Note

Effect of Ca-DTPA and Zn-DTPA on Essential Metal Contents in the Fetal Liver of Mice[†]

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Ca-DTPA or Zn-DTPA was injected subcutaneously to pregnant mice once a day for 5 consecutive days from day 13 to 17 of gestation. Maternal and fetal livers were collected 20 hours after the final injection of DTPA, and essential metal contents in the liver samples were determined by PIXE.

Both Ca-DTPA and Zn-DTPA did not affect essential metal contents in the maternal liver, but Ca-DTPA significantly decreased copper and zinc contents in the fetal liver to 2/3 and 1/2 of the control value, respectively. Zn-DTPA did not affect fetal zinc content, but it decreased that of copper. It could not be determined whether DTPA affected fetal manganese content because the fetal liver contained no detectable amount of manganese.

Key Words : DTPA, essential metal, fetus, liver, PIXE, mouse

1. Introduction

In case internal contamination with radionuclides happens, surgical treatments such as resection of the contaminated skin and/or administration of emetics or purgatives should be done as

soon as possible in order to decrease transfer of the radionuclides to the systemic circulation¹⁾. Several eliminants are also used for enhancing the excretion of incorporated radionuclides from the body. A chelating agent DTPA (diethylenetriaminepentaacetic acid) combines with radioactive metals and removes them via the kidneys²⁾. But this chelating agent has an ability to combine with certain essential metals. In our previous studies, Ca-DTPA increased the urinary loss of manganese, copper and zinc, and induced transitory hypozincemia^{3),4)}. Although Zn-DTPA did not affect any essential metal concentration in the plasma, increased urinary loss of copper was

[†] マウス胎児の肝臓内必須金属含量に及ぼす Ca-DTPA および Zn-DTPA の影響。佐藤 至, 松坂尚典, 津田修治, 小林晴男, 世良耕一郎*, ニッ川章二**, 西村義一***: 岩手大学農学部, 020 盛岡市上田 3-18-8, *岩手医科大学サイクロトロンセンター, 020-01 岩手県岩手郡滝沢村留ヶ森 348, **日本アイソトープ協会仁科記念サイクロトロンセンター, 020-01 岩手県岩手郡滝沢村留ヶ森 348, ***放射線医学総合研究所, 263 千葉県稲毛区穴川 4-9-1

observed^{4),5)}. On the other hand, Ca-DTPA induces fetal injuries such as abortion, fetal death and malformation when injected into pregnant animals^{6),7)}. The cause of these toxic effects of Ca-DTPA is considered an essential metal depletion in fetuses^{8),9)}. Nevertheless effect of DTPA on essential metals has not been reported in fetuses. In the present experiment, therefore, essential metal contents in the fetal liver were measured in pregnant mice which had been treated with Ca-DTPA or Zn-DTPA.

2. Materials and Methods

Adult female ICR mice, 12–14 weeks old, were maintained on a commercial diet (MF, Oriental Yeast) and tap water in a room controlled at 23°C. They were mated overnight, and gestational day was defined as day 0 when a vaginal plug was present. Fifteen pregnant mice were divided into 3 groups of 5 mice each, and 2 groups of them were given subcutaneous injections of Ca-DTPA or Zn-DTPA once a day for 5 consecutive days from day 13 to 17 of gestation. The administered dose of Ca-DTPA and Zn-DTPA was 300 $\mu\text{mol/kg/day}$, which was 10 times as much as the recommended daily dose for humans. The other group was used as controls without any treatment.

Twenty hours after the final injection (day 18

of gestation), the animals were killed under ether anesthesia, and their liver and fetuses were excised and weighed. Five arbitrary fetuses of a litter were dissected to collect the fetal liver. About 100 mg of each liver sample was dissolved in 1 ml of 10 N nitric acid, and indium chloride solution was added to the samples as an internal standard (100 or 200 $\mu\text{g-In/g}$ tissue). The maternal and fetal liver samples were analyzed by PIXE (particle induced X-ray emission), and essential metal contents in the samples were determined by analyzing X-ray spectra using SAPIX¹⁰⁾.

3. Results

Table 1 shows calcium, manganese, iron, copper, zinc, and selenium contents in the liver samples. Calcium, iron, zinc and selenium contents in the fetal liver were similar to those in the maternal liver, whereas fetal copper content was much greater than maternal one. Fetal liver contained no detectable amount of manganese. Although both Ca- and Zn-DTPA did not affect these 6 metal contents in the maternal liver, Ca-DTPA decreased copper and zinc contents in the fetal liver to 70% and 50% of the control value, respectively. Zn-DTPA decreased copper content in the fetal liver, but it did not alter that of zinc.

