Basic Examination of C-11 1-Pyruvate Synthesized in Tohoku University

III. 4 Basic Examination of C-11 l-Pyruvate Synthesized in Tohoku University

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Introduction

Pyruvate is the end-product in glycolysis. There are several metabolic pathways from pyruvate. For example it is metabolized to CO2 through the tricarboxylic acid (TCA) cycle, to lactate by reduction and to alanine by transamination. In the normal tissue, the main metabolite is CO2, but in the tumor tissue where anaerobic metabolism is active, the main metabolite is lactate. In the tumor-bearing rat, radioactivity in the normal tissue rapidly decreased immediately after injection of C-14 2-pyruvate which was metabolized to C-14 CO2, on the other hand, radioactivity in the tumor tissue remained for a while because of the C-14 2-lactate production.[1] In the experimental myocardial ischemic rabbit injected C-14 l-pyruvate, C-14 radioactivity accumulated more in the reversible ischemic myocardium than in the normal myocardium.[2] Using C-11 pyruvate, positron emission tomography (PET) study would be useful for metabolic diagnosis of cancers and ischemic lesions. Clinical studies of brain tumors and other diseases using C-11 l-pyruvate by PET have already made in Nakano Hospital.[3,4]

In this paper C-11 l-pyruvate was synthesized by Hara's method[3,4] and was tested for its clinical applicability in Tohoku University.

Materials and Methods

C-11 CO2 was produced by the proton bombardment to nitrogen gas target using Tohoku University Cyclotron. C-11 CO2 gas trapped by bubbling with 2.7 ml of 0.05 N NaOH solution in the form of C-11 NaHCO3. The C-11 NaHCO3 solution was air-tightly mixed with the reagent solution (Table 1) and pyruvate-ferredoxin oxidoreductase made of cell body extraction of Clostridium butyricum. The mixture was incubated for 15 minutes at 40°C. For this procedure carboxyl group of pyruvate is labeled with Carbon-11 by replacement with C-11 CO2. C-11 l-pyruvate was separated by sublimation as follows. After incubation the mixture was put into the special glass container (Fig. 1 A, The outer container), and dried up in vacua at 100°C. Then the container was cooled with water, and a small quantity of 50% phospholic acid was sprayed into the residue to make the pyruvate volatile acidic form. The inner

container (Fig. 1 B) was slided into the outer one free from contact of both surfaces, and both were locked up. Dry ice-acetone was put into the inner container to cool it. The outer container was evacuated at 100°C for 5 minutes, and then the volatile pyruvate became a frost which attached to the outer surface of the inner container. The inner one was slided out of the outer one free from contact of both surfaces. The frost was dissolved in 3.5 ml of 1% NaHCO3 solution and was filtrated with the Milli-pore filter.

The synthesized C-11 l-pyruvate (Fig. 2) was analyzed by the high performance liquid chromatography (HPLC). We also studied the tissue distribution of C-11 l-pyruvate in Donryu rats.

Results

The synthesis time of C-11 l-pyruvate, the amount of radioactivity and radiochemical yield were shown in Table 2. The radiochemical purity was more than 99%, and its specific activity was 2.13 mCi/mmol at the end of the synthesis. But the end-product solution contained several non-radioactive materials (Fig. 3) and it had ill odor which was different from that of pyruvate. It was contaminated with another materials which we did not identify.

The tissue distribution of the C-11 l-pyruvate was shown in Fig. 4. Radioactivities were high in the liver and kidney, but decreased rapidly after injection in the blood, brain, myocardium and muscle.

Discussion

Because operations of C-11 l-pyruvate synthesis require great skill and pure enzyme activity, we could not produced so much amount of C-11 l-pyruvate as Hara and others could. They produced 20 mCi of C-11 l-pyruvate whose radiochemical yield was about 80%.[3,4] We need 20 mCi of it for clinical application, but produced only a few mCi of it.

The end-product solution contained unknown materials, and we also could not deny but that it might be contaminated with enzyme proteins in the sublimation process. Because of these reasons we concluded that for clinical use of C-11 l-pyruvate the end-product solution must be purified with chromatographic separation, and that the radiochemical yield must be increased.

References

1) Busch H., Cancer Res. 15 (1985) 365.
2) Goldstein R. A., J. Nucl. Med. 21 (1980) 1101.
3) Hara T., Eur. J. Nucl. Med. 11 (1985) 275.
4) Izuchi R., Hara T., Saikurotoron igaku niyoru tyuusuu shinkei shyougai no hatsugen kizyo ni kansuru kenkyuu (1985) 22.

