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Chelation in metal intoxication XXXVI: Effect of substituted piperazine dithiocarbamates in lead-exposed rats

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ABSTRACT Chelation is most acceptable means of managing heavy metal poisoning. Piperazine hexahydrate dithiocarbamate (PHD), *N*-methyl piperazine dithiocarbamate (MPD), *N*-3-chloro-phenyl piperazine dithiocarbamate (CPD), and *N*-benzyl piperazine dithiocarbamate (BPD) were investigated for their efficacies to mobilize lead and restore Pb-induced hematopoietic alterations in experimentally intoxicated rats. MPD and CPD were effective in enhancing the urinary excretion of Pb. While PHD and MPD were successful in lowering hepatic Pb, CPD, and BPD were effective in doing so from renal tissue. All the chelating agents decreased the blood level of Pb. The lowering of Pb body burden had no influence on the restoration of biochemical changes which indicates that the latter may not be directly related to the metal mobilizing potential of the chelating agents. None of the compounds caused excessive depletion of body Cu or Zn. No relationship between efficacies of the chelating agents and their structures was seen. However, MPD and CPD appeared to be promising antidotes of Pb poisoning.

KEY WORDS lead poisoning; piperazines; thiocarbamates; chelation therapy

The chelating agents possessing sulfhydryl groups are most effective antidotes of heavy metal poisoning[1,2]. However, substitution at different positions, number and vicinity of thiol groups seem to greatly influence the metal mobilization. The presence of hydroxyl groups on the organic moieties attached to the N atom in dithiocarbamates significantly increase the biliary excretion of Cd[3]. Further, insertion of methyl or benzyl groups along with a polar group derived from glucose renders substituted dithiocarbamates very effective antidote of Cd intoxication. *N*-benzyl-*D*-glucamine dithiocarbamate (NBGD) was a very effective Cd antidote[4] that NBGD was the current standard for the evaluation of potential Cd antagonists. The dithiol chelators containing vicinal -SH groups mobilise far more Cd from liver than the compounds with non-vicinal SH groups or a single SH group[5]. The relative abilities of various dithiocarbamates to increase the excretion of Cd vary considerably with their structure[6]. We have recently found that the presence of hydroxyl or carboxyl groups on the substituted moieties in dithiocarbamates to be advantageous in mobilizing Pb from intoxicated rats[7]. In view of the role of thiol groups and variation in metal mobilization as a consequence of substitution on the parent system, the present study was undertaken to evaluate the influence of piperazine nucleus and the substitutions thereupon in dithiocarbamate system, on Pb mobilization from the exposed rats.

MATERIALS AND METHODS

Twenty-five ♂ Wistar rats (150± 5 g) bred in ITRC colony maintained on a standard pellet diet with metal content of feed (ppm dry wt): Cu 10, Mn 55, Co 5, Zn 45, Fe 70 (Lipton, India), and water *adlib*, were given 0.1% lead acetate in drinking water for 10 wk. Five rats received no treatment and served as normal group. The Pb-exposed rats were equally divided into 5 groups.

Each group was given intragastrically either of the 4 chelating agents: piperazine hexahydrate dithiocarbamate (PHD), *N*-methyl piperazine dithiocarbamate (MPD),

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N–3–chlorophenyl piperazine dithiocarbamate (CPD), and *N*–benzyl piperazine dithiocarbamate (BPD), synthesized and characterised (Fig 1). The chelating agents were dissolved in propylene glycol, at a dose of 400 μmol (2.5 ml) • kg^{-1} daily for 4 d. The 5th group received 2.5 ml of propylene glycol alone and served as control. During 4 d of chelation therapy, 24–h urine were collected daily. On d 5, the rats were killed under light ether anesthesia, the blood drawn by cardiac puncture, and liver, kidney, and femur removed. Standard procedures were used to determine blood δ–aminolevulinic acid dehydratase (ALAD), zinc protoporphyrin (ZPP), hemoglobin (Hb), hematocrit, urinary ALA, and plasma ceruloplasmin[8]. The Pb was estimated in blood, soft tissues, and urine following wet acid digestion, complexation with ammonium pyrrolidine dithiocarbamate and extraction in methyl isobutyl ketone. The femur was dried at 120℃ to a constant weight, acid digested, and processed as above for Pb content[9]. The Cu and Zn levels in urine were measured after acid digestion. The readings were recorded at 282.3 nm for Pb, 213.9 nm for Zn, and 324.8 nm for Cu on an atomic absorption spectrometer (Perkin–Elmer–5000) using suitable similarly processed standards.

