In Vivo Distribution and Identification of ^{11}C-Activity After Injection of [Methyl-^{11}C]Thymidine in Wistar Rats

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[Methyl-^{11}C]thymidine and PET offer an in vivo, noninvasive quantitative approach for studying nucleoside uptake in cells on the condition the fraction of [methyl-^{11}C]thymidine (in deoxyribonucleic acid [DNA] or as DNA precursors) versus the total accumulated activity is known. **Methods:** In a group of normal (n = 6) and a group of tumor-bearing (n = 3) Wistar rats, the biodistribution of ^{11}C-activity was studied dynamically. In a second group of rats (n = 6), the animals were killed at 20 min postinjection and the organs and tissues of interest (liver, heart, brain, duodenum and tumor) were measured for activity and then homogenized. ^{11}C-activity in each fraction (cell debris, protein/DNA-fraction, lipids and supernatant) was measured. The supernatant was analyzed by high-performance liquid chromatography (HPLC)-radiochromatography for identification of different ^{11}C-labeled compounds. **Results:** After venous injection, most of the ^{11}C-activity was rapidly trapped in the liver and in fast-dividing tissue (e.g., duodenum); minor activity was located in the bladder, kidneys, heart and brain. HPLC separation showed that the ^{11}C-activity of the liver tissue consisted of metabolites only. For the duodenum and tumor, at least 55% of the ^{11}C-activity was precipitated in the protein/DNA-fraction and about 60% as DNA precursors (thymidine, 2'-deoxythymidine 5'-monophosphate and 2'-deoxythymidine 5'- triphosphate) in the supernatant. **Conclusion:** Despite the in vivo metabolism, major ^{11}C-activity in rapidly dividing tissue consists of [methyl-^{11}C]thymidine incorporated in the DNA. Catabolism takes place mainly in the liver where the degradation products are stored. PET quantification data using [methyl-^{11}C] thymidine can give information about thymidine incorporation in DNA and cell proliferation of tumors.

Key Words: [methyl-^{11}C]thymidine; PET; cell proliferation; tissues

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P ET offers a unique opportunity for studying biochemistry noninvasively and quantitatively. Physiologic imaging with naturally occurring tracer molecules is possible with PET in contrast to SPECT. The latter technique uses halogenated analogs (e.g., ^{123,131}I labeled) of which the in vivo behavior is different in comparison to the biologically natural compounds (e.g., thymidine).

Quantification of functional processes with PET requires not only the accurate measurement of migration of radioactivity in tissues, organs and fluids but allows chemical identification of the accumulated activity. PET is based on the registration of positron-electron annihilation processes, independent of the chemical species in which the positron emitter is incorporated at the moment of detection. Associating the PET quantitative data (Bq/mL; mCi/mL) with biochemical parameters requires a knowledge of the nature of the measured activity.

Thymidine labeled with ^{11}C in the methyl- or 2-position has been tested extensively by different groups (1–5) in the study of tumor behavior. Preliminary studies have demonstrated that the tracer is a potential candidate for evaluating tumor proliferation and response to therapy. However, interpretation of the PET data is complicated due to the rapid metabolism of the tracer.

Both animal and human studies have shown that, independent of the ^{11}C position, thymidine is rapidly cleared from the blood pool, whereas most of the residual activity consists of ^{11}C-labeled metabolites (6). Although thymidine, labeled in either position, is incorporated and metabolized in the same manner, the labeled metabolites (β-aminoisobutyric acid versus carbon dioxide) are not chemically identical, resulting in a different in vivo behavior.

In a previous study that used Wistar rats, an indirect way was followed to gain insight on the influence of the metabolites on the PET image. This study showed that, despite rapid in vivo catabolism, 69%–91% of the ^{11}C-activity retained in the intestine came from the originally accumulated [methyl-^{11}C]thymidine (7). Being aware that the proposed approaches only permitted determining the range (lower and upper limits), the best method is to search for a more direct way to find out how much [methyl-^{11}C]thymidine is incorporated into deoxyribonucleic acid (DNA). A previous study by Poupeye et al. (8), in which the DNA of duodenum cells was isolated according to the Qiagen method, showed that at least 30% of the tissue ^{11}C-activity was incorporated in the DNA. However, the proposed separation method was not quantitative and was also time consuming (the separation takes more than 2.5 h)

in comparison to the short physical half-life of ^{11}C (20.4 min). This resulted in a decrease of the activity by a factor of 160, giving a ^{11}C signal too low to obtain reproducible results within an acceptable SD.

