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Effect of Liposome Encapsulated Meso-2,3-Dimercaptosuccinic Acid (DMSA) on Biochemical and Trace Metal Alterations in Cadmium Exposed Rats

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Cadmium a well known occupational and environmental contaminant, causes damage to body organs, particularly to kidneys (Nriagu, 1981). Considerable attention has been paid towards the development of safe and effective chelation therapy in the management of cadmium poisoning. Among chelating agents used diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), triethylenetetraminehexaacetic acid (TTHA), sodium-2,3-dimercaptopropane sulfonate (DMPS), 2,3-dimercaptosuccinic acid (DMSA), N-benzyl-D-glucaminedithiocarbamate (NBG-DTC) and N-methyl-D-glucamine dithiocarbamate (NMG-DTC) exhibited an increased potential for the mobilization of cadmium (Basinger and Jones, 1988; Kojima et al, 1989). However the effectiveness of the chelating agents decreases as the interval between cadmium and the chelator administration increases (Cantilena and Klaassen 1982). The ability of the chelating agents to penetrate the cell membrane could be the limiting factor for the mobilization of intracellularly bound cadmium. Liposomal encapsulation of various drugs has been demonstrated to be an attractive method for the delivery of drugs to intracellular sites. Administration of chelating drugs when encapsulated in liposomes exhibit enhanced efficiency of removing metals from the target sites (Behari et al 1986). Liposomal encapsulation also reduces various side effects due to controlled release of drugs as well as protects it from enzymatic degradation during transport (Gregoriadis 1985). The present communication deals with the use of liposome encapsulated DMSA, a known thiol chelator for the treatment of cadmium intoxicated rats which was found to be effective in restoring cadmium mediated biochemical and trace metal alterations.

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MATERIALS AND METHODS

Egg phosphatidyl choline and cholesterol (Sigma) (1:1), 64.8 μ mole each) dissolved in chloroform were taken in a round bottom flask and a thin film of lipid was prepared at 37°C in a water bath under the stream of nitrogen until traces of chloroform were removed. The film was suspended in water (2 ml) and subjected to probe sonication in ice cold conditions for 3 minutes followed by 1 minute rest (10 cycles) using Vibronics Ulrtrasonic Processor P_2 to obtain small unilameller vesicles. The material was contrifuged at 1500 rpm for 5 minutes to remove the titanium particles. This suspension (1 ml) was mixed with meso 2,3- dimercapto-succinic acid (Sigma) (50 μ moles in 1 ml) and the mixture after gentle shaking was flash frozen in liquid nitrogen to give a shell which was subjected to lyophilization. The dry material was carefully rehydrated with 0.1 ml water followed by slow addition of phosphate buffered saline (PBS) to raise it to a volume of 8 ml. It was centrifuged at 14000 rpm for 45 minutes in a cold centrifuge. The pellet so obtained was resuspended in 8 ml of PBS and centrifuged for 2 more cycles. The dehydration rehydration vesicles (DRV) in the form of pellet were resuspended in 1 ml of PBS (Kirby and Gregoriadis, 1984). The average size of the vesicles as determined by electron microscope was found to be 250 nm. DMSA content in the DRV and in all the supernatants collected was determined using 5,5'-dithiobis-2-nitrobenzoic acid (Sigma) and measuring the absorbance of yellow colour at 412 nm in a Bausch and Lomb spectrophotometer. While supernates were used directly , 0.1 ml of 1% Triton X-100 was used with DRV aliquots to rupture the vesicles. Upto 20% DMSA was entrapped in liposomes in repeated runs. The encapsulated material (90%) was found to be retained in vesicles upon incubation with rat plasma at 37° C upto 2 hrs.

Male albino rats from Industrial Toxicology Research Centre Colony (weighing 150 ± 10 g) housed in an air conditioned room, maintained on pellet diet (Hindustan Lever Ltd. Bombay) and waer ad libitum were used in the study. They received intraperitoneal (ip) injection of cadmium 0.2 mg/kg b.wt. as $CdCl_2$ daily for 3 days. Six animals were injected with the equivalent volume of physiological saline to serve as normal controls. The cadmium treated animals were divided into three groups and administered intravenous (iv) injection of DMSA (33 μ mole/kg in 1 ml volume with the help of 24 G_1 needle attached to a 1 ml disposable syringe), liposome

