LETTERS TO THE EDITOR

Influence of Size of Liposomes in Potentiating the Efficacy of Encapsulated Triethylenetetramine-hexaacetic Acid (TTHA) Against Cadmium Intoxication.

Abstract: Polyaminocarboxylic acids have widely been used as antidotes in heavy metal intoxication, however their hydrophilic nature renders them to be mostly distributed extracellularly. To facilitate the intracellular delivery of such chelating agent, triethylenetetramine-hexaacetic acid (TTHA) was encapsulated in small unilamellar vesicles (SUV) or dehydration rehydration vesicles (DRV) and its effect was examined in the amelioration of cadmium toxicity. Mice were administered cadmium (0.2 mg/kg B.wt.) as CdCl₂ intraperitoneally daily for five days. After a period of four weeks rest, they were given two intravenous injections of TTHA as free material or encapsulated in liposomes (0.16 m mole /kg) at a gap of 48 hours. Urinary and fecal elimination of cadmium and its distribution in the liver, kidney and spleen was monitored after TTHA treatment. The results indicate the efficacy of TTHA in removing cadmium from the body organs of preexposed animals and its excretion through urine and feces was maximum when it was encapsulated in SUV liposomes.

Key words: Cadmium—Chelation—Liposomes—Small unilamellar vesicles (SUV)—Dehydration- rehydration vesicles (DRV).

Handling of chronic cadmium poisoning requires decorporation of the metal from the intracellular binding sites by chelating agents followed by elimination of stable and diffusable metal-chelate complex from the body\textsuperscript{1,2}). In order to develop a successful therapeutic protocol for cadmium intoxication, approaches have been made mainly towards development of newer chelating agents. Diethylene-triamine-pentaacetic acid (DTPA), cyclohexane-diamine-tetraacetic acid (CDTA) and triethylene-tetramine-hexaacetic acid (TTHA) have successfully been used in decorporating cadmium from the body of exposed animals\textsuperscript{3}). Since most of the potent chelating agents are hydrophilic in nature they are distributed extracellularly and thus are unable to remove the intracellularly bound metal\textsuperscript{2,4}). Recent studies including those conducted by us have shown that the efficacy of the chelating agents viz DTPA, dimercaptopropanesulfonate (DMPS) and dimercaptosuccinic acid (DMSA) in the amelioration of cadmium toxicity could be enhanced by their encapsulation in liposomes\textsuperscript{5–7}). It has been further observed that liposomal encapsulation of drugs results in its prolonged circulation in the blood\textsuperscript{6–8}) and enhanced uptake by the tissue resulting in the reduced toxicity due to its sustained release in the biological system. Further, carrier potential of
liposomes is influenced by several factors viz. the size of liposomes, their lipid composition and presence of ligands on the surface of liposomes. Taking these considerations into account we investigated the effect of TTHA encapsulated in liposomes of different sizes, viz. small unilamellar vesicles (SUV 80–100 nm) and large sized vesicles prepared by dehydration rehydration technique (DRV 250–300 nm) to evaluate its efficacy in mobilizing cadmium from the body organs of poisoned animals. The results demonstrate that TTHA encapsulated in SUV was most effective in decorporating cadmium from the storage site(s) and in enhancing its excretion/elimination from urine and feces compared to either TTHA encapsulated in DRV or free drug.

Cadmium (II) chloride, cholesterol and TTHA from Sigma (USA) and other routine chemicals of analytical grade from Qualigens, India were used in the experiment. Egg phosphatidyl choline (PC) was isolated as reported earlier. PC and cholesterol (1:1 molar ratio) in chloroform was taken in a round bottom flask and subjected to rotatory evaporation under reduced pressure at 37°C to obtain a thin film of lipids which was flushed with nitrogen until traces of solvent was removed. To the lipid film 4 ml of water was added and processed for the generation of SUV as reported earlier. SUV suspension together with TTHA (50 μ moles in 2 ml, neutralized with NaHCO₃) were freeze dried and subjected to controlled rehydration. The resulting suspension was raised to 2 ml volume with phosphate buffered saline (PBS) and used directly (DRV-TTHA). For the preparation of TTHA encapsulated in SUV 50 μ moles of TTHA dissolved in water was added to the lipid film and then processed for SUV generation (SUV-TTHA).

