Study on the Biodistribution of Deuterated Biomolecules in Mice Aiming at New Diagnostic Radio-Imaging Agents

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Deuterated compounds (^{2}H-compounds) labeled with ^{14}C prepared from deuterated algae, *Chlorella ellipsoidea*, were examined for their time-coursed distribution in mice after intravenous administration. The ^{14}C-^{2}H-compounds were fractionated and isolated from algae grown in practically 100 mol% ^2H_2O in the presence of ^{14}C-bicarbonate. The fractions obtained were the "basic" and "acid" fractions, composed mainly of amino acids and sugar phosphates, respectively, and glucose, galactose, and lipid fractions. All fractions were examined for their biodistribution in mice bearing Ehrlich solid tumor in comparison with the fractions isolated from ordinary *Chlorella* (^{1}H-*Chlorella*). ^{2}H-Compounds thus examined showed some behaviors different from ^{1}H-compounds. The ^{2}H- "basic" fraction distributed more slowly in heart, lung and liver than the ^{1}H-fraction. The ^{2}H-specific large distribution in tumor was also observed on this fraction. The ^{2}H-dependent characteristics in the distribution of glucose and galactose differed. The ^{2}H-glucose level was lower in blood and higher in brain, resulting in a brain/blood ratio approximately twice that of ^{1}H-glucose, while ^{2}H-galactose did not show such a characteristic. These findings may be useful for the application of ^{2}H-biomolecules to functional radio-imaging agents for nuclear medicine.

Keywords deuteration; isotope effect; radiopharmaceutical agent; *Chlorella ellipsoidea*

Recent progress in nuclear medicine such as positron emission computed tomography (PET) and single-photon emission computed tomography (SPECT) has called for a change in *in vivo* radiopharmaceuticals; agents which not only figure the site of a lesion in tissues but also reflect a change of tissue or cellular functions (function-imaging agents) are required. A positron-nuclide-labeled glucose analog, ^{18}F-2-fluoro-2-deoxy-D-glucose (^{18}F-2FDG) is an example of this sort of agent.[1] This compound is taken up by cells, even in brain, and phosphorylated to form ^{18}F-2-FDG-6-phosphate which is not further metabolized.[2] Utilizing this metabolic specificity, ^{18}F-2FDG is applied to the imaging diagnosis of human brain tumor, *etc.*, although not as successfully as ^{18}F-3-fluoro-3-deoxy-D-glucose (^{18}F-3FDG),[3] ^{11}C-2-deoxy-D-glucose (^{11}C-2DG)[4] or ^{11}C-3-*O*-methyl-D-glucose.[5] Development of new function-imaging radiopharmaceuticals is, therefore, eagerly awaited. The most promising compounds may be those transported into cells and causing metabolic trapping, as seen in the example of ^{18}F-2FDG. The analogs of biomolecules may, hence, be worth investigating.

We have studied the cellular isotope effect of deuterium (^{2}H) using photosynthetic algae, *Chlorella ellipsoidea*, grown in deuterium oxide (^2H_2O).[6] Concerning nonexchangeable ^{2}H such as that in C–^{2}H bonds in biomolecules, we found that several of ^{2}H-compounds were remarkably metabolically trapped in the pathways of photosynthesis and sugar metabolism.[7] Deuterated compounds thus seem to be one of the candidates for function-imaging agents, although further labeling with positron- or γ-emission nuclide is needed. This idea was also suggested by Gatley *et al.*,[8] who investigated ^{2}H-glucose. Here, we present the biodistribution and its time course in tumor-bearing mice of ^{2}H-compounds fractionated from deuterated *Chlorella* (^{2}H-*Chlorella*) cells; the behavior of some ^{2}H-compounds was found to be different from that of ^{1}H-compounds.

Materials and Methods

Cultivation of ^{2}H-*Chlorella* The cells of *Chlorella ellipsoidea* GERNECK (C-27) were cultured in Myer's 4N medium[9] prepared with practically 100 mol% ^2H_2O under the light of 18000 lux at 25 °C and continuous bubbling with dry air (0.04% CO_2) for 10 d.[6]

^{14}C-Labeling of ^{2}H-*Chlorella* The ^{2}H-*Chlorella* cells (1×10^9 cell) were further cultivated with 1 mM $NaH^{14}CO_3$ (740 MBq/mmol, Amersham) for 4 d in the medium containing 100 mol% ^2H_2O under the same condition described above and collected by centrifugation.

