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Effect of Hypothermia on the Disposition of Thiourea in Mice[1)]

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The effect of hypothermia on the disposition of thiourea, which is considered to bind covalently with protein or peptide in the body, was investigated in hypothermal mice. The results of whole blood level analysis and gel filtration chromatography of liver and lung homogenates following intravenous administration of ^{14}C-thiourea showed that the metabolic clearance as well as the renal clearance of thiourea might be lower in hypothermal mice than in normal mice. This might be due to the suppression of covalent bond formation between thiourea and protein or peptide in the body.

Keywords——thiourea; hypothermal mouse; whole blood level; whole-body autoradiography; renal clearance; metabolic clearance; covalent binding; disposition

In a previous paper,[1)] we reported the effect of hypothermia on the dispositions of creatinine and urea, elimination of which is mainly dependent on kidney excretion. Thiourea is a small molecule and water-soluble compound like creatinine and urea. Maloof and Soodak[2)] reported the cleavage of disulfide bonds in the macromolecules followed by the formation of irreversible covalent (disulfide) binding between the macromolecules and thiourea. The formation of such covalent binding was considered to be the major elimination route of thiourea administered intravenously to rats[3)] and mice.[4)]

In the present paper, the disposition kinetics of thiourea in hypothermal mice was studied to clarify the effect of hypothermia on the formation of covalent bonds between thiourea and protein(s) or peptide(s) in the body. For the evaluation of thiourea disposition, plasma level analysis for unchanged thiourea and metabolites (covalent binding products of thiourea and protein or peptide), whole-body autoradiography, and gel filtration chromatography of liver and lung homogenates were carried out following intravenous administration of ^{14}C-thiourea. The data obtained were compared with those for normal mice.[4)]

Experimental

Chemicals——^{14}C-Thiourea (specific activity, 52.0 mCi/mmol) was purchased from New England Nuclear, Boston, Mass., U.S.A. The radiochemical purity was more than 97%. All other chemicals were of analytical grade and were used without further purification.

Animals and Induction of Hypothermia——Male mice of ddY strain were purchased from Shizuoka Agricultural Cooperative for Laboratory Animals, Hamamatsu, Japan, and were used for the experiments at 8 weeks old (28.9—38.1 g). The rectal temperature of mice was reduced to 25—27 °C by ether anesthesia and ice-cooling, and then ^{14}C-thiourea was administered. Ether anesthesia and ice-cooling of mice were carried out when it was necessary to maintain the rectal temperature at 25—27 °C.

Whole Blood Levels of ^{14}C-Thiourea and Its Metabolites Following Intravenous Administration——Mice were given 100 μCi/kg of ^{14}C-thiourea (0.16 mg/kg as thiourea) intravenously into the tail vein, and were sacrificed at 5, 15, 30, 60, 120 and 180 min by cutting the jugular vein to obtain a blood sample (50 μl). Each blood sample was used to

determine unchanged thiourea and metabolites by gel filtration chromatography as mentioned below.

Gel Filtration——Blood samples obtained by the above procedure and the homogenates (about 200 μl) of the liver and the lung obtained at 60 min following intravenous administration of ^{14}C-thiourea (100 μCi/kg, 0.16 mg/kg as thiourea) were applied to a 1 × 15 cm column packed with Sephadex G-10 (Pharmacia Fine Chemicals, Uppsala, Sweden) at room temperature and then eluted with distilled water. The eluates were fractionated by means of an automatic fraction collector.

Whole-Body Autoradiography Following Intravenous Administration——Mice were given 100 μCi/kg of ^{14}C-thiourea (0.16 mg/kg as thiourea) intravenously into the tail vein and sacrificed at 60 min following the administration by soaking them in dry ice–acetone (−78 °C). Sections (40 μm) were obtained with a microtome (Yamato 1111, Tokyo, Japan) at about −25 °C, and attached to Salotape (Hisamitsu Pharmaceutical Co., Ltd., Tosu, Japan). After being dried in a freeze-dryer for a few days, the sections were placed in contact with X-ray films (No. 150, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 20 d at 4 °C.