Therefore, the specific aims of this study were to investigate the biodistribution of the activity (dynamic PET study); to identify the nature of the different labeled compounds in different organs, tissues and fluids, therefore, making a chemical procedure that included fast and complete destruction of the tissues, purification of the homogenized tissue solution (extractions) and high-performance liquid chromatography (HPLC) separation of the water soluble components necessary; and to evaluate the benefits of the use of [methyl-^{11}C]thymidine for the study of cell proliferation.

MATERIALS AND METHODS

Biodistribution Studies in Wistar Rats

The animal experiments were performed according to the "Guiding Principals in Care and Use of Animals" (American Physiological Society), the Belgian Law for Animal Protection and Welfare and the guidelines of the European Community for the use of animals in laboratory investigations.

All studies were performed on healthy male Wistar rats (210–260 g), about 3 mo of age. The animals were anesthetized with sodium pentobarbital (0.05–0.1 mL). The right jugular vein was catheterized for intravenous injection of [methyl-^{11}C]thymidine.

Ten normal female Wistar rats (40 d of age) received twice the 12.5-mg dose of dimethylbenzantracene within a time interval of 10 d. This carcinogenic polycyclic aromatic hydrocarbon was dissolved in 1 mL of sesame oil and administered by gastric intubation. Three rats developed a tumor within a period of 3 mo.

In 2 Wistar rats, 5 min before the injection of [methyl-^{11}C]thymidine, osmotic opening of the blood-brain barrier (BBB) was performed with an infusion of a hyperosmolar manitol solution (25% w/v) (9).

To destruct the organs and tissues rapidly, ultrasonification was performed with short bursts (3 s, period of 5 s, 25 times) of a Vibra Cell, 25-W homogenizer (20 kHz). The destruction of the cell and nucleus membranes was controlled by microscopic examination.

PET Studies

The PET camera used was an ECAT 951/31 (Siemens Medical Systems, Inc., Iselin, NJ). The 16-ring system detects the annihilation photons in 31 tomographic slices over a distance of 10 cm (slice thickness of 3.3 mm). The rats were positioned in the gantry of the PET camera, using the laser beam (corresponding with the nose-tail line). After the transmission scan of 10 min, 37–55 MBq (1.0–1.5 mCi) [methyl-^{11}C]thymidine, with a specific activity of 3.4 GBq/μmol (9), was injected over a 10-s period into the jugular vein, followed by the infusion of 0.5 mL isotonic solution. Immediately after injection, a preprogrammed sequence of 25 frames (10 × 1 min and 15 × 2 min) was started. For the dynamic study, regions of interest (ROIs) were drawn on the reconstructed PET images for the whole body (via transmission), liver, left kidney, brain, heart and bladder. For each ROI, total ^{11}C-radioactivity was calculated, corrected for decay and plotted versus time.

Tissue Radioactivity Analysis

In a group of normal Wistar rats (n = 6), 20 min postinjection of 370 MBq (10 mCi) [methyl-^{11}C]thymidine, the animals were killed

using an overdose of intravenous injected sodium pentobarbital. Organs and tissues of interest were immediately removed by dissection, washed three times with cooled isotonic saline solution (0°C) to remove coagulated blood clots from the surface and the cavities and treated as described in detail in Figure 1. The whole procedure, cell destruction, solvent extraction and HPLC separation, took less than 40 min (twice the physical half-life of ^{11}C). Identification of the different radioactive signals in the radiochromatograms was based on the retention times of standard reference solutions. Finally, all HPLC acquisition data (radiochromatograms) were saved and analyzed with a computer program (Accuspect 3.0, Canberra Packard Benelux NV/SA, Belgium). Only the radioactive signals were registered; in the different ultraviolet chromatograms, the signals corresponding with the metabolites of thymidine were masked by the water-soluble components of the plasma.

The heart tumor tissue was cleaned, cut into small pieces, frozen in liquid nitrogen and immediately pulverized with a pestle and a mortar both precooled to −15°C. Thereafter, the same procedure as for soft tissues was started.

