Measurement of [Methyl-Carbon-11]Thymidine and Its Metabolites in Head and Neck Tumors

Patrick Goethals, Marc van Eijkeren, Wald Lodewyck and Richard Dams

Laboratory of Analytical Chemistry, Institute for Nuclear Sciences, University of Ghent, Ghent, Belgium and Department of Radiotherapy and Nuclear Medicine, University Hospital, Ghent, Belgium

We made routine measurements of [methyl-^{11}C]thymidine and its metabolites in human venous blood in 13 patients with head and neck tumors. **Methods:** Patients underwent PET imaging 10–30 min after bolus injection. Venous samples were collected at various intervals and water-soluble ^{11}C-metabolites were analyzed with semipreparative HPLC on a C18 column with isotonic phosphate solution as an eluant. **Results:** After rapid clearance, total radioactivity remained constant at a level of about 1.2% of the initial injected activity. **Conclusion:** In this group of patients with head and neck cancers, our results show that kinetic differences in various ^{11}C-metabolites are small.

Key Words: positron emission tomography; [methyl-^{11}C]thymidine; in vivo metabolism; head tumors; neck tumors

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PET's ability to quantitate physiologic and metabolic parameters is important for basic and clinical research. Conventional imaging techniques such as diagnostic radiology, x-ray computed tomography or magnetic resonance imaging only detect tumors by its morphological identity.

PET enables tumor imaging based on tumor metabolism. Preliminary studies have shown the possibility of monitoring treatment response by PET with [^{18}F]FDG (*1*) or [^{11}C]methionine (*2,3*) as radiotracers. More recent work has concentrated on the use of [methyl-^{11}C]thymidine as a tumor cell proliferation marker (*4,5*).

Thymidine, an essential building block of DNA, is incorporated during the S-phase of the cell cycle. [Methyl-^{11}C]thymidine is presumed to be a suitable candidate for noninvasive in vivo studies of cell proliferation. Since PET's ultimate goal is to provide useful quantitative clinical data, radiotracer kinetics must be investigated.

For quantitative assessment of thymidine utilization with PET, the ratio of labeled thymidine in total plasma activity should be followed as an input function during the PET study.

Although the metabolic pathway of thymidine is well known (Fig. 1), specific time constants for appearance and disappearance of each metabolite in vivo are not available. In this paper, we investigate the metabolic fate of [methyl-^{11}C]thymidine in human plasma of patients with head and neck tumors.

MATERIALS AND METHODS

No-carrier-added [methyl-^{11}C]thymidine was produced as previously described (*6*). Starting with 44–56 GBq (1.2–1.5 Ci) [^{11}C]carbon dioxide, 7.4–9.3 GBq (200–250 mCi) of chemically and radiochemically pure (>99.5%) [methyl-^{11}C]thymidine in 8 ml of isotonic phosphate solution (pH 4.5; 3% ethanol) were routinely produced within 45 min. The specific activity was calculated to be 7.4–9.3 GBq μmol^{-1} (0.20–0.25 Ci μmol^{-1}) at the time of radiopharmaceutical administration to the patient.

Thirteen patients (age range 25–79 yr) were injected intravenously with [methyl-^{11}C]thymidine (mean activity: 0.9 GBq; range 0.6–1.1 GBq) by a 30-sec infusion. Venous blood samples of about 5 ml were obtained at different time intervals between 1 and 40 min postinjection from the antecubital vein of the arm not used for tracer injection to avoid radiochemical contamination. Total blood activity was measured on 0.3-ml samples with a NaI detector (well type, 2 × 2-inch type) to obtain blood-pool versus time-activity curves (Fig. 2).

Two to four blood samples of six patients were analyzed for plasma-soluble ^{11}C-metabolites. Four milliliters of venous blood were collected in cooled vials (0°C) and centrifuged 1500–2000 rpm for 5 min. To 1 ml of cooled plasma, 50 μl of a standard solution containing 1 μmole of different metabolites (thymidine, thymine, dihydrothymine, beta-ureidoisobutyric acid and beta-aminoisobutyric acid) was added as carrier. Precipitation of plasma proteins was made according to the TCA method (3 ml of 20% trichloroacetic acid, cooled 0°C). After mixing the solution for 1 min, centrifugation at 8000 rpm for 2 min resulted in a protein-free supernatant. Prior to HPLC separation, 1 ml of supernatant was treated with 2 ml of freon/amine solution (1,1,2-trichlorotrifluoroethane/tri-n-octylamine 43:7) to eliminate fatty acids. One milliliter of protein-fatty acid free solution was directly transferred to a C18 semipreparative column (RSIL, 25 × 1 cm, 10 μm; RSL, Eke, Belgium).

