a reduction in thyroid hormone synthesis.

3) Effect of $HgCl_2$ on the thyroid function in male and female Wistar rats:

The results are shown in Table 3. The data in the table indicated that the thyroid weight of the $HgCl_2$ intoxicated male rats was similar to that of the control rats. The mean values of thyroid^{131}I-uptake and serum^{131}I-levels were higher, while PB^{131}I, conversion ratio and T/S ratio were lower in the $HgCl_2$ treated males. The kidney weight significantly increased in the $HgCl_2$ treated male rats.

The data in female Wistar rats are also indicated in Table 3. Similar results were observed in the females as shown in the males, except the T/S ratio was not altered by the administration of 1.3 mg/kg of $HgCl_2$.

4) Effect of $HgCl_2$ on serum T_3- and T_4-levels, Ht value, Hb content and BUN level in male Wistar rats:

The findings in male Wistar rats are shown in Table 4. It can be seen in the table that the serum T_4-levels were significantly depressed in the $HgCl_2$ treated groups, while the serum T_3-levels were not altered by the administration of $HgCl_2$. The Ht values and the Hb contents in rats injected with $HgCl_2$ were similar to the values observed in control animals. The BUN levels were significantly elevated in the $HgCl_2$-treated groups.

DISCUSSION

It has been reported that certain metallic ions interfere with the iodine uptake in the thyroid gland[4,8,9,10], but little is known about the effect of mercury or its salts on the thyroid function.

TABLE 1. Effect of $HgCl_2$ on the thyroid function in male Sprague-Dawley rats

Treatment	No. of animals	Body weight g	Thyroid mg	Thyroid ^{131}I uptake %	Serum ^{131}I %/ml	PB ^{131}I %/ml	Conversion ratio %	T/S ratio	Kidneys g
Control	5 (0/5)[a]	290±13[b]	16.0±0.9	15.2±0.9	0.06±0.005	0.03±0.01	45.4±10.0	15.6±2.0	1.84±0.11
Hg^{++} 1.3 mg/kg body weight	4 (1/5)	260± 5	12.7±1.0	7.2±1.6**	0.11±0.015*	0.01±0**	10.1± 1.9	6.3±2.4*	2.57±0.66
Hg^{++} 2.6 mg/kg body weight	2 (3/5)	290	11.5	2.7	0.52	0.01	2.2	0.5	2.49

a) No. of death/No. of treated animals b) Mean±S.E.

* Significantly different from controls, $p<0.05$

** Significantly different from controls, $p<0.01$

TABLE 2. Distribution of ^{131}I percent in chromatograms of thyroid hydrolysates of male Sprague-Dawley rats treated with $HgCl_2$

Treatment	No. of animals	Origin	MIT	DIT	T_4	T_3	I^-	MIT/DIT
Control	5	0.7±0.1	31.1±1.9	38.2±2.3	24.9±1.8	1.8±0.2	3.6±0.8	0.83±0.08
Hg^{++} 1.3 mg/kg body weight	4	0.5±0.3	40.6±1.1**	30.7±4.2	21.5±1.7	2.2±0.8	4.5±1.8	1.43±0.20*
Hg^{++} 2.6 mg/kg body weight	2	1.1	43.5	27.3	16.1	3.2	8.8	1.62

MIT: monoiodotyrosine, DIT: diiodotyrosine, T_3: triiodothyronine, T_4: thyroxine, I^-: iodide

Filter paper, Toyo-Roshi NO. 51

Solvent system, n-butanol : ethanol : 2N NH_4OH (5 : 1 : 2, v/v/v)

TABLE 3. Effect of $HgCl_2$ on the thyroid function in male and female Wistar rats

Treatment	Sex	No. of animals	Body weight g	Thyroid mg	Thyroid ^{131}I uptake %	Serum ^{131}I %/ml	PB ^{131}I %/ml	Conversion ratio %	T/S	Kidneys g
Control	♂	5	296±19	16.8±1.9	16.3±1.3	0.12±0.04	0.02±0.004	28.2±7.1	11.3±2.5	1.88±0.13
Hg^{++} 1.3mg/kg body weight		5	304±12	17.6±2.5	17.7±0.7	0.22±0.03	0.01±0.002	5.1±0.7*	4.9±0.9	2.63±0.10**
Hg^{++} 2.6mg/kg body weight		4	326±14	16.3±1.7	19.1±1.7	0.23±0.02	0.01±0**	4.5±0.5*	5.2±1.4	2.77±0.16**
Control	♀	5	196± 5	14.2±0.9	10.2±0.9	0.09±0.01	0.02±0	23.2±1.7	9.3±1.7	1.38±0.02
Hg^{++} 1.3mg/kg body weight		5	208± 5	14.4±1.7	15.8±2.1	0.23±0.07	0.02±0.004	19.0±1.0	9.9±4.2	1.70±0.11*
Hg^{++} 2.6mg/kg body weight		5	212± 6	15.4±1.2	8.5±1.4	0.30±0.07*	0.01±0.002**	7.4±0.4**	3.1±1.5*	1.97±0.10**

