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Comparative Effects of Chelating Drugs on Trace Metal and Biochemical Alterations in the Rat

Manoj Misra, Mohammad Athar, Syed K. Hasan, and Ramesh C. Srivastava*

Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Post Box No. 80, Lucknow 226001, India

Chelation therapy is the most successful modality for the management of heavy metal poisoning (Kostyniak and Clarkson, 1981; Williams and Halstead, 1982-83). The success of these drugs stem from their multidentate polyfunctional chelating behaviour (Basinger et al. 1980; Dwivedi et al. 1986; Kaur et al. 1984; Kostyniak and Clarkson 1981). The therapeutic mechanism of chelating drugs involves their interaction with toxic metals leading to their rapid excretion from the body. However, because of their indicriminate affinity for various metal ions, the potential interaction between these drugs and endogenous trace metals is of concern (Cantilena and Klaassen, 1982; Tandon et al. 1984). Thus the toxic manifestations of chelating drugs may partly be the result of their interaction with essential trace metals which may ultimately lead to various characteristic biochemical and pathological alterations (Cantilena and Klaassen 1981; Davidson et al. 1977; Hurley 1981; Sigel 1983). It was, therefore, of importance to define new chelating drugs which in addition to being effective as an antidote in metal poisoning may possess low undesirable toxicity. In this respect our recent studies have indicated that 1,4,8,11 tetraazacyclotetradecane (Cyclam) is capable of serving as a specific antidote for nickel poisoning (Athar et al. 1987). In the present communication we compare the acute effect of Cyclam with those of other conventional chelating drugs namely, triethylenetetramine (TETA), reduced glutathione (GSH), ethylenediamine tetraacetic acid (EDTA), cyclohexanediamine tetraacetic acid (CDTA). diethylene triamine pentaacetic acid (DTPA) and hydroxyethylenediamine triacetic acid (HEDTA) on (i) serum levels of Cu, Zn, lactate dehydrogenase (LDH), glutamyloxaloacetic transaminase (GOT) and ceruloplasmin (CP); (ii) hepatic and renal levels of Cu, Mn, Zn and Fe and (iii) hepatic and renal levels of GSH, glutathione-S-transferase (GST) and phosphoglucomutase (PGM) at various time intervals (16, 24 and 72 hrs) after their administration to rats.

MATERIAL AND METHODS

Cyclam (Alfa Inorganic, USA), TETA (Aldrich, USA), GSH, EDTA, CDTA, DTPA and HEDTA (Sigma, USA) were obtained and used as such. The animals were six weeks old female albino rats of the Industrial Toxicology Research Centre Colony, weighing 110 ± 10 gm and were housed in an air-conditioned room and had free access to pellet diet (Hindustan Lever Ltd., Bombay, India) and water. The animals were divided into eight groups consisting of eighteen rats in each. The animals of the first seven groups received a single subcutaneous (sc) injection of