Elution was made with isotonic phosphate solution (NaH_2PO_4, pH 4.5 with 3% of ethanol) at a flow rate of 4 ml min^{-1}. The elution profile was controlled with a radioactive monitor (NaI 1 × 1-inch crystals) and an UV detector. The effluent fractions containing the ^{11}C-metabolites were collected and measured with a high-efficiency NaI detector (2 × 2 inch, well-type).

Prior to intravenous injection of the patients with [methyl-

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For correspondence or reprints contact: P. Goethals, PhD, Laboratory of Analytical Chemistry, Institute for Nuclear Sciences, Universiteit Gent, Proeftuinstraat 86, B-9000 Gent, Belgium.

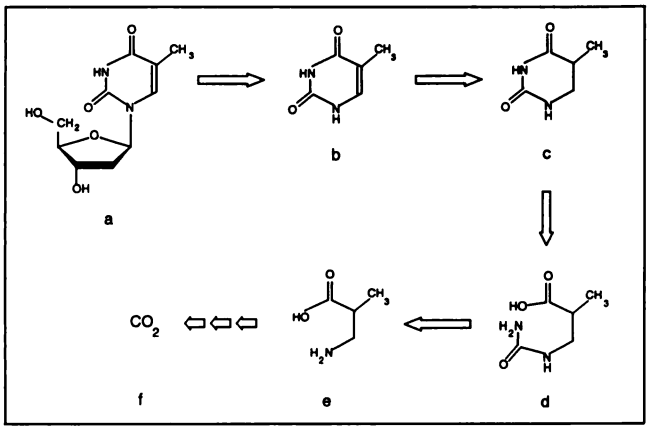

FIGURE 1. Metabolization scheme of thymidine (a) thymidine; (b) thymine; (c) dihydrothymine; (d) beta-ureidoisobutyric acid; (e) beta-aminoisobutyric acid; (f) carbon dioxide.

^{11}C]thymidine for the PET study, venous blood samples were taken and incubated at 37°C after adding 0.9 MBq of [methyl-^{11}C]thymidine (in vitro metabolization tests). After incubation periods of 10 and 20 min, the blood samples were treated as in vivo samples and analyzed for metabolites.

Carbon-11 dioxide appeared 30 min after injection. Supplementary 1-ml blood samples were taken (after 30, 40, 50 and 60 min) and diluted with 2 ml of isotonic phosphate solution (pH 4.0). The solution (tube 1) was vortexed and air was bubbled through each sample for 10 min using a vacuum line system. Carbon-11-dioxide was evacuated from the blood and collected in a NaOH solution (tube 2: 3 ml of 2 *M*) with 3% of n-butanol. Quantitative removal of [^{11}C]carbon dioxide usually took 7 min. Finally, both tubes were measured in the same geometry with a NaI well detector. The amount of labeled carbon dioxide exhaled by the lungs was measured online in a specially designed technical set-up where the CO_2 was trapped quantitatively on an ascarite absorber (Merck; particle size: 0.75–1.5 mm). One-minute breath samples were obtained 30, 40, 50 and 60 min after injection.

Finally, at the end of the study (40–50 min postinjection), urine was collected from three patients and total radioactivity was measured with a calibrated ionization chamber.

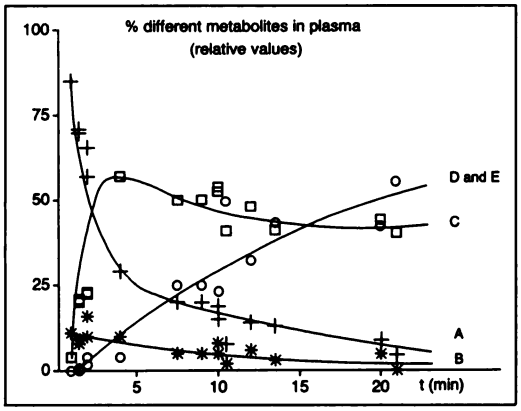

FIGURE 3. Percentage of difference of ^{11}C-metabolites in human plasma versus time (a) thymidine; (b) thymine; (c) dihydrothymine; (d and e) open-chain metabolites.

