Positron Imaging Feasibility Studies. I: Characteristics of [^{3}H]Thymidine Uptake in Rodent and Canine Neoplasms: Concise Communication

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Uptake [^{3}H]thymidine was studied in BALB/c mice with EMT-6 sarcoma, in Buffalo rats with Morris 7777 hepatoma, and in nine dogs with spontaneous neoplasms: four lymphomas, two osteosarcomas, two soft-tissue sarcomas, and a thyroid carcinoma. High tumor-to-tissue ratios were observed for all tumor types assayed, and absolute uptakes, when computed as percent dose per gram tumor normalized for body weight, were similar for transplanted and spontaneous tumors. In the rodent tumors, radiothymidine was retained for at least 3 hr in the tumor without appreciable loss. In canine neoplasms, although the highest uptakes were observed in cellular tumors with many mitotic figures, tumor uptake showed significant variability that did not correlate with any obvious histologic change, and thus may reflect true biologic differences in metabolism among tumors at different sites in the same animal. These studies provide additional experimental evidence that the ratios of neoplastic to normal tissue and the kinetics of thymidine uptake by tumors are suitable for positron emission tomography of neoplasms in small and large animals, including both transplanted and spontaneous tumors.

J Nucl Med 22: 869–874, 1981

The rapid development of positron emission tomography (PET) (*1*) and parallel developments leading to the convenient syntheses of positron-labeled metabolic substrates (*2*) have ushered in a new era in diagnostic imaging, an era in which we can foresee the measurement, in vivo and noninvasively, of biochemical processes of tissues deep within the body.

In a preliminary report (*3*) we described the potential usefulness of certain substrates of glycolysis and nucleic-acid synthesis for the diagnostic imaging of tumors by PET. We studied the uptake of [^{3}H]thymidine, [^{3}H]uridine, and [^{14}C]2-deoxyglucose by the EMT-6 sarcoma of BALB/c mice. Tumor-to-blood ratios and absolute uptakes in tumors were as high 1 hr after injection as the comparable maxima achieved for Ga-67 citrate at 48 hr after injection. These studies suggested that an accelerated metabolism of key substrates by tumors may serve as a basis for detecting tumors and monitoring therapeutic response using PET.

Thymidine tracer labeled with carbon-11, a positron emitter with $T_{1/2}$ 20 min, would be suitable for positron emission tomography. [^{11}C]thymidine has been prepared by Christman et al. (*4*) and used for external imaging of VX2 carcinoma in rabbits using a planar gamma camera (*5*).

Compared with planar scintigraphy, positron emission tomography offers a major improvement in image quantitation because of an inherent superiority of contrast resolution. It has been estimated that, for the uptake of [^{18}F]2-fluoro-2-deoxyglucose by the brain, changes in absolute amounts of radioactivity as small as 10% can

Received Feb. 25, 1981; revision accepted June 3, 1981.

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be reliably quantitated between regions of 1.5 cm^2 on individual tomographic slices with 95% accuracy (*6*). Similar accuracy has been noted for studies of blood flow through the heart (*7,8*). This degree of statistical reliability applies to body regions greater than twice the resolution limits (FWHM) of the PET system (*9*).

In order for PET imaging of tumors with [^{11}C]thymidine to be widely applicable, ratios of tumor to surrounding tissue must be high enough for a variety of tumors so that the radioactivity contained in the tumor can be detected by external imaging. Tumors of sufficient size, containing 20% more radioactivity than surrounding tissue, should be suitable for imaging. Obviously, the greater the uptake of thymidine by the tumor, compared with surrounding tissues, and the higher the tumor-to-tissue ratios, the more readily will the lesions be detectable and quantifiable. Also, the retention of thymidine by the tumor should last long enough to permit the collection of enough counts for quantitative tomographic reconstruction.