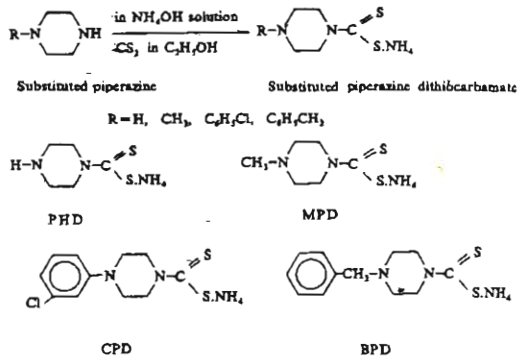

Fig 1. Synthesis of substituted piperazine dithiocarbamates

RESULTS

Exposure to Pb for 10 wk caused a significant inhibition of blood ALAD activity and a decrease in Hb content. These effects were reflected by an enhanced excretion of urinary δ–aminolevulinic acid (ALA) and an interruption of normal heme biosynthetic pathway. None of the substituted piperazine dithiocarbamates had virtually any effect on Pb–inhibited ALAD activity and Hb levels. The accelerated excretion of urinary ALA was attenuated significantly by PHDTC and MPDTC (Tab 1). The blood ZPP level which increased upon Pb exposure, returned to near normal on therapy with test compounds. The plasma ceruloplasmin level, though not

Tab 1. Effects of chelating agents on lead–induced biochemical alterations. $n=5$ **rats.** $\bar{x}\pm$ **SD.**
$^{*}P>0.05$, $^{***}P<0.01$ *vs* **Pb exposed propylene glycol treated control by** *t* **test.**
$^{+}P>0.05$; $^{++}P<0.05$, $^{+++}P<0.01$ *vs* **normal rats.**

Rats	Blood ALAD, μmol ALA • min^{-1} / erythrocyte	Blood Hb, g • L^{-1}	Hematocrit, L • L^{-1}	Urine ALA, mg • L^{-1}
Normal	6.7 ± 0.4	135 ± 18	0.402 ± 0.052	0.6 ± 0.2
Control	3.6 ± 0.4^{+++}	106 ± 11^{++}	0.354 ± 0.047^{+}	5.9 ± 0.4^{+++}
PHD	3.4 ± 0.7*	91 ± 16*	0.380 ± 0.025*	3.0 ± 0.7***
MPD	3.3 ± 0.4*	96 ± 7*	0.385 ± 0.016*	2.4 ± 0.4***
CPD	3.8 ± 0.7*	123 ± 11*	0.410 ± 0.047*	5.0 ± 0.4*
BPD	3.7 ± 0.7*	125 ± 22	0.396 ± 0.042*	4.6 ± 1.8*

Tab 2. Effects of chelating agents on lead-induced alterations in Zn / Cu-dependent Biochemical parameters. $n=5$ rats. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{***}P<0.01$ *vs* Pb exposed propylene glycol treated control by *t* test. $^{+}P>0.05$; $^{+++}P<0.01$ *vs* normal rats.

	Blood ZPP, $\mu g \cdot g^{-1}$ Hb	Plasma Ceruloplasmin, O.D. Change of $0.01 \cdot min^{-1} \cdot ml^{-1}$
Normal	1.1 ± 0.09	26.2 ± 1.6
Control	$2.2 \pm 0.11^{+++}$	$29.5 \pm 3.4^{+}$
PHDTC	$1.5 \pm 0.22^{***}$	$29.4 \pm 4.0^{*}$
MPDTC	$1.4 \pm 0.49^{***}$	$26.6 \pm 3.6^{*}$
CPDTC	$1.3 \pm 0.27^{***}$	$22.9 \pm 1.3^{***}$
BPDTC	$0.7 \pm 0.69^{***}$	$22.6 \pm 2.0^{***}$

influenced by Pb exposure, was significantly lowered following treatment with CPD and BPD (Tab 2).

The blood, liver, kidney, and femur Pb increased significantly on Pb exposure. The elevated blood Pb level was lowered by all the chelating agents, suggesting their potential to complex circulating Pb. While PHD and MPD reduced hepatic Pb, CPD and BPD decreased its renal level. Inspite of the ability of chelating agents to lower circulating and soft organ Pb burden, they were ineffective inreducing femur burden (Tab 3).