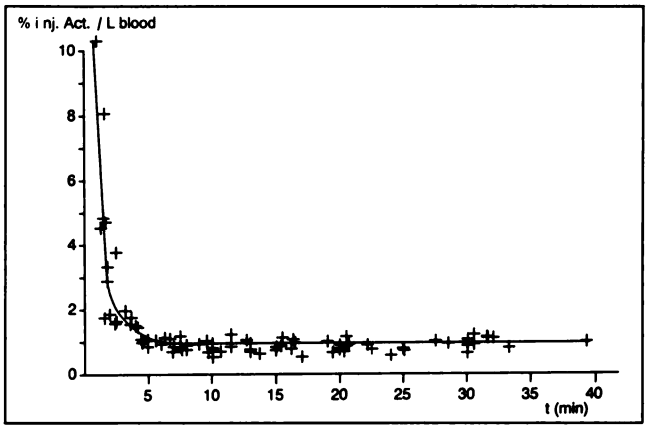

FIGURE 2. Carbon-11 blood-pool activity versus time in 13 patients after intravenous injection of [methyl-^{11}C]thymidine.

RESULTS

The blood clearance of total ^{11}C activity after a bolus injection of [methyl-^{11}C]thymidine is shown in Figure 2. After an initial rapid decrease, total ^{11}C activity remained nearly constant at a level of about 1.2% of the original injected dose L^{-1} (%ID/liter) venous blood. This rapid blood clearance did not seem to be associated with significant renal excretion but is attributed to tracer and/or metabolite incorporation in the extravascular compartments. Total cumulative ^{11}C activity in the urine 1 hr after injection was <1% of the injected dose.

After intravenous injection, [methyl-^{11}C]thymidine accumulated in extravascular compartments, but it also rapidly metabolized (Fig. 3). [Methyl-^{11}C]thymidine itself disappeared rapidly from the blood pool where, soon after injection, [^{11}C]dihydrothymine became the most dominant metabolite. Carbon-11-thymine at any moment was <10% of the total plasma activity and the open-chain metabolites became more dominant as time after injection increased. The in vitro studies (ex vivo) showed a totally different pattern. After 20 min, only 30% of the original activity was found in metabolic form: 25% as thymine, 4% as dihydrothymine and less than 1% as open-chain metabolites.

The results for [^{11}C]carbon dioxide in the blood pool and the excretion by exhalation are summarized in Table 1. Labeled carbon dioxide appeared in the blood pool from 30 min postinjection and increased to about 10% of the blood activity after 1 hr (0.12% ID). At the same time, an increase in exhaled [^{11}C]carbon dioxide was noticed, which accumulated to 1% ID after 1 hr.

TABLE 1
Percentage of [^{11}C]Carbon Dioxide in Blood and Exhaled Air versus the Original Injected Dose (ID)

Time (min)	%ID L^{-1} blood	%ID min^{-1} in exhaled air
30	0.08	0.020
40	0.10	0.037
50	0.15	0.034
60	0.14	0.048

DISCUSSION

[Methyl-^{11}C]thymidine with PET has proven to be an effective tool for tumor visualization (*4,5,7*). Quantitative assessment of thymidine utilization by tumor metabolization, the amount of labeled thymidine in the blood pool, should be used as an input function in kinetic analyses during PET studies.

After intravenous tracer injection, the activity was rapidly distributed from the blood pool throughout the body and accumulated in tumor tissue as well as different organs and tissues. It was apparent that the time-activity blood curves (venous) of the different patients were similar. After a rapid decrease of activity, from 5 to 7 min postinjection, the activity then remained constant at a level of 1.2% ID L^{-1} blood (Fig. 2). Relative differences between venous and arterial blood-pool activity (total and composition) were not studied since there was no authorization by the Ethic Commission to perform arterial blood sampling.

High accumulations of activity were found in liver and kidney parenchyma (*5*). The liver appeared to be the origin for metabolite production and the blood-activity curve reflected the sum of the different labeled metabolites. Analyses of venous blood-pool activity showed that at least 95% of the activity was plasma-soluble. Less than 5% could not be removed, even after three successive washes with isotonic saline solution. Optimization of the proposed purification method resulted in a yield of at least 90% of the different metabolites. The proposed HPLC-separation method (C18 column; isotonic phosphate buffer with 3% ethanol) permitted baseline separation of all metabolites except the open-chain compounds (Fig. 1d and e), which appeared directly after the front peak. Reduction of the ethanol content to 0.5% in the eluant allowed complete separation of both metabolites but resulted in a strong increase in the retention time of other components (e.g., thymidine increased from 18 to 26 min) and lower ^{11}C count statistics (less precision). Only the response of the radioactive detector was useful in detecting the different ^{11}C-metabolites because the UV signal was masked by responses of other plasma-soluble components.