Because of the need to obtain broader experience with the time-varying biodistribution of thymidine, we have extended our initial observations on thymidine assimilation to determine the kinetics of uptake in two rodent tumors: EMT-6 sarcoma (BALB/c mice) and Morris 7777 hepatoma (Buffalo rats). Also, to compare tumor uptake with that of normally active tissues in larger animals with spontaneous tumors, we studied outbred dogs (pets) with osteosarcomas, soft-tissue sarcomas, thyroid carcinomas, or diffuse lymphomas. In addition, the variability of thymidine uptake within a tumor was assessed with autoradiography to determine the pattern of distribution of [^{3}H]thymidine in multiple, separate tumor sites in the liver of a dog with spontaneous lymphoma.

MATERIALS AND METHODS

Inbred 5-wk-old BALB/c mice were held for one week before a study. Their weights averaged 20 g. A solid subcutaneous tumor weighing 50–200 mg was produced by techniques previously described (*3*). The tumor-bearing mice were randomly allocated to study groups of four mice. Each mouse received 25 μCi (0.925 MBq) of [methyl-^{3}H]thymidine (sp ac = 2 Ci/millimol (74 GBq/millimol)) intravenously. Animals were killed by cervical dislocation at 0.5, 1, and 3 hr after intravenous injection of radiothymidine. Immediately before sacrifice, 100 μl of blood was removed from the retro-orbital plexus of each mouse. The H-3-labeled radiotracer in the blood, liver, spleen, kidney, muscle, heart, small intestine, brain, lung, and tumor was assayed after the radioactive tissues were oxidized in an automatic sample oxidizer.* The samples were counted in a liquid-scintillation counter.

Inbred Buffalo rats, approximately 8 wk old and weighing 180 g, received subcutaneous injections of 5×10^5 Morris 7777 hepatoma cells, prepared as described previously (*10*). When tumors weighed about 2 g (14 days after innoculation), four animals were randomly allocated to each study group for sacrifice at 0.5, 1, and 3 hr after intravenous administration of 125 μCi of thymidine(H-3). Animals were killed and tissues assayed as described above, except that 200 μl of blood was taken for counting, and the animals were anesthetized with ether just before sacrifice.

Dogs with spontaneous tumors were referred by veterinarians, with the permission of the owners, to the Fred Hutchinson Cancer Research Center. Further details regarding this dog referral program are described elsewhere (*11–14*). For biodistribution studies, dogs were studied when their tumors were not amenable to further therapy. Animals received 1 to 2 mCi of [^{3}H]thymidine intravenously one hour before being killed with intravenous sodium pentobarbital. The organs listed above for mice were removed and weighed, and tissue specimens obtained. Multiple specimens of primary and metastatic tumor tissue were also removed. Specimens were divided into two parts: one for histologic examination and one for liquid-scintillation counting. Normal tissue and tumor samples were processed and counted as described above. Data were expressed as percent injected dose per kilogram of tissue.

In some dogs, tissue (less than 500 mg) was fixed in phosphate-buffered formalin, embedded in paraffin, and cut into 4-μm sections for autoradiography. The sections were mounted on a slide, deparaffinized, and dipped in nuclear emulsion.† After drying, the sections were stored in light-tight boxes at 4 °C for 4–6 wk, and were developed and stained with hematoxylin and eosin. The "labeling index" (i.e., percent labeled cells per total cells counted) was determined by counting 1000 cells if possible, and the percentage of labeled cells was computed. We chose "labeling index" because, in qualitative terms, this parameter should be roughly related to percent dose per gram as measured by liquid-scintillation counting. Obviously, labeling-index measurement is only a semiquantitative technique, since cells are recorded as positive or negative without regard to the degree of labeling. Nonetheless, in the same animal a low or high autoradiographic labeling index should correspond roughly to a low or high percent dose per gram for tumor radiothymidine uptake.