Fractionation of ^{14}C-Labeled ^{2}H-Compounds (^{14}C-^{2}H-Compounds) The ^{14}C-labeled ^{2}H-*Chlorella* cells (1×10^9 cell) were hydrolyzed in 2 N HCl (10 ml) with 60 min refluxing. The hydrolysate mixture was passed sequentially through a cation exchange column (Dowex 50W-X8, H^+ form, 200—400 mesh, 1×5 cm) and an anion exchange column (Dowex 1-X8, $HCOO^-$ form, 200—400 mesh, 1×5 cm), and the "neutral" fraction which passed through both columns was obtained. The "basic" fraction, composed primarily of amino acids,[10] was obtained by eluting the cation-exchange column with 2 M NH_4OH, and the "acid" fraction, mainly of sugar phosphates,[10] was gotten by eluting the anion-exchange column with 4 M HCOOH. Glucose and galactose were isolated from the neutral fraction by high performance liquid chromatography using HPX-87P column (Bio-Rad Laboratories) by eluting with water at 80 °C at a flow rate of 0.6 ml/min.

On the other hand, the lipid fraction was extracted from the intact cells by the method of Bligh and Dyer[11] with some modifications. The cells (1×10^9) were suspended in 4.75 ml of physiological saline–MeOH–$CHCl_3$ (1 : 2.5 : 1.25), shaken well for 2 min and left for 10 min at room temperature. To this mixture, 1.25 ml of $CHCl_3$ and then 1.25 ml of physiological saline were added, mixing each for 0.5 min. The $CHCl_3$ layer was separated by centrifugation ($2500 \times g$, 5 min), and the lipid fraction in this layer was obtained by the evaporation of $CHCl_3$. The lipid fraction was solubilized in physiological saline containing 1% Triton X-100 (Sigma Chemical. Co.) for animal experiments.

Tissue Distribution Male ddY strain mice (body weight 20—30 g, Japan SLC Ltd.) were transplanted with Ehrlich ascites carcinoma cells (2×10^7 cell/mouse) into the left hindleg by subcutaneous inoculation and fed for 7 d to grow the solid tumor to approximately 1 cm diameter. To the solid tumor-bearing mice thus obtained, a ^{14}C-^{2}H-compound (*ca.* 100000 cpm) was injected through the lateral tail vein. The mice were sacrificed at 15, 30, 60 and 120 min after the injection, and the blood and organs were assayed for ^{14}C-distribution; about a 50 mg aliquot of each organ was solubilized in 0.5 ml of Protosol (NEN Research Products) by incubation at 50 °C for 12 h in counting vials. In the case of blood, the solubilized samples were then decolorized with 0.05 ml of 31% H_2O_2. A scintillation cocktail (20 ml) of the toluene system was added to each vial and the radioactivity was determined in a liquid scintillation counter (Aloka LSC 661). The biodistribution assay was also performed on ^{14}C-labeled ordinary compounds (^{14}C-^{1}H-compound), which were obtained from ^{14}C-^{11}H-*Chlorella* in the same manner as ^{14}C-^{2}H-compounds, for

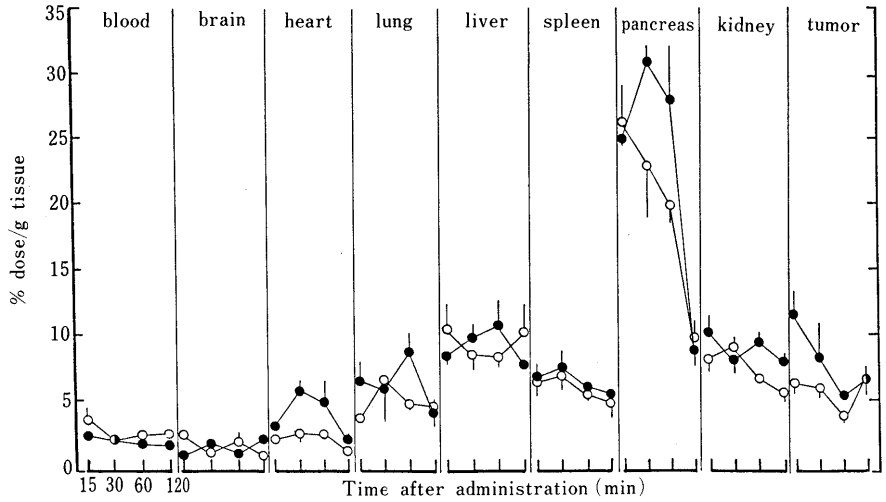

Fig. 1. Distribution of ^{1}H- and ^{2}H- "Basic" Fraction in Mice

Data represent means±S.E. for four mice. (○) ^{1}H-basic fraction; (●) ^{2}H-basic fraction.

comparison. The data were expressed as % of injected dose per g of tissue which normalized 25 g body weight.