Table 2 shows the number of fetuses and their body weight. There were no significant differ-

Table 1 Essential metal concentrations in the mouse liver on gestational day 18 ($\mu\text{g/g}$ wet tissue)

	Maternal liver			Fetal liver		
	Control	Ca-DTPA	Zn-DTPA	Control	Ca-DTPA	Zn-DTPA
Ca	49.9 $\pm$ 6.7	52.3 $\pm$ 7.1	45.5 $\pm$ 10.0	52.3 $\pm$ 8.1	54.6 $\pm$ 6.2	58.5 $\pm$ 9.0
Mn	1.6 $\pm$ 0.4	1.4 $\pm$ 0.4	1.3 $\pm$ 0.3	ND	ND	ND
Fe	64.2 $\pm$ 24.6	46.3 $\pm$ 8.8	65.4 $\pm$ 21.6	86.6 $\pm$ 22.1	72.0 $\pm$ 10.9	85.0 $\pm$ 19.8
Cu	4.6 $\pm$ 0.8	4.2 $\pm$ 0.5	3.8 $\pm$ 0.3	26.1 $\pm$ 6.7	18.6 $\pm$ 3.0*	14.1 $\pm$ 2.9*
Zn	33.7 $\pm$ 5.2	33.0 $\pm$ 1.2	31.3 $\pm$ 2.5	32.8 $\pm$ 5.0	16.1 $\pm$ 1.6*	27.9 $\pm$ 2.1
Se	0.9 $\pm$ 0.1	0.9 $\pm$ 0.0	0.6 $\pm$ 0.2	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4 $\pm$ 0.1

Mean $\pm$ SD, *Significantly different from control ($p<0.05$)

Table 2 Number of fetuses and their weight

	Number (a litter)	Weight (g/fetus)	Total weight (g/litter)
Control	13.8±1.3	1.43±0.13	19.6±1.5
Ca-DTPA	14.6±2.1	1.29±0.14	18.6±1.3
Zn-DTPA	11.6±1.5	1.45±0.18	16.9±2.7

Mean±SD

ences between the control and DTPA-treated groups. As for fetal abnormalities, a case of cerebral hernia and a case of fetal death were recognized with naked eyes in the control and Ca-DTPA group, respectively.

4. Discussion

The results of this experiment showed that Ca-DTPA decreased zinc and copper contents in the fetal liver without inducing zinc depletion in the maternal liver when injected into pregnant mice. As DTPA can not penetrate placental barrier¹¹⁾, the fetal zinc depletion is due to decrease of placental transfer of zinc. Because Ca-DTPA is less stable than Mn-, Cu- and Zn-DTPA¹²⁾, Ca-DTPA may dissociate its calcium, and combine with manganese, copper and/or zinc in the body to form more stable chelates. In our previous studies, Ca-DTPA markedly increased the urinary excretion of zinc, and caused transitory hypozincemia in rats^{3), 4)}. Therefore, the fetal zinc depletion induced by Ca-DTPA is considered to be caused by maternal hypozincemia. In adults, Ca-DTPA-induced urinary loss of zinc, which was estimated to be 1.1 mg/30 μ mol Ca-DTPA³⁾ can be compensated by homeostatic mechanisms such as increased intestinal absorption and controlled excretion of zinc. But fetuses are entirely dependent on the maternal blood for their nutrition, so that maternal hypozincemia will easily induce fetal zinc deficiency. Zinc is essential for many metalloenzymes such as RNA polymerase and superoxide dismutase, and the lack of zinc

induces growth retardation, skeletal disorders, dermatitis, diarrhea, and immunodeficiencies¹³⁾.

Although plasma copper concentration is similar to that of zinc, most of the plasma copper is firmly bound to ceruloplasmin and only a small part of the copper is loosely associated with albumin¹³⁾. Therefore, Ca-DTPA hardly combines with copper in the plasma even though copper has a higher affinity to DTPA than that of zinc¹²⁾. In our previous studies^{3), 4)}, Ca-DTPA-induced urinary loss of copper was estimated to be merely 1/70 that of zinc, and this chelating agent did not lower plasma copper concentration. In the present experiment, however, Ca-DTPA decreased copper content in the fetal liver. This result suggests that Ca-DTPA decreased albumin-bound copper in the maternal plasma without changing ceruloplasmin-bound copper concentration which accounts for the greater part of plasma copper. Ceruloplasmin-bound copper may not be concerned with placental transfer of copper. Copper is an essential element for superoxide dismutase, cytochrome c oxidase, lysyl oxidase, and several other enzymes. Anemia, bone and cardiovascular disorders, hypercholesteremia and impaired keratinization occur in copper-deficient animals¹³⁾.

We have reported that Ca-DTPA enhances urinary excretion of manganese³⁾, but it could not be determined whether Ca-DTPA decreased fetal manganese content because the fetal liver contained no detectable amount of manganese. As manganese is essential for human and animals, more detailed experiments should be carried out in further studies.

Zn-DTPA is less toxic to fetuses than Ca-DTPA because Zn-DTPA does not induce zinc deficiency^{14), 15)}. In the present experiment, Zn-DTPA was confirmed not to induce fetal zinc depletion, but this chelating agent decreased fetal

copper content to a degree similar to that of Ca-DTPA. As Zn-DTPA also increases urinary excretion of copper⁵⁾, the fetal copper depletion is considered to be induced by the same mechanism as described above.

4. Conclusion

Ca-DTPA decreased copper and zinc contents in the fetal liver when it was injected into pregnant mice. Although Zn-DTPA has been reported not to induce essential metal deficiency, it was confirmed to decrease copper content in the fetal liver. Because copper and zinc are essential elements for human and animals, particular attention should be given to the administration of these chelating agents, especially to pregnant women.

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