RESULTS

The uptakes of tritiated thymidine by EMT-6 sarcoma (BALB/c mice) and Morris 7777 hepatoma (Buffalo rats) are shown in Fig. 1. The data are expressed as tumor-to-blood ratios and also percent dose per gram of tumor. The uptake patterns are similar for the two tumor types. Tumor-to-blood ratios increase dramati-

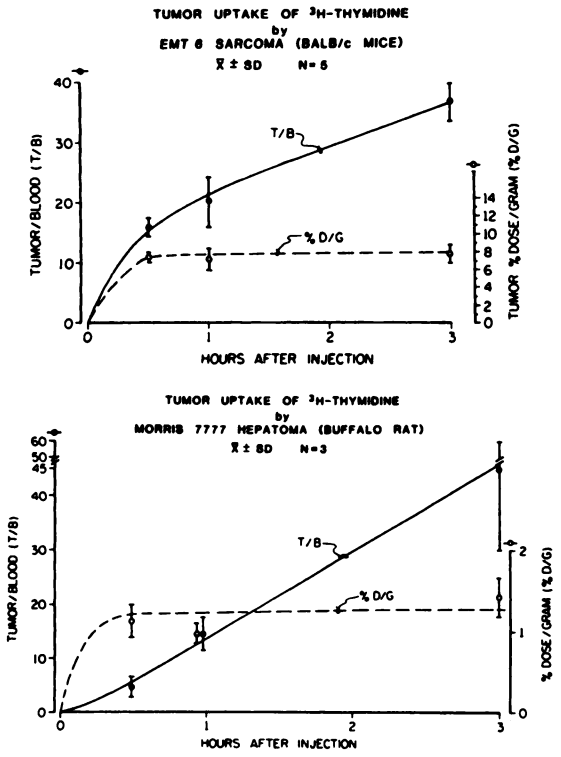

FIG. 1. Time course of uptake of [³H]thymidine by transplanted rodent tumors: percent dose per gram of tumor (dashed line) and tumor-to-blood ratios (solid line). A: EMT-6 sarcoma (BALB/c mice); B: Morris 7777 hepatoma (Buffalo rat). There is progressive increase of tumor-to-blood ratios with time, while the percent dose per gram of tumor reaches a plateau by 0.5 hr after injection.

cally over the 3-h period of observation. The absolute uptake in tumor, as measured in terms of percent dose per gram, reaches a plateau soon after injection, so that by 30 min most of the radioactivity destined for the tumor has already reached that tissue. Over the 3-hr period of observation, the tissue radioactivity is retained by both tumor types. The continued rise in tumor-to-blood ratios reflects decreasing concentration in peripheral blood.

Nine dogs with spontaneous neoplasms were studied, and the results are shown in Table 1. Four lymphomas, two osteosarcomas, one papillary thyroid carcinoma, and two soft-tissue sarcomas were studied. All of these tumor types showed significant concentration of radiothymidine in tumor relative to other tissues. All of the dogs had one or more primary or metastatic lesions, with tumor-to-blood ratios significantly greater than one, except for one dog with an osteosarcoma, primary in the proximal humerus. This was a large tumor, and four different areas within the tumor were sampled. None of these showed significant concentration of thymidine. Histologically, this tumor was relatively hypocellular, with well-differentiated areas consistent with a mixed osteosarcoma/chrondrosarcoma. In contrast, the other dog osteosarcoma in this series contained a portion that was one of the most thymidine-avid tumor specimens in the series, with tumor-to-blood ratios of 11.0 and tumor-to-muscle ratios of 17. Histological examination of this animal's tumor showed that the area of high uptake was highly cellular, with numerous mitotic figures.

Within each dog there was marked variation in the concentration gradients between tumor and other tissues. Tumor-to-blood ratios varied by as much as seven fold (e.g., T-945). Some of this variability could be explained from the histological appearance of the tumors. For example, the inguinal metastasis Dog T-927 had low uptake of thymidine associated with extensive necrosis. However, the individual lymph nodes of the dogs with lymphoma were markedly similar histologically, yet significant variability in uptake was noted. Two of these dogs were treated before the time of study, but had relapsed and were in relatively poor condition when studied (T-829, T-856). It is possible that the prior treatment affected the degree of uptake observed. Other dogs (T-942, T-970) were untreated, and in these dogs some underlying biologic process must account for these differences. Nonetheless, for all tumor types studied there were areas of tumor that concentrated the tritiated thymidine markedly by one hour after injection, and these tumor sites were sufficiently active to have been visualized by PET imaging.