In some cases, the bladder was punctured by a syringe with a thin needle, and the urine samples (0.1–0.25 mL) were measured for total activity and prepared (diluted to 1 mL with isotonic saline solution) for HPLC analysis.

RESULTS

PET Studies

Several PET images obtained with [methyl-^{11}C]thymidine from a normal Wistar rat are shown in Figure 2, illustrating the ^{11}C-activity distribution. The body contours of the
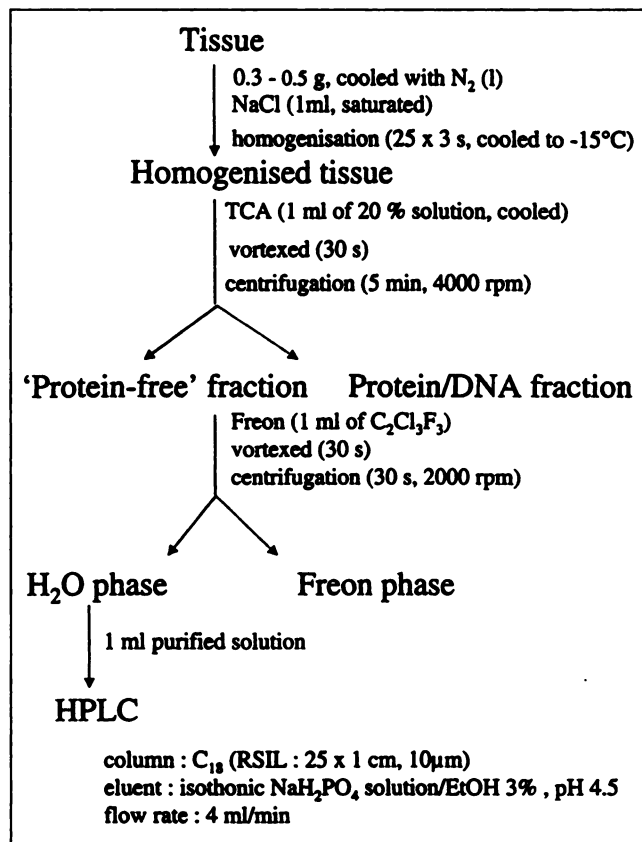

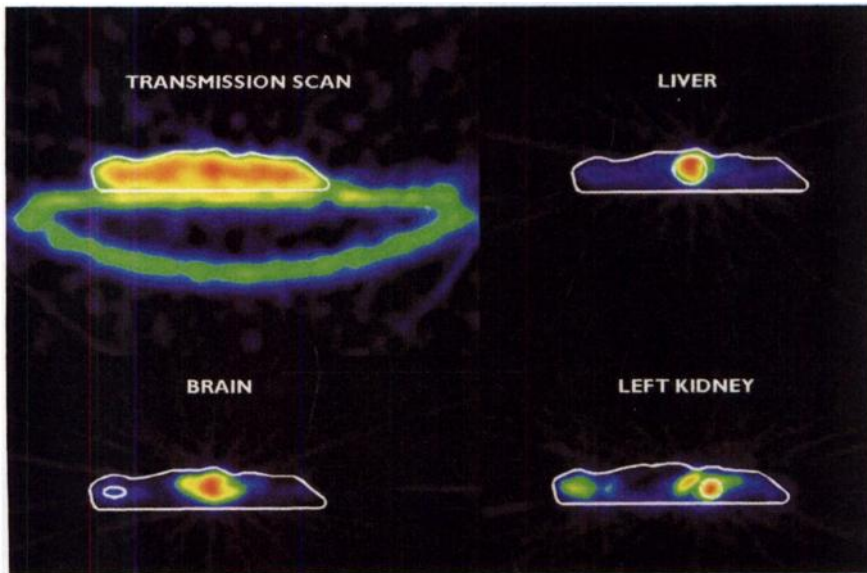FIGURE 1. Destruction and purification pathway for tissue.

FIGURE 2. Color-coded PET image shows transmission and distribution of activity in tomographic section through nose-tail line after intravenous injection of 10 mCi [methyl-^{11}C]thymidine. ROIs are marked on body, liver, brain and left kidney.

previously taken transmission scan, corresponding with the real dimensions of the rat, are superimposed on the emission pictures to facilitate the linking of hot spots of activity to different organs and tissues. ROIs are drawn automatically to correspond with total body, liver, brain, left kidney, heart and brain. The last two are not shown in Figure 2.

