Effects of Chelating Agents on Distribution and Excretion of Terbium in Mice

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When terbium chloride ($TbCl_3$) was intravenously injected to mice, terbium (Tb) was mainly distributed into the spleen, lung and liver. Thus, the effects of five chelating agents on the distribution of Tb to the spleen, lung and liver of mice were examined. The treatments with diethyldithiocarbamate (DDTC), *N*-*p*-methoxybenzyl-D-glucamine dithiocarbamate (MeOBGD) and 2, 3-dimercaptopropanol (BAL) reduced the content of Tb in the spleen. The treatments with D-penicillamine (D-PEN), ethylenediaminetetraacetic acid (EDTA) and MeOBGD reduced the content of Tb in the lung. However, BAL treatment enhanced the content of Tb in the lung, indicating the redistribution of Tb to the tissue. Although the biliary excretion of Tb was significantly increased in mice treated with EDTA and MeOBGD, these increases were negligibly small, and the metal was not detected in the urine. The limited data of this study demonstrated that well-known chelating agents such as D-PEN, EDTA, DDTC, MeOBGD and BAL have little ability to excrete Tb into the bile and urine. Further studies are necessary to develop new type chelating agents to remove Tb effectively from the body.

Key words: chelating agent; distribution; excretion; terbium.

The rare earth metals have been used in metallurgical operations, in chemical synthesis as catalysts, and in the manufacturing of tempered glass and carbon arcs (Venugopal and Luckey 1978). These metals have been moved into the spotlight as new and promising materials in the pioneering technological industries such as basic matter for superconductive materials, ceramics and amorphous substances (Venugopal and Luckey 1978; Gerhardsson et al. 1984).

Among the rare earth metals, terbium (Tb) has often used as a substitutional probe for Ca (II) in purified biomolecules (Luk 1971; Epistein et al. 1974; Furie et al. 1976; Horrocks et al. 1984) because of its strong fluorescence after forming complexes with ligands of biological macromolecules (Mikkelsen and Wallach 1974; Sommerville et al. 1985, 1986). Although the use of Tb is increasing, there are very few reports on the biological effects of the metal. Previously, we reported that Tb administration causes an increase of pulmonary lipid peroxidation and affects oxidative defense systems, such as superoxide dismutase, catalase and glutathione peroxidase (Shimada et al. 1996). Several studies also indicate that administration of Tb can alter the Ca content in tissues, including liver, kidney, lung, spleen and testes (Shinohara and Chiba 1991; Kojima et al. 1994; Shimada et al. 1996; Shinohara et al. 1997; Nagano et al. 2000). These findings suggest that Tb has some biological effects and may affect the human health.

A number of investigators have demonstrated therapeutic effects of various chelating agents in metal intoxication. For example, D-penicillamine (D-PEN) and 2, 3-dimercaptopropanol (BAL) are used clinically to treat poisoning with either inorganic or elemental mercury (Klaassen 1985). Ethylenediaminetetraacetic acid (EDTA), D-PEN and BAL are used clinically for lead intoxication (Klaassen 1985). Diethyldithiocarbamate (DDTC) is effective as an antidote for nickel poisoning in rats (Horak and Sunderman 1976)

and is used in the therapy of nickel carbonyl poisoning in human (Sunderman 1979). *N*-*p*-Methoxybenzyl-D-glucamine dithiocarbamate (MeOBGD) is effective in decreasing cadmium concentrations in tissues of laboratory animals (Jones et al. 1988). However, clinically satisfactory method for enhancing Tb mobilization from deposits in tissues has not been reported.

The present study was undertaken to develop the effective chelating agents to mobilize Tb from the body. For this purpose, the effects of five chelating agents, D-PEN, EDTA, DDTC, MeOBGD and BAL, on the distribution and excretion of Tb was examined in mice.

MATERIALS AND METHODS

Chemicals

Terbium chloride ($TbCl_3$), D-PEN, BAL and EDTA-Ca2Na were purchased from Nacalai Tesque (Kyoto, Japan). DDTC was purchased from Wako Pure Chemicals (Osaka, Japan). MeOBGD was synthesized according to the procedure of Jones et al. (1988). Other chemicals were of reagent grade.

Animals

Male ddY mice, weighing 25-30 g, were purchased from Kyudo (Kumamoto, Japan) and housed in metabolic cages with drinking water and diet (CE-2, Clea Japan) provided *ad libitum*.

Tissue distribution of Tb

Mice were injected intravenously with 5% glucose (10 ml/kg) or $TbCl_3$ (200 μmol/kg) in 5% glucose. The animals were killed by decapitation 24 h after i.v. administration of $TbCl_3$, and the various tissues were removed for measurement of Tb concentration.

Distribution and excretion of Tb by chelating agents

Mice were intraperitoneally injected with saline or chelating agent (400 μmol/kg) 24 h after i.v. administration of $TbCl_3$ (200 μmol/kg). The animals were anesthetized with urethane (1 g/kg) and the bile duct was exposed through a midline abdominal incision and cannulated with a polyethylene tubing. Bile and urine samplers were collected for an experimental period of 5 h. Then the animals were killed and the spleen, lung and liver were removed for measurement of Tb concentration.