The variability within tumors was confirmed by autoradiography. In a dog with lymphoma (T-856 in Table 1), it was possible to compare multiple tumor nodules within the liver. This dog was treated, so the variability in labeling index may have been related in part to therapy. Twenty-four individually distinct microscopic foci were assessed; all of these consisted of at least 100 cells, and many contained more than 1,000 cells. All radioactivity appeared localized over the nuclei. The labeling index varied from 1 to 13%, with a median of 5.0%. No obvious histologic differences were observed among the tumor foci in the liver to explain such a wide range of labeling indices (Fig. 2). Histologic sections suggested viability of the cells in terms of intact nuclei and cell contours, with markedly similar appearance between regions, while the assessment of thymidine uptake indicated marked variation in biochemical activity of various regions within the tumor.

When the means of the tumor uptakes (percent dose per gram) were normalized for body weight by multiplying by the weight of the animals, the tumor uptakes varied over a similar range. The highest normalized uptakes were actually observed in some of the spontaneous dog neoplasms, although (as expected) the rapidly proliferating transplanted rodent tumors ranked towards the upper end of the range of values. The highest uptakes were seen in untreated tumors, but the overall variability in uptakes made it difficult to draw definite conclusions regarding the effect of treatment. Also, thymidine incorporation seemed to be roughly comparable in primary and metastatic lesions (Fig. 3).

TABLE 1. UPTAKE OF [^{3}H]THYMIDINE BY SPONTANEOUS CANINE NEOPLASMS

Dog no. (Weight)	Diagnosis	Rx	Tumor site	% dose/kg	Tumor-to-tissue ratios* T/Blood	T/Muscle	T/Duodenum	T/Marrow
T-829 (5 kg)	Lymphoma	Φ	*Nodes:* prescapular, submandibular, mesenteric, popliteal, inguinal, mediastinal	17.2 (14.7–23.5)	3.4 (2.9–4.7)	6.9 (5.9–9.4)	1.7 (1.4–2.3)	0.7 (0.6–1.0)
			Organs: spleen, lung	10.5 (10.1–11.1)	2.1 (2.0–2.2)	4.2 (4.0–4.4)	1.0 (1.0–1.1)	0.4 (0.4–0.4)
T-942 (29 kg)	Lymphoma	†	*Nodes:* cervical, prescapular, axillary, celiac node	3.8 (3.0–4.4)	3.2 (2.5–3.7)	4.4 (3.5–5.1)	1.4 (1.1–1.6)	0.4 (0.3–0.4)
			Organs: lung, spleen, liver	6.9 (3.5–11.9)	5.8 (2.9–9.9)	8.0 (4.1–13.8)	2.6 (1.3–4.4)	0.7 (0.4–1.2)
T-856 (24 kg)	Lymphoma	Φ	*Nodes:* popliteal, prescapular, axillary	4.0 (1.8–6.0)	4.0 (1.8–6.0)	10.0 (4.5–15.0)	1.1 (0.5–1.7)	— —
			Organs: liver, spleen	3.7 (3.4–4.1)	3.7 (3.4–4.1)	9.2 (8.5–10.3)	1.1 (1.0–1.2)	— —
T-970 (7 kg)	Lymphoma	†	*Primary:* large mediastinal mass. 12 sites within tumor	11.1 (5.2–14.4)	3.8 (1.8–4.9)	7.9 (3.7–10.2)	0.8 (0.4–1.1)	0.4 (0.2–0.5)
T-940 (80 kg)	Osteosarcoma	†	*Primary:* 4 sites within tumor (proximal humerus)	0.7 (0.5–0.9)	0.9 (0.7–1.2)	1.4 (1.0–1.8)	0.3 (0.2–0.4)	0.2 (0.1–0.3)
			Metastases: lung (microscopic)	0.8	1.1	1.6	0.4	0.3
T-945 (75 kg)	Osteosarcoma	†	*Primary:* 3 sites within tumor (distal radius)	7.2 (1.6–10.0)	7.7 (1.7–11.0)	12.2 (2.7–16.9)	1.4 (0.3–2.0)	2.0 (0.4–2.8)
			Metastases: none					
T-927 (26.2 kg)	Undiff. soft-tissue sarcoma	§	*Primary:* popliteal fossa			removed		
			Metastases: inguinal soft tissue, liver, kidney	3.9 (1.0–8.8)	2.2 (0.6–4.9)	5.6 (1.4–12.6)	1.2 (0.3–2.8)	0.2 (.06–0.5)
T-968 (32.5 kg)	Fibrosarcoma	§	*Primary:* mouth, 8 sites within tumor	1.1 (0.9–1.3)	1.0 (0.8–1.2)	0.8 (0.6–0.9)	0.3 (0.2–0.3)	0.3 (0.2–0.3)
			Metastases: cervical lymph node	3.5 (3.3–3.7)	3.2 (3.0–3.4)	2.5 (2.4–3.4)	0.8 (0.7–0.8)	1.1 (1.0–1.2)
T-333 (19.5 kg)	Papillary thyroid cancer	†	*Primary:* 4 sites within neck mass	9.7 (8.4–10.9)	4.4 (3.8–5.0)	8.8 (7.6–9.9)	2.7 (2.3–3.0)	1.5 (1.3–1.7)
			Metastasis: lung nodule	6.0	2.7	5.5	1.7	0.9