The time course of the normalized radioactivity concentration expressed as percentage of injected dose per milliliter of tissue (%ID/mL) in total body, organs, tissues and fluids of interest (liver, brain, left kidney, heart and bladder) in the same rat, representative for the whole group (n = 6), is shown in Figure 3.

After intravenous administration of [methyl-^{11}C]thymidine, the dynamic PET study showed a rapid clearance of the blood-pool activity (heart) ranging from 1.15 %ID/mL immediately after injection to 0.42 %ID/mL from 5 min
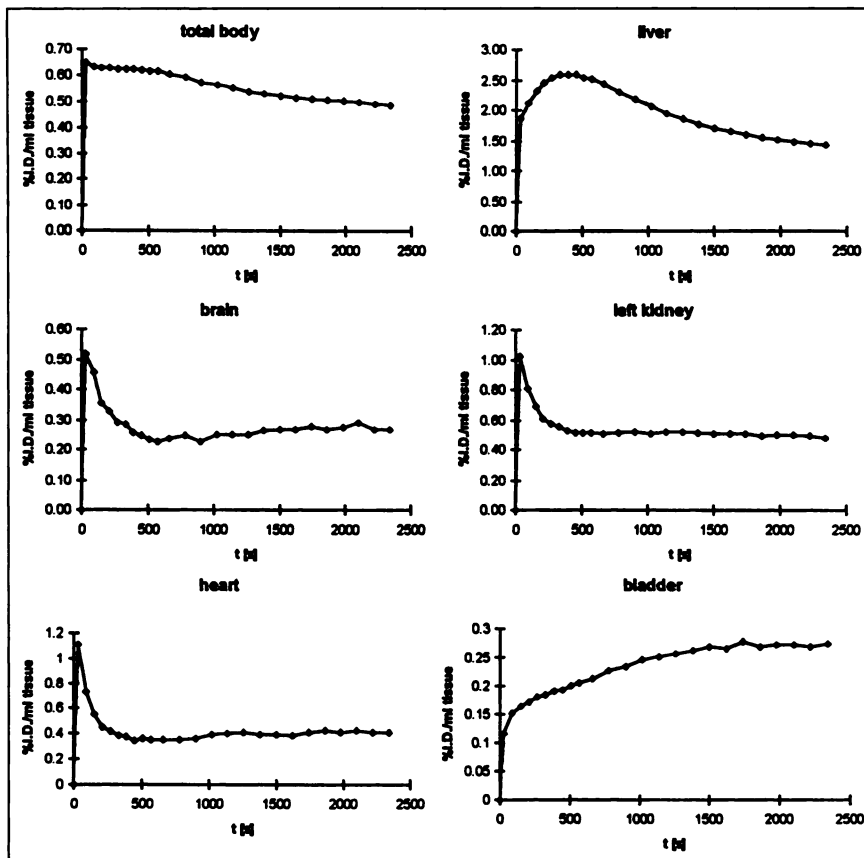

FIGURE 3. Time-activity curves of ^{11}C-activity in %ID/mL in total body, liver, brain, left kidney, heart and bladder.
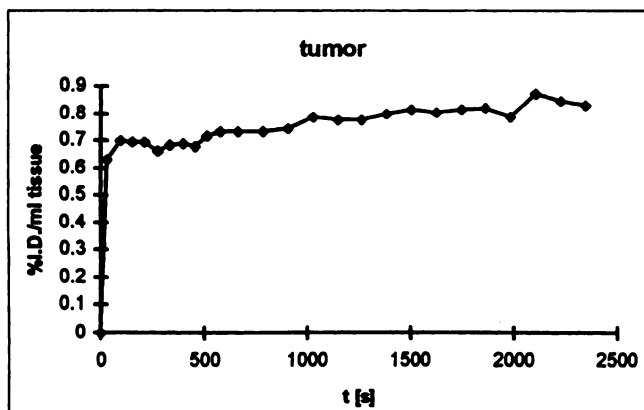
FIGURE 4. Time-activity curve of ^{11}C-activity in %ID/mL in tumor.

postinjection on. Simultaneously, a strong rapid accumulation of activity is noticed in the liver. The dynamic profile shows a plateau from 10 min postinjection on at a level of about 1.50 %ID/mL

The other hot spots of activity identified are the brain, left kidney and bladder. For the brain and left kidney, the dynamic profiles showed an analog pattern. For both curves, the initial high values (0.52 and 1.05 %ID/mL, respectively) decreased rapidly and stabilized from 5 min postinjection on at levels of 0.25 and 0.50 %ID/mL, respectively (about half the initial values). For the bladder, the total activity increased immediately after injection, reaching a level of 0.27 %ID/mL at 25 min postinjection.

