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Removal of thallium by combining desferrioxamine and deferiprone chelators in rats

Asghar Amiri[1], S. Jamilaldine Fatemi[1,*] & S. Nabilaldine Fatemi[2]
[1]*Chemistry Department, Shahid Bahonar University of Kerman, Kerman, 76169, Iran;* [2]*Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, 76169, Iran; *Author for correspondence (E-mail: fatemijam@mail.uk.ac.ir)*

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Abstract

The hypothesis that two known chelators deferiprone (1,2-dimethy1-3-hydroxypyrid-4-one, L_1) and desferrioxamine (DFO) might be more efficient as combined treatment than as monotherapies in removing thallium from the body was tested in rats. Six-week-old male Wistar rats received chelators: L_1 (p.o.), DFO (i.p.) or L_1 + DFO as 110 or 220 mg/kg dose half an hour after a single i.p. administration of 8 mg Tl/kg body weight in the form of chloride. Serum thallium concentration, urinary thallium and iron excretions were determined by graphite furnace atomic absorption spectrometry. Both chelators were effective only at the higher dose level, while DFO was more effective than L_1 in enhancing urinary thallium excretion, L_1 was more effective than DFO in enhancing urinary iron excretion. In the combined treatment group, L_1 did not increase the DFO effect on thallium and DFO did not increase the effect of L_1 on iron elimination. Our results support the usefulness of this animal model for preliminary *in vivo* testing of thallium chelators. Urinary values were more useful because of the high variability of serum results. Result of combined chelators treatment should be confirmed in a different experimental model before extrapolation to other systems.

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 includes ligand formation with blood proteins (Xiao *et al.* 2004; Peter & Viraraghavan 2005). People are poisoned by intake of rat poisons (homicidal and suicidal attempts) by chronic exposures in occupations to T1 such as the workers in cement factories or workers handling pyrites and by contact to ash from coal-combustion power plants (Thompson 1981; Ewers 1988). Thallium is a cumulative poison and the retention in various tissues increases with age. Symptoms of T1 intoxication in humans include nausea, vomiting, abdominal pain, hair loss, alopecia, tachycardia and cardiac arrhythmias. Death may result from cardiac failure (Anderson 1984) or respiratory failure (Mayfield *et al.* 1983). 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 (Heim *et al.* 2002).

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 (Schoer 1984). Activated charcoal, British antilewsite (BAL), calcium salts, cystine, dithiocarb, dithizone, histamine and theophylline

were recommended as antidotes against acute thallium poisoning (Zitko & Carson 1975). Hoffman *et al*. (1999) 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 as a potential adsorbent.

A study by Ghezzi and Marrubini (1979) showed that the patients, including a newborn baby with transplacentar intoxication, were successfully treated with Prussian blue. Moeschlin (1980) recommended Berlin-blue (ferrihexacyanate) and sodium iodine in 1%.

Desferrioxamine (DFO) has been the most widely used chelator for the treatment of iron overload. Its use is restricted due to its oral inactivity and therefore subcutaneous administration is usual but intravenous is also possible. It has numerous side effects and a high production cost. DFO was also found to be a maternal, embryo and terato-toxic agent in some animal species (Bosque *et al*. 1995; Kontoghiorghes 1995). Kontoghiorghes and Sheppard (1987) described the simple synthesis of a new chelator L_1 for iron overload. Although the stability constant to bind iron is higher than that with thallium, L_1 was also tested in thallium overload (Clarke & Martell 1992). These human studies showed that L_1 efficiency was comparable to that of DFO (Kontoghiorghes 1995). L_1 is water soluble and can be given orally. These two chelators have different transport abilities through the organism (Berdoukas *et al*. 1993).

This kind of therapy by combining two chelators is based on the assumption that various chelating agents mobilize toxic element from different tissue compartments and therefore better results in mobilizing are expected. Recent studies with chelators having different lipophilic properties given in combination have shown favorable efficiencies to mobilize lead (Flora *et al*. 1995), mercury (Kostial *et al*. 1997) and cadmium (Kostial *et al*. 1996).