Results

Figure 1 shows the time-coursed distribution of the basic fraction, which may be mainly composed of amino acids,[10] in mice bearing Ehrlich solid tumor. On the whole, no dramatic difference was observed between ^{2}H- and ^{1}H-compounds. With both compounds the highest ^{14}C-radioactivity expressed as % dose/g tissue appeared in the pancreas. However, the time-course of pancreatic radioactivity differed between ^{2}H- and ^{1}H-compounds; the distribution of ^{2}H-compounds reached a peak at 30 min after administration and then decreased, while the decrease of ^{1}H-compounds occurred from 15 min. Such a delay of maximal distribution in ^{2}H-compounds was also observed in heart, lung and, seemingly, in liver. In addition to this kinetic difference, the radioactivity of ^{2}H-compounds was higher than that of ^{1}H-compounds at 30 and/or 60 min in the organs mentioned above. In tumor, a markedly higher distribution of ^{14}C-^{2}H-compounds, *ca.* 13% dose/g which was approximately double that of the ^{1}H-compounds, was found at 15 min.

The biodistribution of the acid fraction is shown in Fig. 2. This fraction predominantly contains sugar phosphates, *e.g.*, glucose-6-phosphate and fructose-6-phosphate.[10] Essentially no difference was found between ^{2}H- and ^{1}H-compounds in distribution, except that the initial distribution in tumor of ^{2}H-compounds was lower than that of ^{1}H-compounds, in contrast to the basic fraction.

Glucose (Fig. 3a) and galactose (Fig. 3b) obtained from the hydrolysates of ^{2}H- and ^{1}H-*Chlorella* showed their distribution profiles slightly differently. The radioactivity of ^{2}H-glucose in the blood as well as kidney was remarkably lower and was a little higher in brain than that of ^{1}H-glucose. It is noteworthy that the concentration of ^{14}C-^{2}H-glucose in brain was higher than in blood particularly at 15—60 min. This is clearly seen in Fig. 4 representing the brain/blood ratios calculated for ^{2}H- and ^{1}H-glucose. Furthermore, the lower blood and higher brain concentrations of ^{2}H-glucose lead to a much higher brain/blood ratio than with ^{1}H-glucose (Fig. 4). The distribution of ^{2}H-galactose in

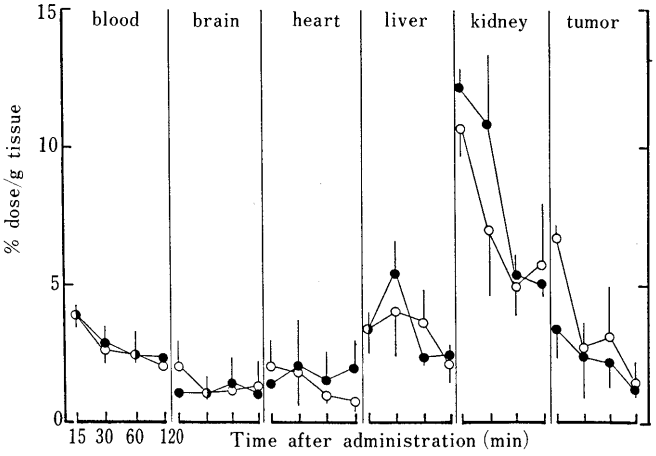

Fig. 2. Distribution of ^{1}H- and ^{2}H- "Acid" Fraction in Mice

Data represent means±S.E. for four mice. (○) ^{1}H-acid fraction: (●) ^{2}H-acid fraction.

comparison with ^{1}H-galactose was some what different from that of glucose. Contrary to ^{2}H-glucose, the blood of ^{2}H-galactose was a little higher than ^{1}H-galactose. The lower distribution of ^{2}H-galactose was not observed in kidney but was in lung, spleen, pancreas and tumor.

With respect to the lipid fraction (Fig. 5), no remarkable difference in the organ distribution was observed between ^{2}H- and ^{1}H-compounds so far examined, while the blood clearance of ^{2}H-compounds was slower than that of ^{1}H-compounds.

Discussion

The deuteration of biomolecules causes some change in their metabolic kinetics, particularly in the enzymatic hydrogen transfer reaction due to the double mass ratio of ^{2}H to ^{1}H.[12] This change appears remarkably in some cases; we observed several metabolic trappings in the pathway of photosynthetic sugar metabolism in deuterated *Chlorella* cells.[7] It therefore seemed worth while to investigate the application of ^{2}H-compounds for functional radio-imaging agents like ^{18}F-2FDG.