Table 1. Contents of the reagent solution.

0.2 mol/l Sodium pyruvate

10 mol/l Coenzyme A

0.2 mol/l Mercaptoethanol

50 mol/l Vitamin B12

in 0.2 mol/l Potassium phosphate buffer 2ml, PH 6.1.

Table 2. Synthesis of C-11 pyruvate. The synthesis time started at the time when C-11 CO2 was trapped with NaOH solution. Radioactivity and radiochemical yield were measured at the end of synthesis.

No.	Synthesis Time(min)	Radioactivity (mCi)	Radiochemical Yield (%)
1	36	0.47	12.65
2	44	2.00	20.20
3	42	3.80	15.30
4	36	2.60	6.70
5	38	0.96	3.38

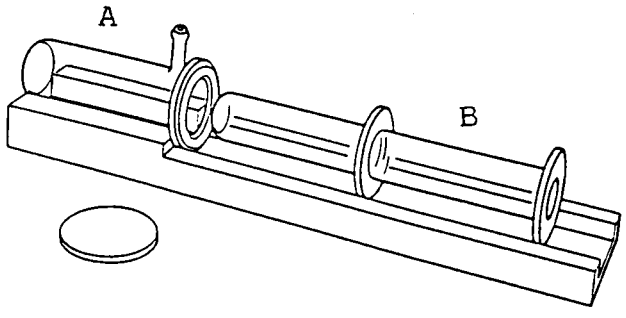

Fig. 1. The special glass containers. A is the outer one, B is the inner one. (Hara and others, 1985)

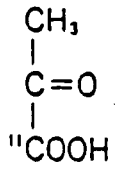

Fig. 2. C-11 1-pyruvate.

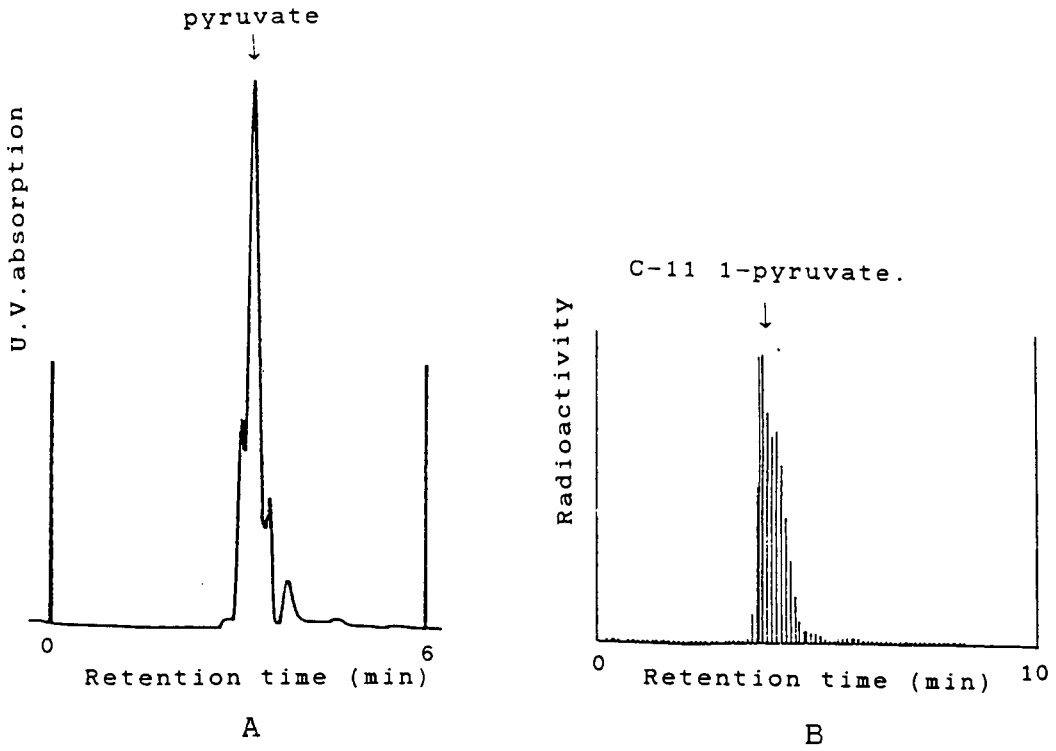

Fig. 3. HPLC analysis.
Colume: Partisil 10 SCX. Colume temperature: 20°C.
Eluent: Potassium phosphate buffer, 1 ml/min.
Detector: A — U.V. 254 nm, B — Radioactivity.
There is a lag time between A and B.
A — There are another peaks besides the peak of pyruvate.
B — There is one peak of C-11 1-pyruvate.