*To whom all correspondence should be made

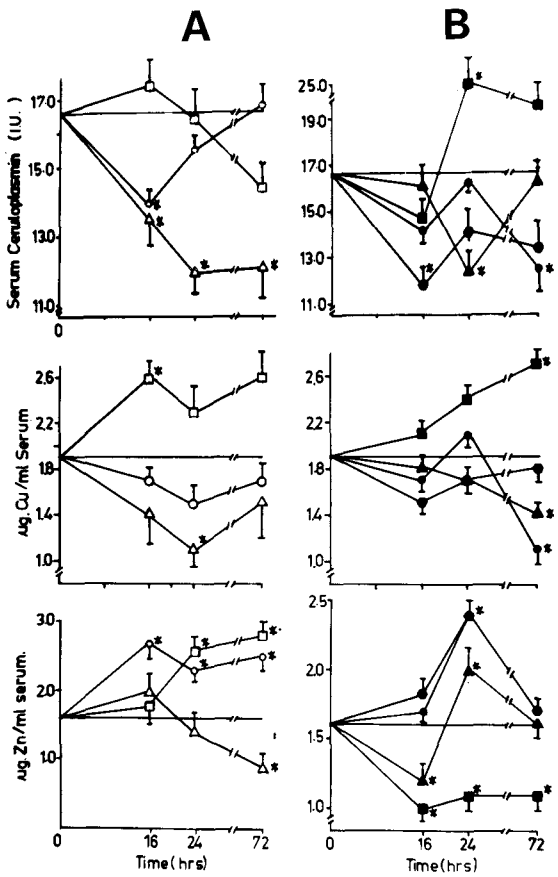

Figure 1. Effect of A, Cyclam ○—○, TETA △—△, GSH □—□ and B, EDTA ●—●, CDTA ▲—▲, DTPA ■—■ and HEDTA ◆—◆ on the levels of serum ceruloplasmin (CP), copper (Cu) and zinc (Zn) at 16, 24 and 72 hrs after their treatment to rats.

freshly prepared solution of the chelating drugs (500 μmole/kg/2 ml). The dose regimen was based on our previous study (Srivastava et al. 1986, 1987) on the criteria of having no or less than 10% mortality in adult rats over a period of 14 days. The eighth group of animals received normal saline and served as control. Six rats from each group were sacrificed by decapitation at 16, 24 and 72 hrs after treatment. The pharmacokinetics of these drugs is not well established however, it is known that these drugs are highly effective in promoting the excretion of toxic

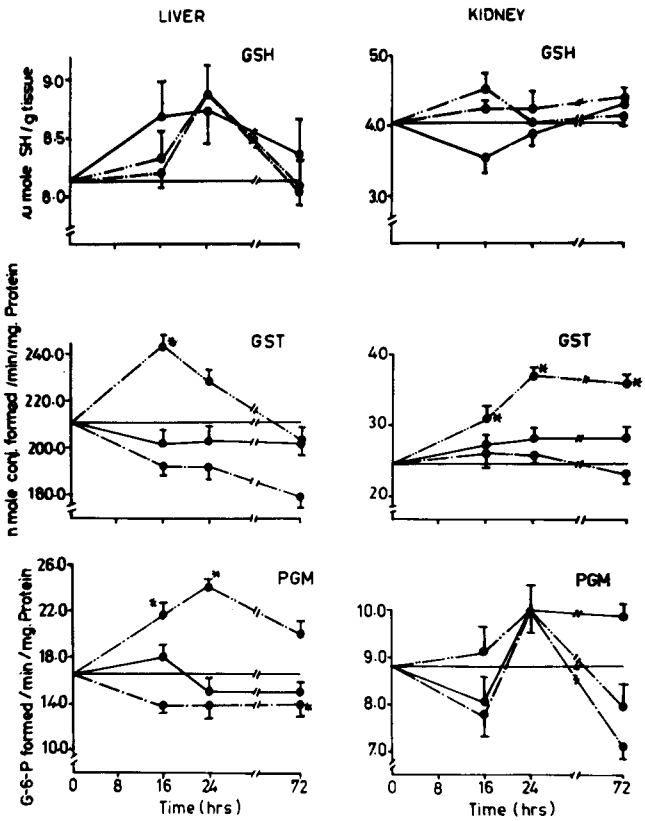

Figure 2. Effect of Cyclam ●— —●, TETA ●-•-● and GSH ●——● on the hepatic and renal levels of GSH, GST and PGM at 16, 24 and 72 hrs after their administration to rats.

metal ions from the body organs over a period of 24 hrs and by 72 hrs the effects become minimal (Athar et al. 1987; Cantilena and Klaassen 1981 and 1982). We, therefore, selected for this study three time points i.e. 16, 24 and 72 hrs after the administration of the drugs. Blood samples were collected and serum was separated by centrifugation. Liver and kidney were immediately removed, cleaned free of blood and extraneous material and processed for either biochemical assays or metal estimation.