Measurement of Tb concentration

After tissues were digested with nitric acid, the concentration of Tb was measured by inductively coupled plasma spectrometry (ICAP-575, Nippon Jarrel Ash). Tb content in tissues was expressed as μg Tb per g wet weight of tissue.

Statistical analysis

Data were analyzed by a one-way analysis of variance. When the analysis indicated significant difference, the treated groups were compared to the controls by Duncan's new multiple range test. $p < 0.05$ was considered as statistically significant.

RESULTS

Figure 1 shows the contents of Tb in the various tissues at 24 h after i.v. injection of $TbCl_3$. The spleen, lung and liver exhibited large Tb contents compared with other tissues. A small amount of Tb was also found in the kidney and heart.

The effects of chelating agents on the distribution of Tb to the spleen, lung and liver of mice were examined. As shown in Fig. 2, the treatments with DDTC, MeOBGD and BAL reduced the content of Tb in the spleen. The treatments with D-PEN, EDTA and MeOBGD reduced the content of Tb in the lung. However, BAL treatment enhanced the content of Tb in the lung, indicating the redistribution of Tb to the tissue. The content of Tb in the liver was not affected by treatments with these chelating agents.

The effects of chelating agents on the biliary and urinary excretions of Tb in mice are shown in Table 1. Bile and urine samples were collected for 5 h after i.p. injection of chelating agents. Control mice excreted 0.025% of the dose into bile during experimental period. On the other hand, control mice had no ability to excrete Tb into urine. Very small but significant increases were observed for the biliary excretion of Tb in mice treated with EDTA and MeOBGD, although the treatments with D-PEN, DDTC and BAL had no significant effect on the biliary excretion of Tb.

DISCUSSION

We have at first demonstrated that Tb injected intravenously into mice is mainly distributed in the spleen, lung and liver. Based on this result, an attempt has been made to evaluate the effects of chelating agents on the distribution and excretion of Tb in mice. Among five chelating agents tested, DDTC, MeOBGD and BAL effectively reduced the content of Tb in the spleen, and D-PEN, EDTA and MeOBGD had the ability to reduce the content of the metal in the lung. Furthermore, the biliary excretion of Tb was significantly increased in mice treated with EDTA and MeOBGD. However, these increases were negligibly small, and the metal was not detected in the urine. Therefore, the tissue content of Tb reduced by chelating agents cannot be explained by enhancement of biliary or urinary excretion of Tb.

The present study has shown that the treatment with BAL causes a significant redistribution of Tb to the lung. This may be because BAL forms a lipophilic complex with Tb. A similar example is BAL-mercury complex. Since BAL forms a lipophilic complex with mercury, the redistribution of the metal to other tissues such as the lung, heart and brain is observed (Aaseth et al. 1982; Kiyozumi et al. 1988). DDTC is also known to form lipophilic complexes with cadmium and nickel and to cause the redistribution of these metals to the brain (Gale et al. 1983; Oskarsson and Tjalve 1980). However, the treatment with DDTC, unlike that with BAL, caused no significant redistribution of Tb to the lung.

D-PEN, EDTA and MeOBGD have the ability to form hydrophilic complexes with metals (Kiyozumi et al. 1988; Gale et al. 1989). In this study, although the treatments with D-PEN, EDTA and MeOBGD reduced the contents of Tb without any redistribution, the enhancement in biliary or urinary excretion of Tb was not observed. The reason for this discrepancy is unclear at this time. However, two possibilities can be proposed: 1)

redistribution of Tb to tissues other than tissues tested in this study (brain, heart, lung, liver, spleen, pancreas, kidney and testes); 2) slow excretion rate of Tb removed from tissues. Shinohara et al. (1997) have reported that i.v. administration of Tb at high doses (25 and 50 mg/kg) can cause the formation of colloidal substances of Tb in plasma, and that the colloidal Tb in plasma is incorporated into the lung and spleen by phagocytosis. The dose (200 μmol/kg) of Tb used in this study corresponds to 32 mg/kg and is taken as a high dose. Thus, Tb administered at a dose of 32 mg/kg may be incorporated into the lung and spleen by phagocytosis through formation of colloidal substances. It is possible that formation of colloidal substances further complicates the effects of chelating agents on the distribution and excretion of Tb in mice.

In conclusion, the limited data of this study demonstrate the possibilities that chelating agents such as D-PEN, EDTA, DDTC, MeOBGD and BAL cause the redistribution of Tb to other tissues and/or have little ability to excrete rapidly the metal into the bile and urine. Thus, usefulness of these well-known chelating agents may not expected for detoxication of Tb. Further studies are necessary to develop new type chelating agents to remove Tb effectively from the body.

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Figure legends

FIG. 1. The content of Tb in various tissues after iv administration. $TbCl_3$ (200 μmol/kg) were intravenously injected to mice. The data represent the mean ± S.D. (n = 6).

FIG. 2. Effects of chelating agents on distribution of Tb to the spleen, lung and liver. $TbCl_3$ (200 μmol/kg) were intravenously injected to mice, and chelating agent (400 μmol/kg) was intraperitoneally injected 24 h after $TbCl_3$ administration. The animals were killed at 5 h after the treatment with chelating agent, and the tissues were removed. Data represent the mean ± S.D. (n = 6). *Significant reduction vs control ($p<0.05$). $^{\#}$Significant enhancement vs control ($p<0.05$).