* Means (ranges); T = tumor.
† No treatment.
Φ Total body irradiation; combination chemotherapy.
§ Amputation, prednisolone.

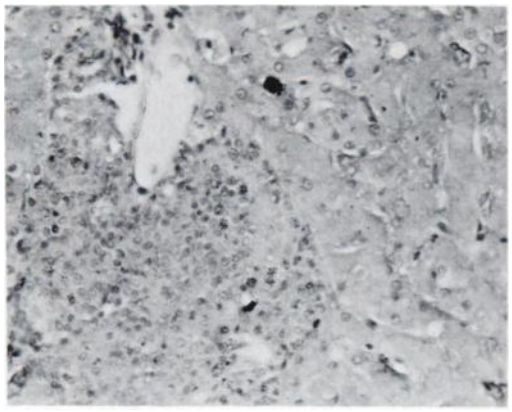

FIG. 2. Microautoradiograph of tear-shaped tumor nodule surrounded by normal liver parenchyma in dog with diffuse canine lymphoma. [^{3}H]thymidine was injected intravenously; tissue sample obtained at 1 hr. Dark spots of exposed silver emulsion can be seen over nuclei of tumor cells, but not normal hepatocytes. A labeling index was obtained by counting cells containing nuclei with five or more silver grains and dividing by total number of cells in section.

The metabolism of thymidine has been studied extensively in normal tissues and in tumors (*15,16*) and it is widely accepted that, in rapidly proliferating tissues, the vast majority of radiolabeled thymidine is rapidly incorporated into DNA by a series of phosphorylation reactions. Retention of radioactivity is prolonged in these tissues. In other tissue types, such as liver, catabolic processes predominate and thymidine is broken down to β-amino isobutyric acid, and ultimately to CO_2 and water, with resultant rapid clearance from the tissue. Within 1 hr, virtually all the radiothymidine taken up by untreated tumors is incorporated into DNA (*17*).

