Asian Journal of Chemistry Vol. 19, No. 3 (2007), 1743-1748

Chelation of Thallium by 1,2-Diethyl-3-hydroxy-4-pyridinone in Rats

S.J. FATEMI*, A. AMIRI, A. BAGHIZADEH† and M. TORKZADEH†
Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, Iran
Tel:/Fax: (98)(341)3222033; E-mail: fatemijam@mail.uk. ac.ir

Thallium was introduced to several groups of weanling male wistar rats by act of drinking and feeding. After a period of 35 d, all the rats administered thallium were severely anaemic and showed toxicity symptoms through loss of hair, and increased thallium and decreased iron levels in blood. Chelation therapy was chosen to remove the toxic element from the body. The ability of 1,2-diethyl-3-hydroxy-4-pyridinone chelating ligand in removing thallium was investigated by use of this chelating ligand for one week to the remaining rats of similar groups. The results showed that the thallium level present in blood was significantly reduced and at the same time, the iron concentration returned to a normal level. It was concluded that 1,2-diethyl-3-hydroxy-4-pyridinone chelating ligand is able to remove thallium from the body and could be used for the treatment of complications and eradication of symptoms of thallium intoxification.

Key Words: Thallium, Desferrioxamine, Chelation therapy, Thallium rats.

INTRODUCTION

Thallium is a toxic heavy metal with lethal dose of 15-20 mg/kg for human and it is quickly distributed from the blood to the tissues. One of the possible toxic mechanisms of thallium include ligand formation with blood proteins[1,2]. People are poisoned by intake of rat poisons (homicidal and suicidal attempts) by chronic exposures in occupations to thallium such as the workers in cement factories or handling pyrites and by contact to ash from coal-combustion power plants[3,4].

Thallium is a cumulative poison and the retention in various tissues increases with age. Symptoms of thallium intoxication in humans include nausea, vomiting, abdominal pain, hair loss, alopecia, tachycardia and cardiac arrhythmias. Death may result from cardiac failure[5] or respiratory failure[6].

It is also a neurotoxin which causes tremor, ataxia, ptosis of the eyelids, painful lower extremities, paresthesias of hands and feet after a few days of intoxication[7].

†International Center for Science, High Technology and Environmental Science, Kerman, Iran.

Thallium excretion *via* the kidney can be increased upon dosage of potassium chloride or employment of diuretics. Hemodialysis and forced diuresis can be an effective means of decreasing the body burden[8]. Activated charcoal, British antilewsite (BAL), calcium salts, cystine, dithiocarb, dithizon, histamine and theophylline were recommended as antidotes against acute thallium poisoning[9]. Hoffman *et al.*[10] demonstrated that activated carbon could adsorb thallium *in vitro* and the similarity between thallium and potassium has led some authors to consider the use of sodium polystyrene sulfonate a potential adsorbent. A study by Ghezzi and Marrubini[11] showed that the patients, including a newly born baby with transplacentar intoxication, were successfully treated with prussian blue. Moeschlin[12] recommended Berlin-blue (ferrihexacyanate) and sodium iodine in 1 %. One way of removing toxic element from the body is chelation therapy. In this method, toxic element is excreted from the rats by a special biological chelating ligand[13]. 3-Hydroxy-4-pyridinones (3,4-HPs) are bidentate chelating compounds under active development, due to their potential pharmacological usefulness, mostly related to removal therapies of unbalanced toxic hard metal ions in the body (*e.g.*, Fe^{3+}, Al^{3+}, In^{3+}, Ga^{3+}) but also to clinical diagnosis and chemotherapy through their complexes with radionuclides[1-5].

3-Hydroxy-4-pyridinones are typically oxyanions with high selectivity for the tribasic metal cations of group III and with ability to form highly stable complexes over a wide range of pH. One of these compounds, 1,2-dimethyl-3-hydroxy-4-pyridinone (L_1) is available for clinical use. Since it does not meet the thermodynamic requirement of high stability under conditions that prevail in physiological systems. In this work a close derivative of L_1 (with ethyl instead of methyl groups) was studied for removal of thallium ions in rat because of its lower toxicity[6].

EXPERIMENTAL

Four groups of weanling male wistar rats (*Novegicus rattus*) were individually caged in stainless steel and plastic cages with girded bottoms. All groups were fed on a diet containing normal levels of iron (35 μg/g). These groups were classified as follows: The first group (control group) was given distilled water to drink. The second and third groups were given water containing 25 μM (as low level drinking) and 165 μM (as high level drinking) of thallium (III) chloride, respectively, the fourth group (food group) had thallium (III) chloride incorporated into their food. Food was prepared by adding 50 mg of thallium salt to 2 kg of normal food.