The experimental animals were male albino mice (25 ± 3 g) of Industrial Toxicology Research Centre breeding colony. They were administered cadmium (0.2 mg/kg B.wt.) as CdCl₂ intraperitoneally (ip) daily for five days followed by 4 week rest and were provided food and water ad-libitum. The animals were divided into four groups of six each. The first-three groups of animals received respectively two intravenous (iv) injections at 48 h gap) of TTHA as free material, SUV-TTHA or DRV-TTHA (0.16 m mole TTHA in 64.8 μ mole lipid content/kg). The fourth group received only equivalent volume of PBS and served as control. Four animals from each group were separately kept in the glass metabolic cages for urine and feces collection at every 24 hour interval up to 144 hours. Animals of all the groups were sacrificed 96 h after the last injection of the chelating drug. Liver, kidney and spleen were immediately removed and washed free of extraneous material. About 0.2 g tissue, urine and feces were subjected to acid digestion with HNO₃, H₂SO₄, HC10₄ (6: 1: 1) followed by analysis of cadmium on Perkin Elmer 5000 Atomic Absorption Spectrophotometer. Statistical significance between the groups was determined using Student’s ‘t’ test.

The results of the present investigation reveal that administration of SUV-
TTHA was most effective in mobilizing cadmium from liver, kidney and spleen of cadmium exposed animals followed by DRV-TTHA and free TTHA (cf. table-1). Enhancement in excretion of cadmium through urine and its elimination via feces was also observed by all the three treatments. Relatively highest cumulative

Table 1. Effect of TTHA encapsulated in liposomes (DRV or SUV) on the mobilization of cadmium from the body organs of preexposed mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd-Sal.</td>
<td>20.04±0.81</td>
<td>17.85±0.41</td>
<td>3.87±0.20</td>
</tr>
<tr>
<td>Cd-TTHA</td>
<td>14.07±0.67*</td>
<td>15.18±0.72*</td>
<td>2.60±0.51</td>
</tr>
<tr>
<td>Cd-TTHA via DRV</td>
<td>12.81±0.56*</td>
<td>14.21±0.92*</td>
<td>2.33±0.19*</td>
</tr>
<tr>
<td>Cd-TTHA via SUV</td>
<td>9.19±0.51*</td>
<td>12.92±0.59*</td>
<td>1.76±0.26*</td>
</tr>
</tbody>
</table>

Cadmium content expressed as µg/g fresh tissue.
Each value represents mean ± S.E. of six animals.
*p<0.05 when compared to cadmium saline.
+p<0.05 when compared to cadmium TTHA

Fig. 1 Cumulative excretion of cadmium in urine of cadmium exposed mice after treatment with TTHA encapsulated in liposomes (DRV or SUV).

*p<0.05 when compared to Cd-Sal
+p<0.05 when compared to Cd-TTHA
excretion of cadmium by SUV-TTHA treatment is in correlation with the pattern of cadmium mobilization from the body organs (cf. Fig. 1 and 2).

The higher efficacy of SUV-TTHA may be due to the fact that the higher curvature of smaller liposomes gives rise to a lower surface pressure than the larger liposomes making them more stable and available for circulation in blood for longer duration\(^{12}\). The delivery of TTHA via SUV to intracellular sites could thus be facilitated by its smaller size (80–100 nm) compared to large sized DRV (250-300 nm)\(^{12}\). The relative ineffectiveness of TTHA given via DRV could thus be due to their retention in body for shorter duration and release of the entrapped material extracellularly. The results of the present investigation demonstrate that encapsulation of chelating agents in liposomes of smaller size considerably improves chelation therapy. Further studies involving modulation in composition of liposomes are in progress which may provide significant information.

Fig. 2 Cumulative excretion of cadmium in feces of cadmium exposed mice after treatment with TTHA encapsulated in liposomes (DRV or SUV).

*\(p<0.05\) when compared to Cd-Sal

**\(p<0.05\) when compared to Cd-TTHA
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REFERENCES


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