Radioactivity Measurement——The radioactivity was determined in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.). All samples were determined with 10 ml of toluene–Triton X-100 liquid scintillator (PPO 5 g, POPOP 300 mg, toluene 700 ml, Triton X-100 300 ml). The counting efficiencies were automatically determined by the ^{133}Ba external standard ratio method and cpm was converted to dpm.

Results and Discussion

Whole Blood Levels of ^{14}C-Thiourea and Its Metabolites Following Intravenous Administration

Our previous work[3,4] demonstrated that the radioactivity contained in the low molecular fraction of the two molecular weight fractions obtained by Sephadex G-10 column chromatography of the whole blood following intravenous administration of ^{14}C-thiourea was due to unchanged ^{14}C-thiourea in the whole blood. Therefore, the determination of unchanged thiourea and its metabolites in whole blood was carried out by the separation of the radioactivity in the whole blood into the low molecular (unchanged thiourea) and high molecular (metabolites) fractions.

Figure 1 shows the whole blood levels of ^{14}C-thiourea and its metabolites following intravenous administration to hypothermal and normal[4] mice. The data for ^{14}C-thiourea

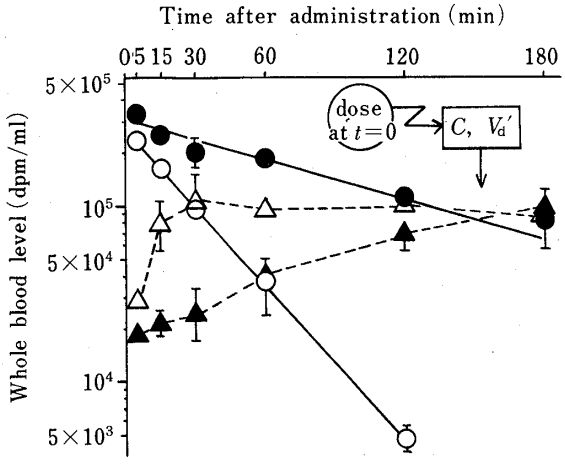

Fig. 1. Whole Blood Levels of ^{14}C-Thiourea (●, ○) and Its Metabolites (▲, △) Following Intravenous Administration to Hypothermal and Normal Mice

Solid and open symbols represent the data for hypothermal and normal mice, respectively. Each point represents the mean ± S.D. for three mice. Pharmacokinetic analyses were conducted on the basis of a one-compartment open model as shown in the figure. The plots for unchanged thiourea are computer-fitted straight lines (weight (i) = 1). The data for normal mice were reported previously.[4]
——, thiourea; – – – –, metabolites.

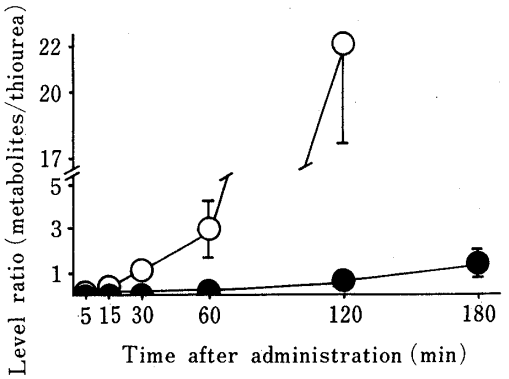

Fig. 2. Level Ratios of Metabolites to ^{14}C-Thiourea in Whole Blood Following Intravenous Administration to Hypothermal (●) and Normal (○) Mice

Each point represents the mean ± S.D. for three mice. The data for normal mice were reported previously.[4]

TABLE I. Pharmacokinetic Parameters for ^{14}C-Thiourea Following Intravenous Administration to Hypothermal and Normal Mice (Value for Parameter ± Standard Error[a])