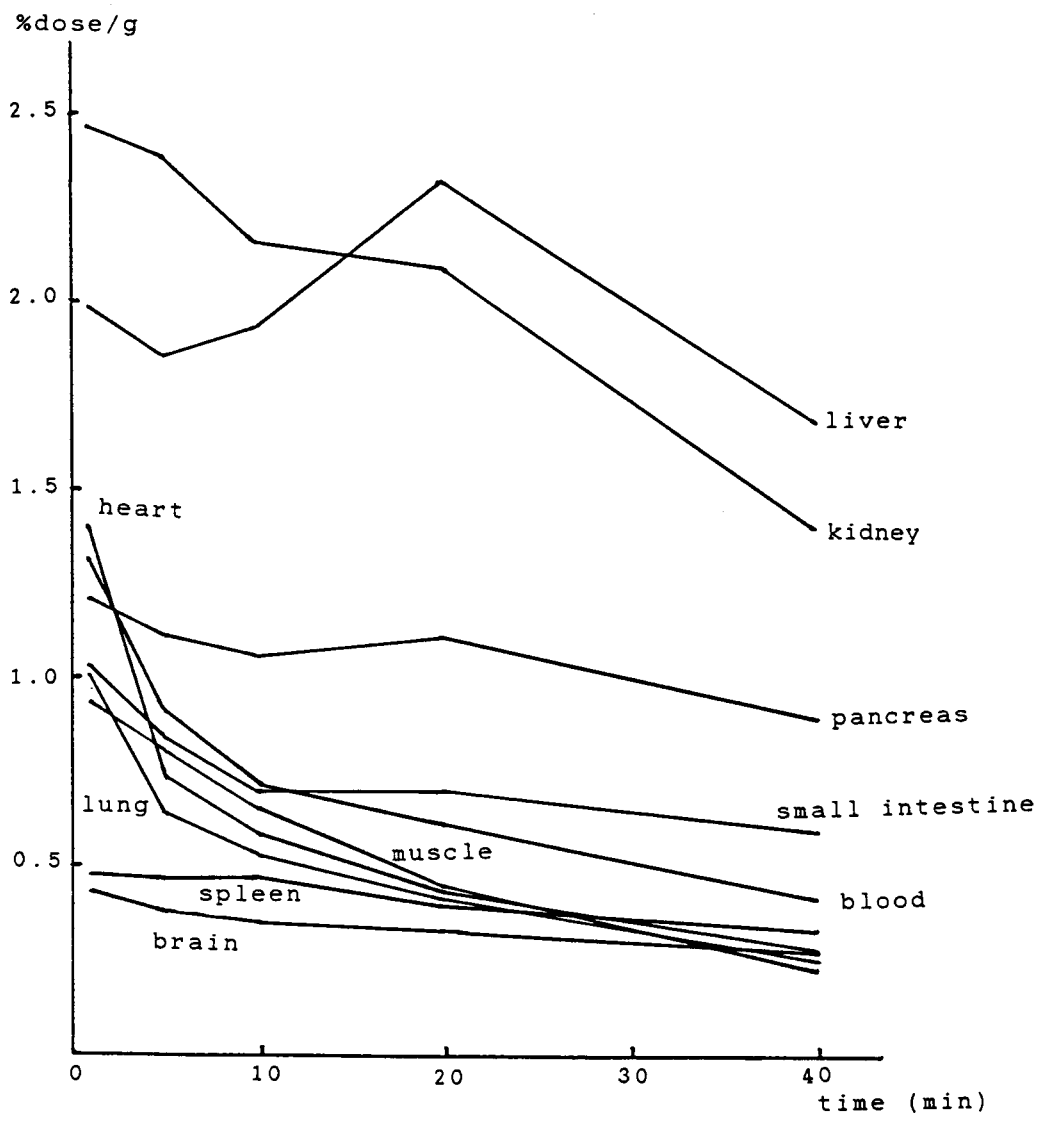

Fig. 4. Tissue distribution of C-11 l-pyruvate. Donryu rat, male.