The animals were weighed at regular intervals. After 35 d, half of the animals in each group were kept for the chelation therapy experiment and the other half of other groups were sacrified and blood was taken by

cardiac puncture. The following analyses were performed: a) serum iron concentration (Technicon RA-1000, America), b) total iron binding capacity (TIBC), c) unsaturated iron binding capacity (UIBC) (TIBC-serum iron) before and after chelation therapy and serum thallium before and after chelation therapy (graphite furnace atomic absorption spectroscopy (GF AAS). The serum samples were prepared after each sampling. The blood was transferred to a centrifuge tube until blood clotting occurred then the samples were centrifuged at 1500 rpm for 10 min. The supernatant fraction (serum) was pipette into a sampling cup. The samples for thallium analysis were prepared by adding 0.5 mL serum to an acid washed, EDTA-washed (100 g/L) polyethylene centrifuge tube and the protein precipitated[14]. To the solution, 0.5 mL water and 50 mL concentrated nitric acid was added and then the solution was placed on a vortex mixer at medium speed for 1 min. The solution was heated for 10 min at 70°C in a water-bath, placed on the vortex mixer for 20 s and then centrifuged for 10 min. The supernatant fraction was pipetted into a teflon sampling cup and the autosampler used for injection. Chelation therapy was performed on the animals in the remaining halves of animals of the above groups in order to remove thallium from their bodies. Rats received orally 1 mL of 100 mg/kg dose 1,2-diethyl-3-hydroxy-4-pyridinone (DEHP) in a day. After one week, the animals were killed and the sample solutions were prepared as described above. Thallium urinary excretion was investigated, in this way animals in high level drinking group were divided into two sub-groups (DEHP group and non DEHP group). Thallium concentration of the sample solutions were determined directly by GF-AAS, the details of which are described below. Thallium standards were prepared by appropriate dilution with doubly distilled water from a stock solution of thallium chloride (1 mg/mL) and a standard curve constructed on GF-AAS. The calibration curve was linear over the range of 0 to 60 μg/L. A deuterium lamp was used to correct the background noise. Operating conditions are shown in Table-1.

TABLE-1
OPERATING CONDITIONS USED FOR DETERMINATION OF THALLIUM CONCENTRATION BY GF-AAS

Step	Temperature (°C)	Ramp (s)	Hold (s)
1 Dry	100	8	20
2 Ash	300	4	20
3 Atomize	1500	4	4
4 Cleanup	2800	0	3
5 Cooling	25	0	20

Sample volume 10 μL; wavelength 276.8 nm; signal mode-peak height; autosampler; argon; pyrolytic coated graphite tube; internal gas flow rate (500 mL/min).

RESULTS AND DISCUSSION

There were no significant difference between the groups in the initial body-weights of the rats (mean 135 g) but at the end of thallium administration experiment, those given thallium in their diet weighed significantly less (Table-2). There was also an effect of dietary treatment on food intake, where by animals given normal diet, consumed more food than those given thallium. Some of thallium toxicity symptoms which appeared during period of thallium uptake were such as: appearance of bloody red spot in their eyes and greenish one in their liver and even losing hair in injection group. The thallium concentration of the diet had a significant effect on iron status as assessed by serum iron (Table-3). These results shown as thallium concentration increases in blood serum, iron level decrease. These animals were severely anaemic. Iron serum concentration is lowest in the group having the highest thallium concentration which is probably due to a significant interference that could take place by thallium through iron uptake mechanism. The results obtained after DEHP administration to similar remaining groups of rats are shown in Tables 4 and 5.

TABLE-2
BODY WEIGHTS OVER 30 DAYS FOR RATS IN DIFFERENT GROUPS (VALUES ARE MEAN FOR NUMBER OF OBSERVATION IN PARENTHESES)

Group	Control	Low level drinking	High level drinking	Food
Final body weight (g)	290 (10)	225 (10)	270 (10)	200 (9)

TABLE-3
SERUM IRON LEVEL, TIBC AND UIBC IN VARIOUS GROUP OF RATS BEFORE DEHP ADMINISTRATION (VALUES ARE MEANS ± SD FOR NUMBER OF OBSERVATION SHOWN IN PARENTHESIS)

Group	Serum iron (μg/dL)	TIBC (μg/L)	UIBC (μg/L)
Control	95 ± 2(5)	379 ± 14	284 ± 14
Drinking (low level)	91 ± 3(5)	420 ± 9	321 ± 9
Drinking (high level)	83 ± 5(5)	319 ± 15	236 ± 7
Food	69 ± 4(5)	213 ± 16	121 ± 17

Iron and thallium level showed that thallium level present in blood was significantly reduced and at the same, iron concentration returned to the normal level and the symptoms also reduced. Interactions between thallium and iron have not previously been reported. It is not clear whether or not thallium interferes with iron absorption and/or subsequent metabolism,

but it may well be that thallium absorption takes place along pathways for essential metals. If this is the case, iron deficiency could result in increased absorption, as is observed for several inorganic elements such as manganese, cobalt, lead[15], gallium and indium[16]. Results of the present study on a diet containing normal level of iron showed a significant effect of thallium level on iron deficiency for diet containing normal level of iron.