encapsulated DMSA or equivalent volume of phosphate buffered saline (PBS) in the tail vein daily for three days. The animals were kept in metabolic cages 48 hrs. after the last injection of the drug. Twenty four hrs. urine was collected afterwhich they were sacrificed (72 hrs. after the last injection). Liver, kidney, spleen and pancreas were taken out, washed free of extraneous material and weighed. All the tissue were digested with acid mixture (HNO_3: $HClO_3$: H_2SO_4; 6:1:1) and after appropriate dilution the concentration of cadmium , copper and zinc was determined by atomic absorption spectrophotometry. The activiy of γ-gultamyl transpeptidase (γ-GT) was determined in kidney homogenate (0.1% in Tris-HCl buffer pH 8.0) and in urine samples (after centrifugation, 1500 rpm, 10 minutes) as described by Roomi and Goldberg (1981). The activity of alkaline phosphatase was determined in diluted serum and urine samples as described by Bessey et al. (1946). Protein content of kidney, serum and urine samples was measured according to Lowry et al (1951).

RESULTS AND DISCUSSION

Decrease in levels of serum alkaline phosphatase (ALP) and concomitant increase in the activity of this enzyme in urine was observed in cadmium exposed-saline treated rats. The inhibition of this enzyme in serum is the manifestation of cadmium toxicity. Treatment with DMSA resulted infurther decrease of this enzyme in serum with significant increase in urine. Administration of liposomal DMSA however was able to restore the loss of ALP through urine (Fig.1).

γ-glutamyl transpeptidase, an enzyme highly concentrated in the renal brush border, predominantly membrane bound and localized on the external surface of proximal renal tubule is a sensitive marker of tubular damage. Heavy metals are known to cause renal dysfunction which leads to considerable decrease of this enzyme in kidney with an excess of activity observed in urine (Tanaka et.al., 1990). DMSA treatment after exposure to cadmium was able to restore the levels of this enzymes in kidney. Treatment with liposomal-DMSA was able to check significant loss of the enzyme through urine and also restore the levels of γ-GT in kidney (Fig.1).

Levels of copper in kidney was enhanced and in pancreas it was depleted after administration of cadmium. Treatment with DMSA has been reported to decrease