The aim of this study was to test the chelation potency of DFO and L_1 in combination given to animals after thallium loading. Testing was performed by using an acute experimental model on rats with single or combined chelators given shortly after thallium application. Thallium and iron urinary excretion and serum thallium concentration were measured after single treatment with chelators.

Material and methods

Maintenance of the animals

Wistar rats bred at department of biology animal house in Shahid Bahonar University were maintained under a controlled light:dark (12:12 h) schedule at 23 ± 1 °C. The animals were kept in well cleaned sterilized cages. The rat feed was purchased from Karaj Institute, Tehran.

Material

Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one, L_1) was synthesized using a previously described method (Kontoghiorghes & Sheppard 1987). DFO and other material were obtained from Sigma Chemicals, St. Louis, Mo.

Methods

In our model, we used a single i.p. administration of thallium follow by an early administration of chelating agents similar to experiments performed by Gomez *et al*. (1998b). Our experiment lasted only 24 h and urinary elimination of thallium and iron and serum concentrations of thallium were determined. Experiments were performed on male, six weeks old wistar rats. Body weights of animals were between 102 and 125 g with mean ± S.E.M. of 113.5 ± 0.9 g.

Two experiments were performed, E_1 and E_2, with two different doses of chelators. In each experiment animals were divided into four groups, Control, L_1, DFO and L_1 + DFO of 10–15 animals in each case. Chelators were given half an hour after thallium application and animals were immediately placed into individual glass metabolic cages for 24 h urine collection. Animals were given deionized water *ad libitum* and no food to avoid possible contamination of urine with thallium originating from food. Before placing the animals into metabolic cages deionized water was splashed and collected as a blank sample of each cage separately. Blank and urine collected samples were analyzed for thallium and iron.

Thallium chloride was given in a single i.p. dose of 8 mg/kg to all groups in a volume of 0.5 ml. Chelators were given orally (L_1) or intraperitoneally (DFO) in the volume of 1 ml as single or combined therapy. Dose of L_1 and DFO was 110 mg/kg and 220 mg/kg body weight in experiments E_1 and E_2, respectively. These were dissolved in deionized water or saline solution. Control group received vehicle. After collection, urine was acidified by adding one drop of concentrated nitric acid to each sample and total volume was measured. Thallium was analyzed by graphite furnace atomic absorption spectrometry (GT AAS) on a Shimadsu instrument. Results are expressed as μg/24 h urine. After this step, animals were killed by exsanguination from the abdominal aorta and blood samples were collected for serum thallium determination. Thallium was measured as described above for urine. The data were statistical analysis by student's t test (Pillai & Sinha 1968). $p < 0.05$ was considered significant.

Results

Results of thallium and iron elimination in urine and serum concentrations of both control groups from E_1 and E_2 experiments were not statistically different. Therefore, the results of thallium and iron urinary excretion and serum thallium from two control groups were pooled and presented as one control value (Tables 1 and 2). At the lower chelator dose in experiment E_1 urinary thallium elimination in treated groups were not statistically different from the control groups (Table 1). At higher dose in experiment E_2, L_1 group was about four times higher and DFO and L_1 + DFO groups about six to seven times higher than the control group. Thallium urinary excretion of treated animals at higher dose was significantly higher in all experimental groups than the corresponding groups at lower dose.

Serum thallium concentrations showed relatively high variability especially in the control group and therefore although lower in all treated groups, significantly lower values were only found in the DFO and combined DFO + L_1 groups given at higher dose (Table 2). In the E_2 experiment the highest excretion of iron in urine was found in L_1 and L_1 + DFO groups five to six times higher than in control.