In the tumor-bearing rats (n = 3), the uptake of activity in the different organs and tissues was similar to the distribution in normal rats. The activity accumulation in the tumor area as a function of the time is shown in Figure 4, and an initial strong uptake within 3 min, followed by a slow increase from 0.70 to 0.84 %ID/mL at 30 min postinjection is also shown.

In the 2 Wistar rats of which the BBB was osmotically opened, no significantly higher uptake of total ^{11}C-activity was noticed in comparison to the normal rats.

Tissue Radioactivity Analysis

The destruction method presented in Figure 1 allows an efficient separation of the water-soluble radioactive metabolites of [methyl-^{11}C]thymidine from the cell debris (pellet), the protein/DNA and the fatty acid fraction. The percentage of activity retained in those three fractions in most cases was about 5%, except for the intestine and tumor samples. In those tissues, the activity of the protein/DNA-fraction, after washing three times with isotonic saline solution, ranged between 55% and 65% of the original activity, decay corrected. The results for the different samples are summarized in Table 1.

The results of the HPLC-radiochromatographical identification of the different labeled metabolites in the different organs, tissues and fluids, representative of all the rats are summarized in Figures 5 and 6 and Table 1.

For all the different organs and tissues, with exception of duodenum, tumor and urine, HPLC radiochromatograms were characterized by the same pattern. The purified supernatant fraction contained practically no [methyl-^{11}C]thymidine but only metabolites. In all of them, radioactive signals corresponding with the open chain metabolites (β-aminoisobutyric acid and ureidoisobutyric acid) were the most dominant. For the liver, the radiochromatogram showed peaks corresponding only with the open chain metabolites. No [methyl-^{11}C]thymidine could be detected. In the brain, the activity associated with [methyl-^{11}C]thymidine was about 10%. For the heart, the activity identified as DNA precursors, thymidine, 2'-deoxythymidine 5'-monophosphate and 2'-deoxythymidine 5'-triphosphate, was about 50%. In the duodenum and tumor samples, the fraction increased to at least 60%.

The urine activity, about 0.15% of the originally injected dose, consisted of 60%–70% thymidine and 30–40% of the open chain metabolites.

DISCUSSION

PET methodology is complex and requires a multidisciplinary approach with a combination of physical, biologic

TABLE 1
Brief Summary of Activity Distribution in Different Organs, Tissues and Fluids

| Organs/ tissues | % Tissue activity | | | % in supernatant | | Σ DNA (fraction and precursors) |
	Pellet	Fatty acid fraction	Protein/DNA fraction	DNA precursors*	^{11}C-metabolites†	% tissue activity
Liver		3–7		—	100	—
Brain		3–5		8–12	88–92	8–11
Heart		2–4		45–53	47–55	44–51
Duodenum	3–6		55–65	60–68	32–40	82–89
Tumor	2–5		57–65	57–67	33–43	82–88
Urine				60–70	30–40	—