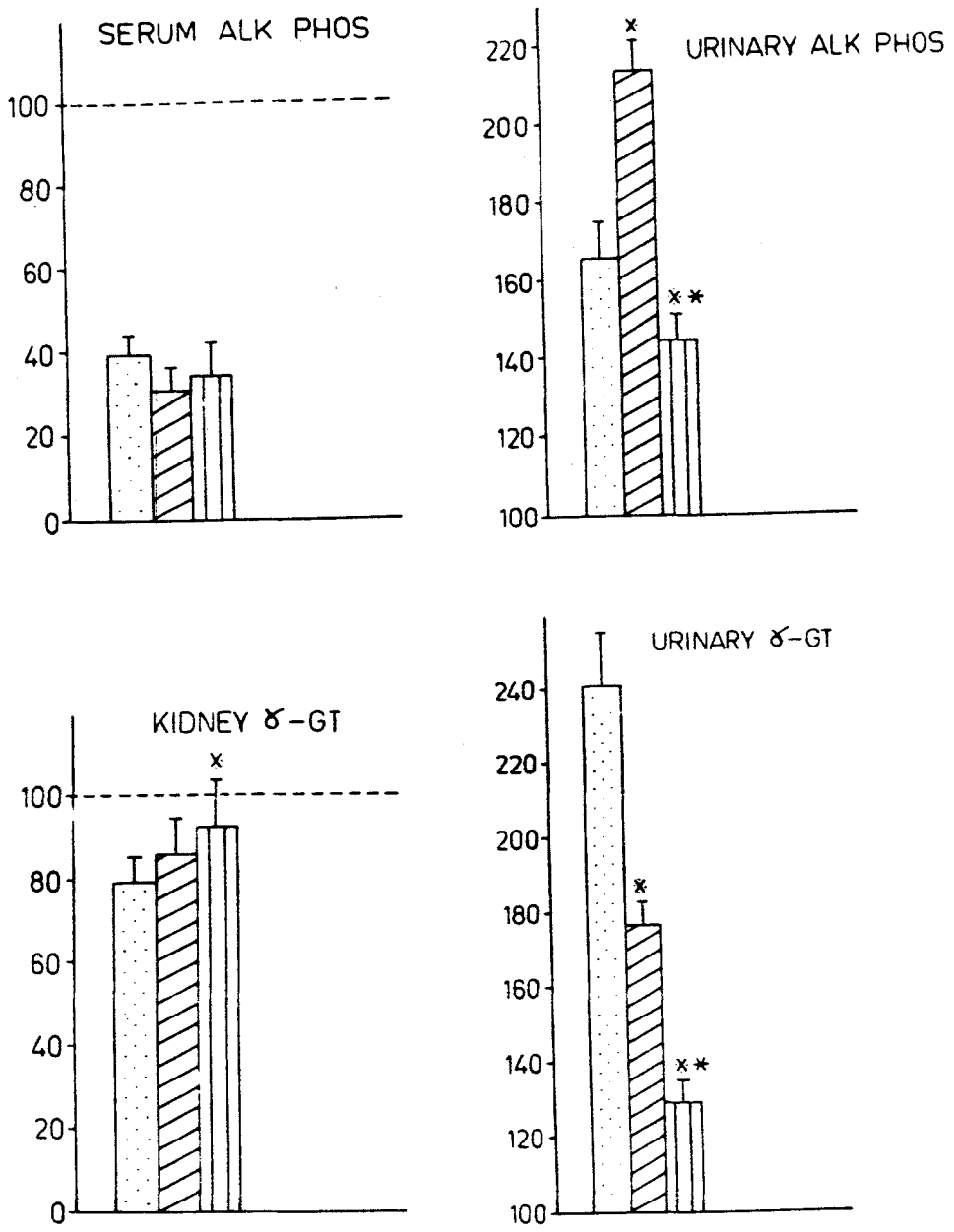

Figure 1. Alterations in the levels of alkaline phosphatase in serum and urine and γ-glutamyl transpeptidase activity in kidney and urine of saline, free DMSA or liposome encapsulated DMSA, in cadmium treated male rats. Values expressed in percent change taking normal control value as 100%. ※ Significantly different $p < 0.05$, compared to cadmium saline. ✱ Significantly different, $p < 0.05$, compared to free DMSA group. Each value represents mean ± S.E. of six rats.

copper from kidneys (Behari et. al, 1984). Liposomal DMSA was effective in maintaining the copper levels in liver and kidney, while it also restored depleted copper levels in pancreas.The administration of cadmium led to a significant increase of zinc in liver and kidney which is expected as these organs actively synthesize metallothionein. DMSA treated group exhibited higher levels of zinc in liver and pancreas and low levels of zinc in kidney as compared to saline treated group. Liposomal DMSA treatment resulted in restoring the zinc lvels in liver and kidney although pancreatic zinc content remained elevated (Table-1).

Table 1. Effect of liposome encapsulated DMSA on cadmium, copper and zinc content (μg/g, wet weight) in the body organs of cadmium exposed male rats

Metal	Organ	N-Control	Cd-Saline	Cd-DMSA	Cd-Lipo-DMSA
Cadmium	Liver	ND	15.40±1.66	14.92±0.90	12.06±1.81
	Kidney	ND	6.48±0.39	5.81±0.22	5.73±0.49
	Spleen	ND	2.88±0.20	1.99±0.13	1.68±0.15
	Pancreas	ND	5.55±0.39	5.12±0.53	5.38±0.52
Copper	Liver	5.48±0.24	4.44±0.59	4.53±0.34	5.56±0.27
	Kidney	9.31±0.12	11.38±0.67	6.36±0.28	7.44±0.22
	Spleen	2.55±0.11	2.63±0.37	2.49±0.15	1.95±0.17
	Pancreas	3.90±0.32	2.78±0.28	2.98±0.29	3.25±0.21
Zinc	Liver	34.49±3.08	55.52±1.35	60.52±2.51	47.63±3.20
	Kidney	21.31±1.19	26.76±0.86	18.98±1.75	21.32±1.82
	Spleen	27.95±2.00	26.59±1.60	26.53±1.80	25.35±1.59
	Pancreas	15.78±0.83	16.43±1.34	19.76±1.71	22.34±2.05

Each value represents the mean ± S.E. of five animals.

The retention of cadmium in the body organs is considerably long due to its strong binding to metallothionein and difficulty in mobilizing it from the storage sites. DMSA an effective antidote against heavy metal poisoning decreases the concentration of cadmium in body organs (Table-1) but its hydrophilicity may restrict its passage inside the cell. Encapsulation of DMSA in liposomes renders it to become lipophilic and be able to cross the membrane barrier. Besides encapsulation of the drug in liposomes prevents

alterations in trace metal levels produced by the free form of DMSA, Liposomal DMSA shows relatively higher restoration of altered biochemical parameters than equivalent amount of free form without affecting the levels of trace metals in the body organs. This may be primarily due to slow release of the drug from the vesicles and its targetted delivery to the storage sites.

Our results with dehydration rehydration vesicles (DRV) having a larger vesicle size might be limiting its entry in the cells thus making relatively less amount of chelator available at the site of cadmium storage. Further investigations with higher dose of chelator in smaller vesicle size may reveal better results.

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