Discussion

In this investigation, a short-term experimental model was used in order to speed up the preliminary testing procedure. When comparing individual efficiencies of chelators in this experiment DFO was more efficient in enhancing urinary thallium excretion than L_1. One of the possibilities to explain this higher efficiency of DFO is the direct chelation of DFO and thallium in the intraperitoneal cavity. However, similar results, i.e. higher efficiency of DFO as compared to L_1 in enhancing urinary elimination of other metals such as aluminum has been also obtained by Gomez *et al.* (1998a) in a different experimental model in which DFO was administered subcutaneously and L_1 orally. This indicates that our finding is not an artefact second to the mode of DFO administration. The effect of chelators on iron urinary

Table 1. Excretion of thallium and iron in urine (μg/24 h) after chelation treatment of Rats with DFO and/or L_1.

Experiment	Treatment (mg/kg b.w.)	Thallium	Iron
E_1 + E_2	Control	10.51 ± 0.61 (20)	3.10 ± 0.53 (15)
E_1	L_1	16.01 ± 2.21 (9)	4.90 ± 0.79 (9)
	DFO	22.51 ± 5.80 (10)	3.21 ± 0.81 (9)
	L_1 + DFO	18.61 ± 3.21 (9)	5.90 ± 0.77 (8)
E_2	L_1	43.21 ± 4.14 (9)	15.84 ± 2.52 (9)
	DFO	65.49 ± 8.41 (9)	9.12 ± 2.51 (8)
	L_1 + DFO	77.02 ± 10.31 (9)	18.41 ± 2.91 (8)

Chelators (L_1, DFO or L_1 + DFO) were given either p.o. (L_1) or i.p. (DFO) in two doses (110 mg/kg in experiment E_1, 220 mg/kg in E_2) 0.5 h after thallium administration (8 mg/kg b.w., given i.p in the form of chloride). Results are presented as arithmetic means ± S.E.M., number of animals in parenthesis. Significant at $p < 0.05$ when compared with control.

Table 2. Serum thallium concentration after chelation treatment of rats with DFO and/or L_1 (see method under Table 1).

Experiment	Treatment (mg/kg b.w.)	Thallium (μg/l)
$E_1 + E_2$	Control	84.37 ± 14.1 (15)
E_1	L_1	62.37 ± 18.2 (7)
	DFO	77.10 ± 12.2 (7)
	L_1 + DFO	67.20 ± 14.1 (7)
E_2	L_1	67.24 ± 14.1 (7)
	DFO	47.63 ± 4.5 (7)
	L_1 + DFO	52.47 ± 14.2 (7)

excretion was more different than thallium since L_1 more efficiently enhances urinary iron excretion than DFO. This can be explained by different stability constant of L_1 with iron than DFO or by the different *in vivo* distributions of L_1 and DFO.

Our results indicate that this procedure might be useful for preliminary testing of the efficiency of chelating agents in removing thallium *in vivo* for several reasons. We obtained dose related increases in thallium excretion and decreases in serum thallium concentration for the two known chelating agents L_1 and DFO as expected. We observed a higher efficiency of L_1 than DFO in enhancing urinary iron excretion as expected in relation to their stability constants for iron (Clarke & Martell 1992). It should be also mentioned that in this experimental model urinary values of thallium showed less variation than serum thallium values. This testing procedure, of course does not provide all the relevant answers for evaluating the efficiency of chelating agents in thallium toxicity like, kinetic data, mobilizing thallium from deeper compartments, thallium dosing etc. In spite of these shortcomings, it does provide results indicating whether a new chelating agent or chelating agent mixtures deserve further testing.

In conclusion, the above results show that the addition of L_1 did not increase the DFO effect on thallium and that the addition of DFO to L_1 did not increase the effect of L_1 on iron elimination. Therefore, these results do not support the hypothesis that DFO and L_1 could be used interactively permitting lower doses of chelators to be effective. However, when given together as combined treatment, a simultaneous increase in both thallium and iron elimination was observed. This applies especially to testing our hypothesis at later time intervals after thallium administration, when differences in lipophylic properties between these two chelates might cause higher thallium tissue depletion than after monotherapies which were not noticeable in urinary elimination in this acute experiment. This might provide additional information on the potential usefulness in using combined chelation treatment of thallium or iron overload.

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