Although the deuterated and ^{14}C-labeled biomolecules

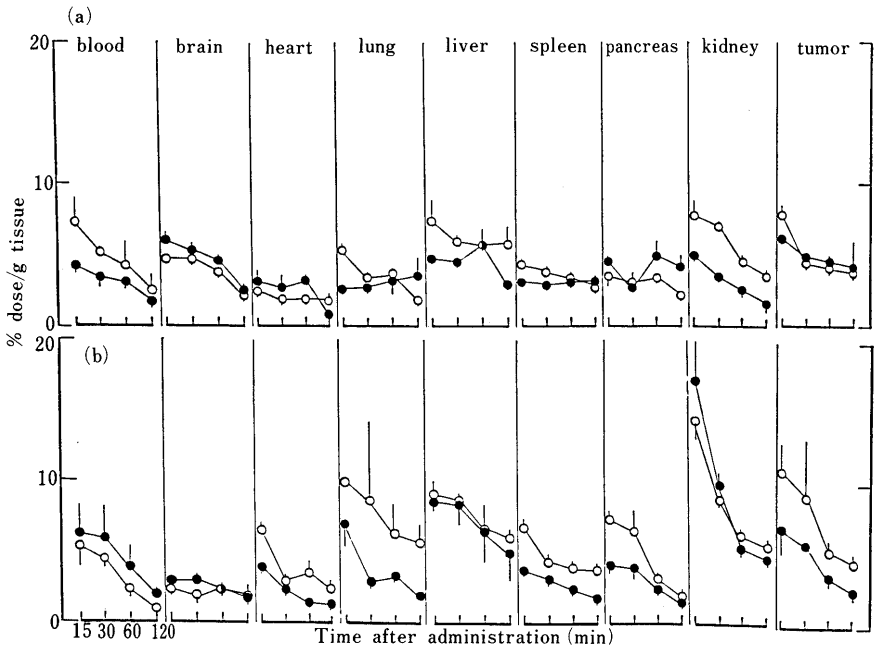

Fig. 3. Distribution of ^{1}H- and ^{2}H-Glucose and Galactose in Mice

Data represent means±S.E. for four mice. (a) (○) ^{1}H-glucose; (●) ^{2}H-glucose. (b) (○) ^{1}H-galactose; (●) ^{2}H-galactose.

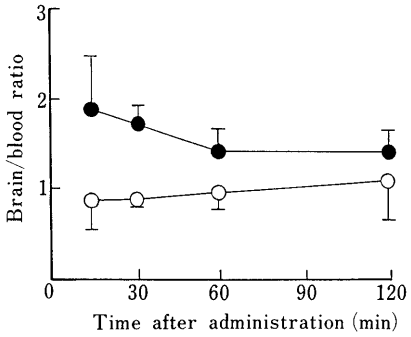

Fig. 4. Brain to Blood Ratio of ^{1}H- and ^{2}H-Glucose

Ratios were calculated from the result of Fig. 3a and represent means±S.E. for four experiments. (○) ^{1}H-glucose; (●) ^{2}H-glucose.

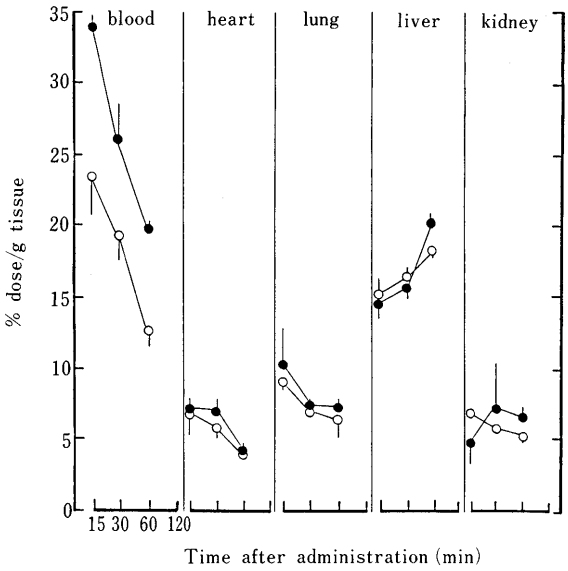

Fig. 5. Distribution of ^{1}H- and ^{2}H-Lipid Fraction in Mice

Data represent means±S.E. for four mice. (○) ^{1}H-lipid fraction; (●) ^{2}H-lipid fraction.