TABLE-4
SERUM IRON LEVEL, TIBC AND UIBC IN VARIOUS GROUP OF RATS AFTER DEHP ADMINISTRATION (VALUES ARE MEANS ± SD FOR NUMBER OF OBSERVATION SHOWN IN PARENTHESIS)

Group	Serum iron (μg/dL)	TIBC (μg/L)	UIBC (μg/L)
Control	95 ± 3(5)	410 ± 5	315 ± 6
Drinking (low level)	94 ± 5(5)	410 ± 6	316 ± 8
Drinking (high level)	93 ± 4(5)	404 ± 7	311 ± 8
Food	82 ± 6(5)	301 ± 13	219 ± 14

TABLE-5
THALLIUM CONCENTRATION (μg/L) IN BLOOD SERUM OF VARIOUS GROUP OF RATS (VALUES ARE MEANS ± SD FOR NUMBEROBSERVATION SHOWN IN PARENTHESIS)

Group	Before DEHP administration	After DEHP administration
Control	1.12 ± 0.2 (5)	1.1 ± 0.2 (5)
Drinking (low level)	11.0 ± 1.7 (5)	2.3 ± 0.7 (5)
Drinking (high level)	24.2 ± 1.9 (5)	4.2 ± 0.7 (4)
Food	31.2 ± 5.1 (4)	7.5 ± 1.2 (4)

Thallium disturbances take place through the interference in iron uptake and transfer mechanism. This idea is in consistent with the results obtained on hepatocytes and intestinal cells as *in vitro* models[17,18] which showed the absorption of thallium by tissues. Moreover, in the presence of thallium, the iron uptake is reduced. This interference has also been confirmed by preliminary studies on the ability of thallium to bind transferrin[15]. It supports the idea that thallium could be carried in plasma predominatly by transferring in which it is firmly bound (probably replacing iron in the specific binding sites) and partly by albumin and citrate in labile association (at relatively high levels of thallium). Our results show that use of DEHP as a chelating ligand is a potential treatment for complication of thallium intoxification. As a chelating agent DEHP reduced serum thallium levels and led to a rise in normal iron level. Thallium urinary excretion was found in DEHP group six times higher than in non

DEHP treatment group so thallium excretion *via* the kindney can increase upon dosage of DEHP. Thallium affected rats do not appear to lose excessive iron during DEHP chelation, which is interesting because DEHP is widely used as a chealting agent for the treatment of both chronic and acute iron overload.

REFERENCES

1. A.L.J. Peter and T. Viraraghavan, *Environ. Int.*, **31**, 493 (2005).
2. T. Xiao, J. Guha, D. Boyle, C.-Q. Liu, B. Zheng, G.C. Wilson, A. Rouleau and J. Chen, *Environ. Int.*, **30**, 501 (2004).
3. D.F. Thompson, *J. Clin. Toxicol.*, **18**, 972 (1981).
4. U. Ewers, *J. Sci. Total Environ.*, **71**, 285 (1988).
5. O. Anderson, *J. Acta. Neurol. Scand.*, **70**, 185 (1984).
6. S.R. Mayfield, D.P. Morgan and R.J. Roberts, *J. Clin. Pedial.*, **23**, 461 (1983).
7. M. Heim, O. Wappelhorst and B. Market, *Ecotoxicology*, **11**, 369 (2002).
8. J. Schoer, Handbook of Environmental Chmistry, 3 (c) New York: Springer-Velag, p. 143 (1984).
9. V. Zitko and W.G. Carson, *Bull. Environ. Contam. Toxicol.*, **13**, 23 (1975).
10. R.S. Hoffman, R. Hoffman, J.A. Stringer, R.S. Feinberg and L.R. Goldfrank, *J. Chem. Toxicol.*, **33**, 833 (1999).
11. A. Ghezzi and B.M. Marrubini, *Vet. Hum. Toxicol.*, **21**, 64 (1979).
12. S. Moeschlin, *J. Clin. Toxicol.*, **17**, 133 (1993).
13. M. Franchini, G. Gandini and M.Gironcoli, *Blood.*, **5**, 2776 (2000).
14. M.V. Hulle, K.D. Cremer and R. Cornelis, *J. Environ. Monit.*, **3**, 86 (2001).
15. W.R. Harris and L. Messori, *Coord. Chem. Rev.*, **228**, 237 (2002).
16. W.R. Harris, Y. Chen and K. Wein, *J. Inorg. Chem.*, **33**, 4991 (1994).
17. D. Ramsden, *Thallium Mol. Death.*, 304 (2002).
18. J.P. Mulkey and F.W. Oehme, *Vet. Hum. Tonicol.*, **35**, 445 (1993).

(*Received*: 13 February 2006; *Accepted*: 13 October 2006) AJC-5175