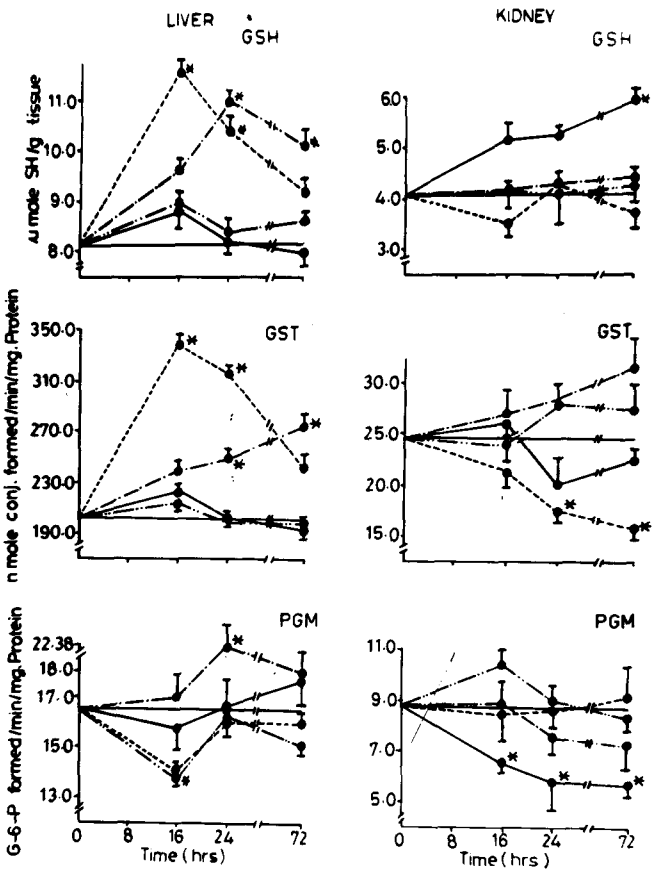

Figure 3. Effect of EDTA●-•-●, CDTA ●——●, DTPA●-•-● and HEDTA●----●on the hepatic and renal levels of GSH, GST and PGM at 16, 24 and 72 hrs after their administration to rats.

The levels of GSH and enzyme activities were assayed in serum, or liver and kidney homogenates according to known methodology: GSH - Ellman (1959) modified by Jollow et al. (1974); GST - Habig et al. (1974); PGM - Najjar (1955); LDH - Kornberg (1955); GOT - Reitman and Frankel (1957); CP - Curzon and Vellet (1960); Protein - Lowry et al. (1951).

The serum samples were diluted (1:1) with double distilled water and metal content was read directly on a Perkin Elmer 5000 atomic

absorption spectrophotometer. Tissue samples were subjected to acid digestion (Dwivedi et al. 1986) for the estimation of trace metals on the atomic absorption spectrophotometer. An analysis of significance of difference between the groups was performed by means of student 't' test (Fisher, 1954).

RESULTS AND DISCUSSION

The effect of acute administration of various chelating drugs (500 μmole/kg) on the levels of serum CP, Cu and Zn at 16, 24 and 72 hrs after their treatment are shown in Fig. 1. Cyclam produced significant decreases in the levels of CP at 16 hrs which returned to normal value by 72 hrs. The drug, however, produced no change in the Cu level but raised the level of Zn at each time interval (Fig. 1A). The effect of TETA was more pronounced than that of Cyclam in depleting serum levels of CP, Cu and Zn. Unlike Cyclam and TETA, GSH exhibited an increase in the levels of Cu and Zn while produced no significant change in the level of CP. Among polyaminocarboxylic acids (Fig. 1B), DTPA significantly increased the levels of CP and Cu at 24 and 72 hrs while decreased the serum Zn level. EDTA, CDTA and HEDTA on the contrary reduced CP and Cu levels with simultaneous increase in the Zn level at one or the other time intervals.

The effect of these chelating drugs on hepatic and renal levels of Cu, Zn, Fe, and Mn at various time intervals is shown in Table 1 and 2. Most of the drugs reduced the concentration of Cu and Zn either at 16 or 24 hrs after their administration but in all the cases values returned to the normal control level at 72 hrs of treatment. Changes produced by polyaminocarboxylic acid drugs were, however, more pronounced than that occurred with Cyclam, TETA and GSH. The levels of Fe and Mn were also found to be reduced after the exposure to these drugs. Among all the drugs, Cyclam produced minimum alterations in the organ and serum levels of trace metals.