Parameter	Hypothermal ($n=6$)[b]		Normal[c] ($n=5$)[b]	
V'_d (ml/kg)	7.20×10^{2} [d]	$\pm5.37\times10^{1}$	8.08×10^{2}	$\pm7.77\times10^{0}$
k_{el} (min^{-1})	8.58×10^{-3} [e]	$\pm1.71\times10^{-3}$	3.46×10^{-2}	$\pm6.42\times10^{-4}$
$t_{1/2}$ (min)	8.08×10^{1} [e]	$\pm1.61\times10^{1}$	2.00×10^{1}	$\pm3.71\times10^{-1}$
$k_{el}\cdot V'_d$ (ml/min/kg)	6.18×10^{0} [e]	$\pm1.31\times10^{0}$	2.80×10^{1}	$\pm5.84\times10^{-1}$

a) W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946.
b) The number of input data, each of which is the mean for three mice.
c) Data in previous report.[4]
d) Significantly different from the value for normal mice at $p<0.05$.
e) Significantly different from the value for normal mice at $p<0.01$.

levels in each mouse were analyzed on the basis of a one-compartment open model, and the estimated pharmacokinetic parameters are summarized in Table I. It is apparent that the elimination of thiourea and the accumulation of metabolites in hypothermal mice were slower than those in normal mice. The elimination half-life of thiourea in hypothermal mice (80.8 ± 16.1 min) was just four times that in normal mice (20.0 ± 0.4 min). However, no remarkable difference was observed in the distribution volume of thiourea in hypothermal (720 ± 54 ml/kg) and normal (808 ± 8 ml/kg) mice. Accordingly, the total body clearance ($k_{el}\cdot V'_d$) of thiourea was quite different in hypothermal (6.2 ± 1.3 ml/min/kg) as compared with normal (28.0 ± 0.6 ml/min/kg) mice. As thiourea administered intravenously to 8-week-old mice was partly excreted into the urine in an unchanged form[4] and the glomerular filtration rate and the renal plasma flow were reduced with decreasing rectal temperature,[1,5] one of the causes of the decreased total body clearance of thiourea in hypothermal mice is considered to be the decrease in renal clearance. Moreover, as shown in Fig. 2, the ratios of metabolites to ^{14}C-thiourea in the whole blood in hypothermal mice were very small at any time, as compared with those in normal mice. This result suggests that another cause of the reduced total body clearance of thiourea in hypothermal mice may be the decrease in metabolic clearance. Namely, the covalent binding of thiourea to protein(s) or peptide(s) in the body is assumed to be suppressed by hypothermia.

Whole-Body Autoradiography Following Intravenous Administration

A whole-body autoradiogram at 60 min following intravenous administration to hypothermal mice is shown in Fig. 3, together with that for normal mice.[4] No difference was observed between the two autoradiograms, and the radioactivities contained in the liver and the lung were much higher than those in the whole blood and the other tissues. To examine the difference between hypothermal and normal mice as regards the radioactive substances contained in the liver and the lung, gel filtration chromatography of the liver and lung homogenates was carried out.

Gel Filtration on Sephadex G-10 of the Liver and Lung Homogenates

Figures 4 and 5 show typical Sephadex G-10 gel filtration chromatography profiles of the homogenates of the liver (Fig. 4) and the lung (Fig. 5) obtained at 60 min following intravenous administration of ^{14}C-thiourea to hypothermal (Figs. 4-A and 5-A) and normal (Figs. 4-B and 5-B)[4] mice. It is apparent that the radioactivity contained in the low molecular fraction, which is considered to be due to the radioactivity of unchanged thiourea,[4] accounted for almost all the radioactivities in these organs in hypothermal mice; however, unchanged thiourea could not be found in these organs in normal mice. These results suggest that the