The HPLC separation method proposed by Shields et al. (*8*) to determine different metabolites in canine blood allows no separation of [^{11}C]thymine and [^{11}C]dihydrothymine. Shields et al. conclude, however, that thymine was the most dominant metabolite. With our method, all cyclic metabolites (Fig. 1) are baseline separations and we found that [^{11}C]dihydrothymine and not [^{11}C]thymine was the more dominant metabolite in human plasma during PET imaging. These results were recently confirmed by Tilsley et al. (MRC Cyclotron Unit, Hammersmith Hospital, London, U.K. *personal communication*). [Methyl-^{11}C]thymine was always less than 10% of the total blood-pool activity and ^{11}C-labeled open-chain metabolites became increasingly dominant as the time after injection increased. It appears that during thymidine metabolization the degradation of dihydrothymine with the formation of open-chain structures is the slowest step.

Because of the short half-life of ^{11}C, only HPLC analyses of the labeled metabolites in venous blood plasma samples taken within 20 min postinjection gave reliable and reproducible results. At later times, accurate determinations would only be possible if the initial injected dose was increased (≥56 GBq); this is not advisable for patient studies.

In comparison to in vivo results, where soon after injection blood-pool activity mainly consists of thymidine metabolites, in vitro studies showed a totally different pattern, proving that catabolism by the liver is dominant in the degradation process in vivo.

The amount of labeled carbon dioxide in the blood pool is inferior to other metabolites. Cumulative excretion of activity as carbon dioxide by exhalation or by the kidneys through the urine is negligible (1%–2% of the initial dose).

CONCLUSION

[Methyl-^{11}C]thymidine is rapidly distributed in different organs and tissues after intravenous injection. In the blood pool, the contribution of [methyl-^{11}C]thymidine to total ^{11}C activity decreases rapidly. From 3 min postinjection, [^{11}C]dihydrothymine becomes the dominant metabolite. In our limited study group, patients appeared to metabolize thymidine in a similar manner. Several issues still need investigating: the contribution of different metabolites (especially dihydrothymine) to the PET image and the correlation of accumulated activity in tumor tissue with real cell proliferation rates.

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REFERENCES

1. Strauss LG, Conti PS. The application of PET in clinical oncology. *J Nucl Med* 1990;32:623–648.
2. Leskinen-Kallio S, Nagren K, Lehikoinen P, Ruotsalainen U, Teras M, Joensuu H. Carbon-11-methionine and PET is an effective method to image head and neck cancer. *J Nucl Med* 1992;33:691–695.
3. Sato S, Karreyama M, Tshiwata K. Dynamic study of methionine uptake in glioma using PET. *Eur J Nucl Med* 1992;19:426–430.
4. Martiat Ph, Ferrant A, Labar D, et al. In vivo measurement of carbon-11-thymidine uptake in non-Hodgkin's lymphoma using positron emission tomography. *J Nucl Med* 1988;29:1633–1637.
5. van Eijkeren M, De Schryver A, Goethals P, et al. Measurement of short-term ^{11}C-thymidine uptake in tumors of the head and neck using positron emission tomography. *Acta Oncol* 1992;31:539–543.
6. Goethals P, Sambre J, Coene M, Castelyn K, Poupeye E. A remote controlled production system for preparation of [methyl-^{11}C]thymidine. *Appl Radiat Isot* 1992;43:952–954.
7. van Eijkeren M, De Schryver A, Schelstraete K, Poupeye E, Goethals P, Lemahieu I. Detection of early ^{11}C-thymidine uptake in human non-lymphomatous tumors using positron emission tomography (PET). In: Höfer R, Bergman H, Sinzinger H, eds. *Radioactive isotopes in clinical medicine and research.* Stuttgart; New York: Schattauer Verlag; 1991:14–17.
8. Shields A, Lim K, Grierson J, Link J, Krohn KA. Utilization of labeled thymidine in DNA synthesis: studies for PET. *J Nucl Med* 1990;31:337–342.