The effect of various drugs on serum LDH and GOT activities is summarised in Table 3. All the drugs with the exception of Cyclam increased the level of LDH either at 16 or 24 hrs which returned to the normal value by 72 hrs. The activity of GOT was, however, less affected as compared to LDH and was only increased by polyaminocarboxylic acid drugs. The effect of these drugs on hepatic and renal levels of GSH, GST and PGM is shown in Figs. 2 and 3. Cyclam increased hepatic PGM and GST activities respectively at 24 and 16 hrs while produced no change in GSH. However, in kidney, it produced an increase in GST activity. TETA and GSH showed no significant alterations on any of these parameters in both the organs under study. Among polyaminocarboxylic acids, DTPA increased the levels of GSH, GST and PGM in liver without producing effect on renal levels. HEDTA increased the hepatic levels of GSH and GST at 16 and 24 hrs while decreased their renal level of GST at 24 and 72 hrs. The other two drugs, i.e. EDTA

Table 1. Comparative effects of the chelating drugs on the levels of hepatic trace metals at 16, 24 and 72 hrs after treatments.

Chelating drug	Time intervals (hr)	Metal content (µg/g wet weight)			
		Copper	Zinc	Iron	Manganese
	16				
Saline	24	2.80±0.12	42.50±1.85	167.5±6.17	2.10±0.11
	72				
Cyclam	16	2.97±0.16	44.68±1.21	158.7±7.87	2.38±0.11
	24	2.17±0.10[a]	42.08±1.12	154.2±5.21	2.03±0.12
	72	2.88±0.59	40.00±1.85	165.0±6.21	2.13±0.08
TETA	16	2.30±0.11[a]	46.07±1.02	131.0±3.18[a]	2.09±1.00
	24	1.88±0.41[a]	45.90±0.19	141.1±1.39[a]	2.06±0.09
	72	2.20±0.18	39.35±1.63	144.0±6.72	2.16±0.40
GSH	16	2.84±0.20	35.50±1.06[a]	121.39±9.26[a]	1.89±0.09
	24	2.37±0.13[a]	29.27±1.74[a]	122.0±5.48[a]	1.72±0.04[a]
	72	2.60±0.18	35.76±0.71[a]	157.8±7.43	1.99±0.08
EDTA	16	2.01±0.34[a]	36.22±1.28[a]	123.1±3.47[a]	2.48±0.24
	24	2.68±0.18	40.75±2.41	133.9±2.48[a]	1.84±0.12
	72	2.70±0.17	41.24±1.79	144.1±2.02	1.97±0.11
CDTA	16	2.64±0.24	24.73±2.11[a]	143.3±3.41	2.53±0.11
	24	2.60±0.19	35.18±1.09[a]	146.8±2.22	2.81±0.09[a]
	72	2.71±0.09	38.17±1.94	153.4±3.24	2.97±0.14[a]
DTPA	16	2.11±0.14[a]	32.57±2.61[a]	150.0±3.14	1.65±0.14
	24	2.64±0.19	33.11±1.78[a]	155.9±2.48	1.90±0.22
	72	2.81±0.18	38.27±1.94	159.3±3.41	2.01±0.34
HEDTA	16	2.31±0.31	28.60±1.31[a]	137.8±2.51[a]	1.85±0.21
	24	2.04±0.17[a]	37.46±1.47	143.8±2.18	1.93±0.19
	72	2.50±0.20	40.28±1.28	149.0±3.17	1.98±0.09

Each value represent mean ± S.E. of six animals
[a] $p < 0.05$, when compared with saline treated rats

Table 2. Comparative effects of the chelating drugs on the levels of renal trace metals at 16, 24, and 72 hrs after treatments