The cumulative urinary excretion of Pb over 4 d showed only MPD and CPD to be effective Pb mobilizing agents Fig 2. An interesting observation was that none of the chelating agents depleted Cu or Zn through urine (data not shown).

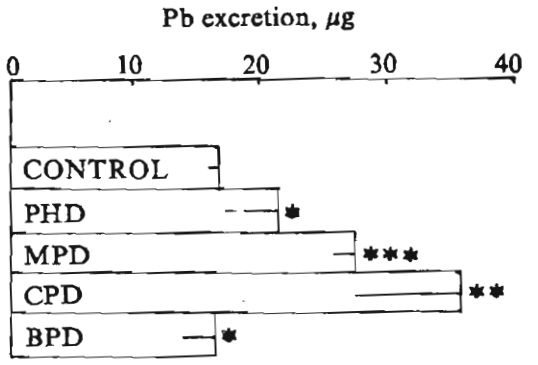

Fig 2. Urinary Pb excretion (4 d) after chelation therapy. $n=5$ rats. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* propylene glycol control by *t* test.

DISCUSSION

Recently we have investigated the efficacy of some structurally different substituted dithiocarbamates for mobilization of lead and reversal of Pb-induced hematopoietic alterations[7]. It was found that the presence of carboxyl and / or hydroxyl group on the substituted moiety at *N*-position of dithiocarbamates might serve as additional electron donating centre[10]. However, in the present study, the effect of linkage of piperazine moiety and substitution thereupon, to *N*-position of dithiocarbamate, on the mobilization of Pb has been investigated. Since all the compounds were able to reduce blood Pb and restore blood ZPP level to nearly normal, it appeared that freely accessible circulating Pb could be effectively complexed and removed

Tab 3. Effects of chelating agents on blood and tissue lead levels. $n=5$ rats. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* Pb exposed propylene glycol treated control by *t* test. $^{+++}P<0.01$ *vs* normal rats.

	Blood, $\mu g \cdot L^{-1}$	Liver, $\mu g \cdot g^{-1}$	Kidney, $\mu g \cdot g^{-1}$	Femur, $\mu g \cdot g^{-1}$
Normal rats	70 ± 13	0.6 ± 0.2	0.9 ± 0.4	10.0 ± 4.9
Control	$1\,252 \pm 121^{+++}$	$3.7 \pm 1.2^{+++}$	$10.4 \pm 6.3^{+++}$	$52.0 \pm 24.4^{+++}$
PHDTC	$818 \pm 136^{***}$	$1.2 \pm 0.2^{***}$	$7.8 \pm 1.6^{*}$	$40.5 \pm 15.0^{*}$
MPDTC	$795 \pm 179^{***}$	$1.3 \pm 0.2^{***}$	$11.5 \pm 9.6^{*}$	$54.5 \pm 14.5^{*}$
CPDTC	$980 \pm 25^{**}$	$2.5 \pm 0.7^{*}$	$5.1 \pm 1.1^{***}$	$43.9 \pm 8.7^{*}$
BPDTC	$920 \pm 72^{***}$	$2.9 \pm 0.9^{*}$	$6.6 \pm 2.7^{**}$	$43.2 \pm 10.1^{*}$

irrespective of their structure. The fact that PHD and MPD but not CPD and BPD could mobilize hepatic Pb indicated that substitution of a bulky group on the piperazine nucleus did not form stable and diffusible chelate with Pb in hepatic tissue. On the other hand, the effectiveness of CPD and BPD but not PHD and MPD in lowering renal Pb did not support this assumption. This might be due to a difference in binding of Pb to bioligands in tissues or the differential permeability of chelating agents to various cell types. The failure of all the compounds in reducing femur Pb may support the proposed mechanism of action. The success of only MPD and CPD in enhancing urinary excretion of Pb suggests that the tissue depletion of Pb may not be related to its urinary elimination. The failure of all the substituted piperazing dithiocarbamates in restoring Pb-induced decrease in blood ALAD activity, Hb, and hematocrit and that of CPDTC and BPDTC in reversing enhanced excretion of urinary ALA may not be related to their Pb mobilizing potential[7,11]. The ability of PHDTC and MPDTC in lowering Pb-induced urinary excretion of ALA without restoring blood ALAD activity is intriguing and merits further investigation.

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