*Thymidine, 2'-deoxythymidine 5'-monophosphate and 2'-deoxythymidine 5'-triphosphate.
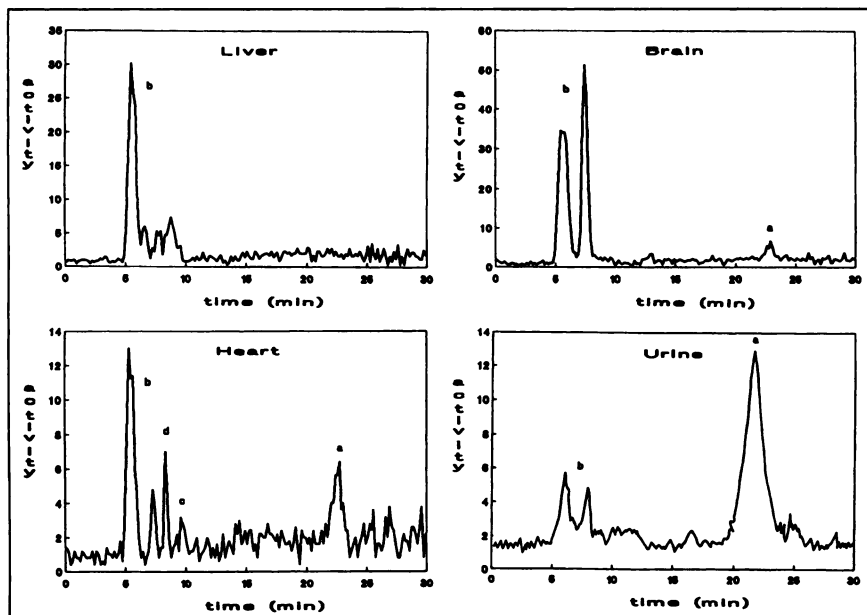†β-Amino isobutyric acid and ureidoisobutyric acid.

FIGURE 5. Radiochromatograms of purified supernatant of homogenized liver, brain, heart tissue and urine. a = [methyl-¹¹C]thymidine, b = open chain metabolites, c = 2'-deoxythymidine 5'-monophosphate and d = 2'-deoxythymidine 5'-triphosphate.

and chemical sciences. The object of this project was to study the in vivo behavior of [methyl-¹¹C]thymidine with the emphasis on the identification of the different radioactive components of the accumulated activity in different organs and tissues. To solve that fundamental problem, a chemical procedure was developed to evaluate the amount of thymidine incorporated in DNA and as DNA precursors.

Previous studies in normal Wistar rats (7) and in human subjects (6) have demonstrated that [methyl-¹¹C]thymidine was rapidly cleared from the blood pool and the contribution of [methyl-¹¹C]thymidine to the total ¹¹C-activity decreased rapidly. From 3 min postinjection on, [¹¹C]dihydrothymine, the second metabolite of thymidine in the metabolization scheme, was the most dominant metabolite. Because the activity is only supplied by blood flow, one could assume that the accumulated activity in the organs and tissues is a reflection of the blood-pool activity.

PET Quantification Data

The results are presented in Figures 2, 3 and 4.

Similar to the strong decrease of the blood-pool activity, as shown by the dynamic heart profile, there is a simultaneous increase of activity in the liver, and a negligible accumulation of activity in the bladder. The dynamic profile of the liver can be considered the result of two physiologic

processes; the first part of the curve (0–10 min) is mainly due to high initial blood-pool activity, the second part shows that once the activity is accumulated practically no migration takes place. It is an advantage of [methyl-¹¹C]thymidine that the majority of the labeled metabolites are blocked in the liver. That is a first indication that the influence of the labeled metabolites on the total activity in organs and tissues with a high cell proliferation rate is negligible. This supposition is confirmed by the dynamic profile of the central tomographic plane, shown in Figure 3 (total body), indicating that, soon after injection, no important migration takes place. Despite its heterogeneous character, which can be considered as representative of the whole body, multiplying the specific activity (0.61 %ID/mL) with the body volume (weight/density: 240/1.07) resulted in a value close to the injected dose (1.37 versus 1.45).

The dynamic profiles of the brain, left kidney and heart (Fig. 3) show the same pattern. The first part of the curves is due to high blood-pool activity, whereas the formation of a plateau indicates incorporation of activity.

Figure 4, illustrating the dynamic profile of the activity in the tumor, is representative of all the tumor-bearing rats (n = 3), indicating that, once the activity is picked up by the tumor, no release takes place. Moreover, one noticed that,
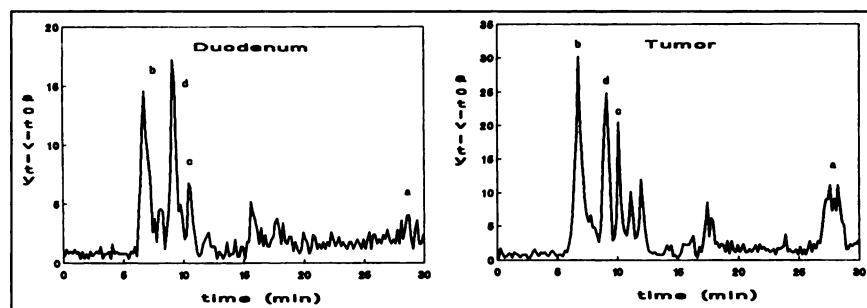