Chelating drug	Time intervals (hr)	Metal content (μg/g wet weight)			
		Copper	Zinc	Iron	Manganese
Saline	16 24 72	4.21±0.22	28.10±0.91	112.0±3.28	1.67±0.10
Cyclam	16	4.01±0.31	26.21±1.11	85.0±4.21[a]	1.48±0.14
	24	3.94±0.20	28.07±0.69	90.2±3.67	1.60±0.27
	72	4.11±0.09	28.10±2.41	104.4±6.33	1.70±0.08
TETA	16	3.32±0.27[a]	25.11±1.21	80.9±4.21[a]	1.41±0.19
	24	3.52±0.24	26.90±2.21	87.9±5.18[a]	1.58±0.31
	72	3.81±0.40	26.99±1.81	95.8±3.47	1.87±0.32
GSH	16	3.11±0.13[a]	30.69±1.31	69.7±4.21[a]	1.28±0.31[a]
	24	3.54±0.81	26.38±2.89	70.1±6.92[a]	1.52±0.11
	72	3.71±0.16	26.64±1.31	94.6±8.25	1.60±0.88
EDTA	16	3.64±0.17	22.19±1.49[a]	80.6±3.14[a]	1.51±0.11
	24	3.60±0.31	24.34±1.08[a]	90.4±5.10[a]	1.48±0.09
	72	3.94±0.12	26.85±1.17	95.5±3.41	1.58±0.14
CDTA	16	3.38±0.41	25.18±1.14	78.6±2.59[a]	1.44±0.13
	24	3.89±0.37	27.10±2.19	85.6±1.99[a]	1.54±0.12
	72	4.01±0.29	27.10±1.74	90.8±2.64[a]	1.55±0.12
DTPA	16	3.04±0.24	20.12±1.28[a]	68.4±3.13[a]	1.10±0.09[a]
	24	3.56±0.31	24.89±1.09[a]	81.0±3.74[a]	1.46±0.07
	72	3.79±0.18	26.14±2.01	85.8±4.18[a]	1.49±0.11
HEDTA	16	3.54±0.19	24.19±1.48[a]	77.4±4.36[a]	1.30±0.11[a]
	24	3.91±0.11	26.28±2.11	84.5±5.10[a]	1.43±0.19
	72	4.02±0.31	27.91±2.36	92.9±3.88	1.49±0.20

Each value represent mean ± SE of six animals
[a] $p < 0.05$, when compared with saline treated rats

and CDTA depleted only the levels of PGM in liver and kidney respectively.

The efficacy of the chelating drugs to remove toxic metals from the biological system stems from their ability to form stable complexes with the toxic metal ions and to enhance their excretion from the body without affecting the levels of essential trace elements. Since most of the chelating drugs are not specific for a particular metal ion, they are also known to interact with the endogenous trace metals leading to their enhanced excretion resulting in the depletion in their organ levels (Cantilena and Klaassen 1981, 1982; Kostyniak and Clarkson, 1981; Tandon et al. 1984). These drugs also form stable complexes with various metal ions *in vitro* (Sillen and Martell, 1971). Among the drugs evaluated in the present study, Cyclam exhibited least potential to interact with endogenous trace metals as evidenced by unaltered level of the trace metals in organs. In our previous study, we reported the specificity of Cyclam as an antidote for nickel (Athar et al. 1987) and also showed that Cyclam produced least alterations compared to other drugs on the levels of endogenous trace metals in the presence of toxic concentrations of nickel. It is quite possible that the poor affinity of Cyclam for endogenous trace metals may be due to its cyclic nature which increases its specificity for a particular metal ion due to the match of its cavity size with ionic radii of metal ions. TETA being linear is non-specific and has an affinity for various metal ions. The significant depletion of Cu in serum and Cu and Fe in hepatic/renal tissue after the administration of TETA in the present study appears reasonable in view of its use in the treatment of Wilson's disease (Walshe, 1973). The high affinity of TETA for Cu may also be due to the high stability constant of Cu-TETA complex in solution (Dubois et al. 1970; Sillen and Martell 1964; Walshe 1973). Similarly, the high stability constant of Fe-TETA complex in solution could explain the cause for the depletion of tissue Fe level after its treatment (Sillen and Martell 1964). The polyaminocarboxylic acid drugs produced comparatively more drastic effects on the serum/organ levels of trace metals as evident from the results of this and previous studies (Brownie et al. 1986; Chapvil et al. 1974; Fisher et al. 1976). These drugs have several highly electronegative oxygen and nitrogen containing functional groups which become charged and solvated in blood/plasma thus it is unlikely that these drugs can cross the cell membrane. It, therefore, appears that the carboxylic acid drugs interact with trace metals during their transit from the site of absorption to the site of their distribution (Foreman 1960). The different pattern exhibited by polyaminocarboxylic acid drugs on the trace metal levels could be due to the difference in their binding affinities for various metal ions.