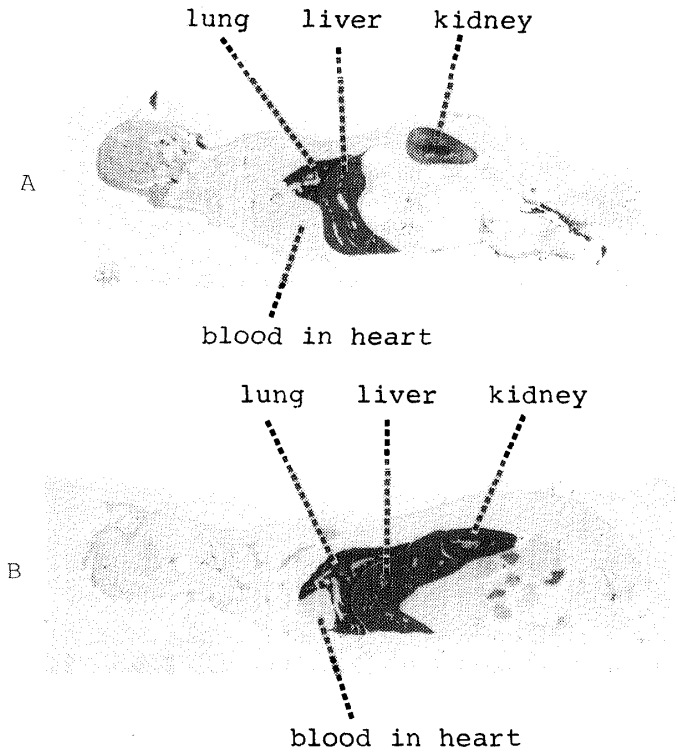

Fig. 3. Whole-Body Autoradiograms Showing the Distribution of Radioactivity (Dark Areas) at 60 min Following Intravenous Administration of ^{14}C-Thiourea to Hypothermal (A) and Normal (B) Mice

The autoradiogram for a normal mouse was reported previously.[4]

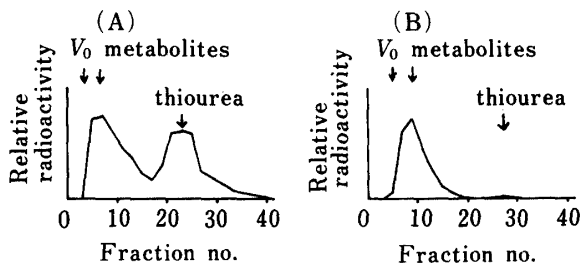

Fig. 4. Typical Sephadex G-10 Gel Filtration Chromatograms of Liver Homogenates at 60 min Following Intravenous Administration of ^{14}C-Thiourea to Hypothermal (A) and Normal (B) Mice

V_0: void volume. Column size, 1 × 15 cm; eluent, distilled water; volume of fractions, 250 μl; sample applied, about 200 μl. Liver tissue was homogenized with an appropriate volume of distilled water. The data for normal mice were reported previously.[4]

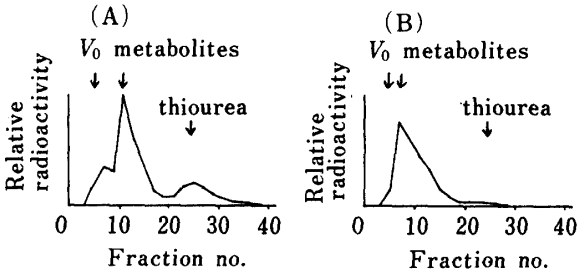

Fig. 5. Typical Gel Filtration Chromatograms on Sephadex G-10 of Lung Homogenates at 60 min Following Intravenous Administration of ^{14}C-Thiourea to Hypothermal (A) and Normal (B) Mice

V_0: void volume. The experimental conditions were the same as mentioned in Fig. 4 for liver.

liver and the lung contain the protein(s) or peptide(s) capable of binding covalently with thiourea, and that the binding formation is suppressed by hypothermia.

In conclusion, hypothermia in mice induces a decrease of thiourea metabolic clearance through the inhibition of covalent bond formation between thiourea and protein(s) or peptide(s) contained in the liver and the lung, as well as the decrease of thiourea renal clearance.

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