TABLE 4. Effects of $HgCl_2$ on serum T_3 and T_4 levels in male Wistar rats

Treatment	No. of animals	Body weight g	Thyroid mg	T_3 ng/ml	T_4 μg/dl	Ht %	Hb g/dl	BUN mg/dl	Kidneys g
Control	5	340± 9	13.8±0.7	0.4±0.05	7.4±1.2	44.6±1.3	15.4±0.3	23.8± 1.1	2.27±0.06
Hg^{++} 1.3 mg/kg body weight	5	310±21	15.2±1.1	0.3±0.05	3.4±0.2*	43.2±4.5	15.3±0.5	123.1± 6.1**	2.14±0.12
Hg^{++} 2.6 mg/kg body weight	5	292±12	14.8±1.6	0.4±0.06	3.4±0.5*	43.8±1.5	15.7±0.6	128.6±13.3**	2.77±0.30

T_3: triiodothyronine, T_4: thyroxine

The synthesis of thyroxine in the thyroid gland is considered to include iodide accumulation, iodination of tyrosine, coupling of monoiodotyrosine and diiodotyrosine to form triiodothyronine and thyroxine, and finally the release of thyroid hormone from the thyroid gland. Each of these steps is controlled by the thyrotrophic hormone (TSH).

Mercuric chloride may affect the iodide accumulation step or any other step in thyroid hormone synthesis in the thyroid gland. It may also affect the pituitary gland, and its action may be manifested through a change in the TSH level.

Our study, however, does not include any analysis of TSH levels in the sera, only the mechanism of action of $HgCl_2$ on thyroxine formation in the thyroid gland will be discussed in this report.

The data obtained in our study show that $HgCl_2$ administered to rats was associated with decreased in the 24-hr thyroid^{131}I-uptake, and are in agreement with Slingerland's[8] *in vitro* observation that heavy metals, including $HgCl_2$, inhibit the *in vitro* uptake of iodine by thyroid slices. The thyroid weight also decreased in the $HgCl_2$ treated rats. These findings are contrary to the results expected from a stimulation of the pituitary gland. Thus it seems likely that the effect observed is due to the direct action of mercuric ion on the thyroid gland, as suggested by Anber *et al*[4]. The observations on the chromatographic analyses of thyroid hydrolysates indicated the increase in the percentage of MIT, the decrease in DIT and T_4, and the incrcase in the MIT/DIT ratio in the $HgCl_2$-treated rats. These data suggested that $HgCl_2$ inhibit the thyroid hormone synthesis in the thyroid gland.

It has been reported that SH containing enzymes participate in the process of thyroid hormone synthesis, and most probably in the step of iodide concentration[4,8]. It has also been reported that a great many enzymes which depend upon SH groups for their activity are sensitive to low concentration of heavy metals, especially of mercury[11]. It is thus probable that the action of mercury in the thyroid tissue is mainly due to its blocking action on SH groups of enzymes.

The susceptibility of thyroid function to $HgCl_2$ in male Sprague-Dawley rats was compared with that in male Wistar rats. It has been suggested from our data that the thyroid function in male Sprague-Dawley rats seemed to be more susceptible to $HgCl_2$ than that in male Wistar rats.

Sandstead[12] observed that *in vivo* depression on ^{131}I-uptake by the rat thyroid and the conversion ratio was produced by chronic lead intoxication, and female rats were more susceptible than male rats. Accordingly, the susceptibility of thyroid function to $HgCl_2$ in male Wistar rats was also compared

with that in female rats of the same strain. Our data, however, did not indicate any significant difference in the susceptibility of thyroid function between the sexes.

Further studies should be carried out to ascertain the differences between the sexes and strains in the susceptibility of thyroid function to mercury exposure.

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