The alterations in serum activities of LDH and GOT have been considered as a tool to study the variation in cell viability and changes in membrane permeability (Gentry et al. 1984; Vonen et al. 1984). The alterations produced by these drugs on the serum levels of LDH and GOT at initial hours in our study may be the result of their interaction

Table 3. Comparative effects of the chelating drugs on serum glutamate oxaloacetic transaminase and lactate dehydrogenase activities at 16, 24, and 72 hrs after treatments.

Chelating drug	Time intervals (hr)	GOT (n moles of pyruvate/min/ml serum)	LDH (n moles of NADH oxidized/ min/ml serum)
Saline		29.50±0.54	416.40±6.18
Cyclam	16	32.80±0.96	390.00±9.52
	24	33.90±0.54	436.60±6.21
	72	31.30±0.21	417.20±8.44
TETA	16	30.70±0.40	432.80±13.99
	24	29.00±0.48	459.30±11.98
	72	27.80±0.68	374.80±5.75[a]
GSH	16	31.20±1.17	460.90±16.88
	24	33.30±1.13	445.80±8.38
	72	32.60±0.35	436.80±12.44
EDTA	16	37.64±1.30[a]	524.16±6.24[a]
	24	32.93±0.91	463.38±5.73[a]
	72	30.56±1.60	422.46±6.96
CDTA	16	40.12±1.04[a]	474.28±6.84[a]
	24	28.96±1.54	426.49±5.47
	72	24.86±0.97	403.95±6.54
DTPA	16	33.37±1.24	477.90±7.07[a]
	24	29.09±0.94	425.87±5.82
	72	32.75±0.76	404.65±6.82
HEDTA	16	39.87±1.11[a]	519.42±5.74[a]
	24	29.75±0.98	435.81±5.97
	72	30.94±1.16	431.30±6.35

Each value represent mean ± SE of six animals
[a]$p < 0.05$, when compared with saline treated rats.

with cell membrane (Cornish 1971; Pool 1981). The more profound effect of polyaminocarboxylic acids as compared to Cyclam and TETA on the levels of these serum enzymes may be attributed to their strong affinity for Zn (Brownie et al. 1986) which is known to maintain the structural integrity of cell membrane (Brownie et al. 1986; Chapvil et al. 1974; Gabard 1974; Fisher et al. 1976). The normalization of enzyme activity by 72 hrs suggest that these drugs produce a temporary and reversible effects.

Glutathione, a sulfhydryl multifunctional tripeptide plays an important role in the detoxification of various chemicals, serves as a γ-glutamyl donor and maintains the cysteine pool of the system. GSH also plays a key role in the excretion of metals (Kawata and Suzuki 1983; Maines and Kappas 1977a,b). GST is an important enzyme which plays crucial role during the metabolic disposition of a variety of xenobiotics through conjugation with GSH (Chatterjee and Bhattacharya 1984; Patridge et al. 1983; Stockstill and Dauterman 1982). The unaltered levels of GST and GSH after the administration of TETA, GSH, EDTA and CDTA suggest that these drugs do not alter the ability of tissue for the biotransformation/elimination of xenobiotics including metals. The induced activity of GST and/or the enhanced level of GSH exhibited by DTPA, HEDTA and Cyclam in the organs indicate that the tissue may corroborate the functions of these chelating drugs to potentiate excretion of toxic metals. The enhancement in the activity of PGM by Cyclam and DTPA might lead to the fast turnover of NADPH, the latter is known to maintain the redox potential including the levels of GSH (Nordenberg et al. 1982)..

In summary, our results suggest that among all the drugs, Cyclam produced least effect on the levels of trace metals and biochemical parameters followed by TETA, GSH and polyaminocarboxylic acids. The Cyclam may find a better place as an antidote for metal poisoning.

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