isolated from ^{14}C-^{2}H-*Chlorella* cells were distributed similarly to the ordinary ones, in some cases ^{2}H-compounds showed several different behaviors from ^{1}H-compounds in tumor-bearing mice when i.v. administered. The significant difference observed was in the basic fraction, *i.e.*, the amino acid fraction,[10] and glucose. That is, ^{2}H-basic fraction showed some delay in the distribution in some organs, *e.g.*, pancreas, heart, lung and liver, compared to that of ^{1}H-basic fraction, suggesting that ^{2}H-amino acids were more slowly transported to these organs and, presumably, more slowly metabolized than ^{1}H-amino acids. Another noteworthy observation is that the ^{2}H-basic fraction showed a much higher tumor distribution (*ca.* 13% dose/g tissue at 15 min) than the ^{1}H-fraction. This suggests that some of ^{2}H-amino acids might be useful for imaging.

On the other hand, glucose gave a more remarkable and interesting change by deuteration; the most notable change was that, at 15 min after i.v. injection, ^{2}H-glucose exhibited much lower blood concentration and higher brain distribution than ^{1}H-glucose, resulting in a brain/blood ratio approximately double that of the latter. This high ratio is of great interest, because current attention in the utilization of PET is focused on the visualization of brain function since Wagner *et al.*[13] in 1983 first succeeded in visualizing a human brain dopamine receptor. Together with the fact that the brain distribution of ^{2}H-glucose was as high as *ca.* 6% dose/g tissue at this time point, ^{2}H-glucose may be worth investigating further as a functional imaging agent for brain. Furthermore, we found that the behavioral change of galactose by deuteration was different from that of glucose, suggesting that some other ^{2}H-sugar might also be valuable to examine.

As mentioned above, the *in vivo* behavior of deuterated biomolecules i.v. dosed was not always identical to ^{1}H-biomolecules. This may arise from not only the high mass ratio of $^{2}H/^{1}H$ but also the difference of zero point

energy.[14] Several metabolic analogs such as ^{18}F-2FDG are currently applied for PET, because these agents are easily transported to target tissues and then cause metabolic trapping. Deuterated biomolecules, *e.g.*, sugars and amino acids, may be candidates as new functional radio-imaging agents for PET and SPECT, although further investigations including their radio-labeling are required.

References

1) B. M. Gallagher, A. Ansari, V. Casella, D. R. Christman, J. S. Fowler, T. Ido, R. R. MacGregor, P. Som, C. N. Wan, A. P. Wolf, D. E. Kuhl and M. Reivich, *J. Nucl. Med.*, **18**, 990 (1977).
2) B. M. Gallagher, J. S. Fowler, N. I. Gutterson, R. R. MacGregor, C. Wan and A. P. Wolf, *J. Nucl. Med.*, **19**, 1154 (1978).
3) T. T. Tewson, M. J. Welch and M. E. Raichle, *J. Nucl. Med.*, **19**, 1139 (1978).
4) R. R. MacGregor, J. S. Fowler, A. P. Wolf, C. Y. Shiue, R. E. Lade and C. N. Wang, *J. Nucl. Med.*, **22**, 800 (1981).
5) G. Kloster, C. Müller-Platz and P. Laufer, *J. Label. Compd. Radiopharm.*, **18**, 855 (1981).
6) K. Unno, H. Busujima, S. Shimba, K. Narita and S. Okada, *Chem. Pharm. Bull.*, **36**, 1828 (1988).
7) S. Shimba, K. Unno and S. Okada, *Plant Cell Physiol.*, **31**, 159 (1990).
8) S. J. Gatley, M. M. Wess, P. L. Govoni, A. Wanger, J. J. Katz and A. M. Friedman, *J. Nucl. Med.*, **27**, 388 (1986).
9) Y. Takechi, "*Chlorella*—the Basis and Application—," Gakken Co., Tokyo, 1971, p. 305.
10) C. A. Atkins and D. T. Canvin, *Can J. Bot.*, **49**, 1225 (1971).
11) E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
12) I. A. Rose and E. L. O'Conell, *J. Biol. Chem.*, **236**, 3086 (1961).
13) H. N. Wagner, Jr., H. D. Burns, R. F. Dannals, D. F. Wong, B. Långström, T. Dueffer, J. J. Frost, H. T. Ravert, J. M. Links, S. B. Rosenbloom, S. E. Lukas, A. V. Kramer and J. M. Kuhar, *Science*, **221**, 1264 (1983).
14) J. F. Thomson, "Biological Effects of Deuterium," Vol. 19, International Series of Monographs on Pure and Applied Biology, Division: Modern Trends in Physiological Sciences, Macmillan, New York, 1963, pp. 2—6.