FIGURE 6. Radiochromotograms of purified supernatant of duodenum and tumor tissue. a = [methyl-¹¹C]thymidine, b = open chain metabolites, c = 2'-deoxythymidine 5'-monophosphate and d = 2'-deoxythymidine 5'-triphosphate.

after 3 min when the blood-pool activity is stabilized at a low value and mainly consisted of metabolites, the total activity slowly increased (about 20%) in the period from 3–30 min postinjection on. This increase is completely due to uptake metabolites and confirms the data of the destructive method (Table 1) that more than 80% of the total tumor activity is incorporated in DNA or as DNA precursor.

Evaluation of the Destruction Method

The main goal of this study was to identify the accumulated activity in the different organs and tissues. The method was optimized using liver samples (soft tissue), followed by an adaptation of the procedure for other, more resistant tissues such as heart and tumor. In several tracer experiments, optimization of parameters, such as amount of tissue, destruction time, sonification time, increment of temperature during sonification, cooling temperature during different steps and destruction time, were studied in detail.

Tracer experiments with [methyl-^{11}C]thymidine (10–20 µCi [370–740 kBq]) added to liver tissue (0.5 g) and treated as described in Figure 1 without cooling, resulted in a complete absence of [methyl-^{11}C]thymidine in the HPLC radiochromatogram and the formation of different ^{11}C-labeled metabolites. On the contrary, cooling the samples during the different steps as described in detail in Figure 1 resulted in a complete blocking of the enzymatic activity. In the HPLC radiochromatogram, only one radioactive signal appeared, corresponding to the retention time of thymidine.

The purification steps, eliminating the proteins/DNA and the fatty acids quantitatively, were optimized with tracer experiments to minimize the adsorption of ^{11}C-activity on biologic material. Additional extractions of the protein/DNA and the freon fraction, with isotonic sodium chloride solution, proved that no absorption on the homogenized tissue took place. The percentage of original activity in the supernatant fraction was at least 90%. Two additional extractions with equal volumes of isotonic saline solution reduced the activity in the pellet, the protein/DNA and the fatty acid fraction to less than 5%, except for the duodenum and tumor tissue. For those tissues, additional extractions of the protein/DNA fraction could not recuperate any activity. At least 55%–65% of the original activity was precipitated in the protein/DNA fraction, indicating incorporation of [methyl-^{11}C]thymidine

For the liver, the organ with the highest accumulated activity, only labeled open chain metabolites were detected, which is not surprising because this organ is mainly responsible for catabolism.

In the brain, the activity associated with [methyl-^{11}C]thymidine was less than 10% because thymidine does not cross the BBB. In the 2 rats with the BBB opened, no significant increase of activity (associated with either the parent molecule or metabolites) could be detected. In normal brain tissue, no cell proliferation takes place.

For tissue with a high cell proliferation rate (Fig. 6), duodenum and tumor, the results are completely different

from the other tissues. The ^{11}C-activity in the supernatant is associated with the DNA precursors and ranged between 55% and 65%. Taking into account the accumulated activity in the protein/DNA-fraction for those tissues (55%–65%), the total activity associated with thymidine, 2'-deoxythymidine 5'-monophosphate and 2'-deoxythymidine 5'-triphosphate and DNA increased to about 80% of the total tissue activity. Those results are in agreement with previously obtained values that used an indirect way (7).

CONCLUSION

A reliable and fast method has been developed to identify tissue activity after injection of [methyl-^{11}C]thymidine. This procedure is sufficiently flexible and can be used for the identification of tissue activity of any radiopharmaceutical.

Despite fast in vivo metabolism of [methyl-^{11}C]thymidine by the liver, at least 80% of the accumulated activity is originally due to thymidine incorporated in DNA or as DNA precursor. PET quantification data are representative of DNA synthesis and a simple kinetic model with two or three compartments allows to calculate cell proliferation factors.

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