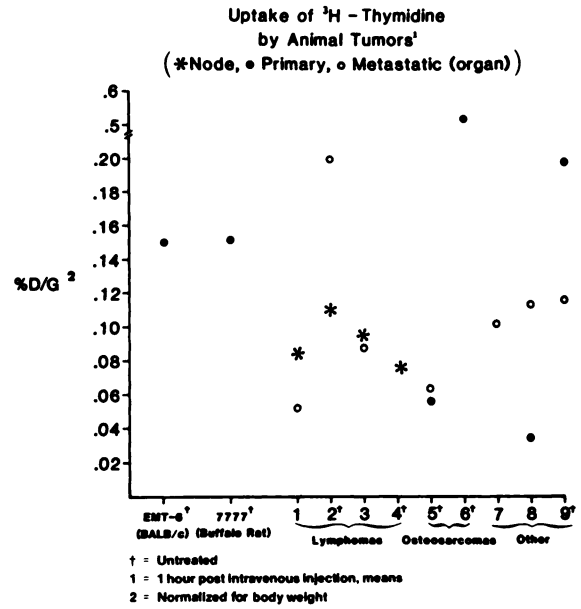

FIG. 3. [^{3}H]thymidine uptake by animal neoplasms. Data have been normalized by multiplying percent dose per gram by kilogram body weight. Computed from mean values for tumor uptake in Table 1, and 1-hr values in Figs. 1A and 1B.

Since the uptake of tritiated thymidine by rapidly proliferating tissue is thought to reflect DNA synthesis, differences in tritiated thymidine concentration between tissues or tumor sites should reflect the relative degree of DNA synthesis by these tissues. In a previous publication (*3*) we discussed the relative amount of incorporation of thymidine into the DNA of various organs and of a tumor in the 6-wk-old mouse. Based on the 1-hr incorporation of thymidine, the relative radioactivity in tissues was: spleen (hematopoietic portion) > small intestine > tumor > liver > kidney > heart muscle > skeletal muscle. In the rat, at 1 hr after intravenous injection, the radiothymidine incorporation into tissues was very similar, the radioactivity levels ranking as: spleen > small intestine > liver > kidney > lung > heart muscle > skeletal muscle > brain. In the dog, this rank order was fairly well maintained, except that the renal cortex was frequently a very active tissue. Approximate ranking for dog tissues was as follows: red marrow $\simeq$ renal cortex $\simeq$ most active tumor $\geq$ duodenum > spleen > liver > normal lung > pancreas > heart muscle > skeletal muscle.

DISCUSSION

Accelerated synthesis of DNA, through rapid incorporation of nucleotides such as thymidine, is one of the hallmarks of the biochemistry of malignant tumors (*16,18*). Furthermore, the rate of incorporation of thymidine corresponds closely to the rate of growth of a tumor, with the more rapidly growing tumors having the greatest rate of thymidine incorporation and DNA synthesis (*19*).

This close coupling between DNA synthesis and growth probably underlies the effectiveness of antitumor agents that inhibit DNA synthesis in human tumors. It seems reasonable, therefore, that PET imaging of tumors based on thymidine assimilation could provide a way to monitor the growth state of individual neoplasms and the response of individual tumors to treatment.

For the rodent tumors that we studied, there was retention of the tritiated thymidine in the tumor once uptake had occurred. The uptake was rapid, so that the plateau uptake had already been reached at 30 min after intravenous injection. Thereafter, tumor-to-blood ratios continued to increase, owing almost entirely to progressive blood clearance. Moreover, in both dog and rodent tumors the absolute uptake of thymidine was relatively high, so that tumor-to-blood ratios above 10 were observed in the rodent tumors, and above 5, in general, in the dog tumors. Uptake of tritiated thymidine by rapidly proliferating normal tissue, duodenum (small intestine), and bone marrow, served as useful compa-

rators. In general, tumor uptake was usually less than that for bone marrow, and equaled or slightly exceeded that for small intestine.

An important finding was that tumor uptake varied considerably from site to site within an individual tumor-bearing animal. In lymphoma the involved lymph nodes and tumor specimens varied by a factor of 2, whereas in osteosarcoma this variation was almost tenfold. We emphasize that the variability was observed in specimens shown histologically to be malignant. There was a general correspondence between the degree of necrosis and reduced uptake. Thus, the most viable areas of tumor tended to be those with the highest uptake. However, particularly in dogs with lymphoma, the nodes appeared histologically identical, yet they varied over a factor of 2 in absolute uptake of tritiated thymidine. Whether this represented a difference in metabolism or a difference in some other tumor function, such as blood flow, remains to be determined.

This study demonstrates that both the ratios of tumor to normal tissue and the kinetics of thymidine uptake are suitable for applying C-11-labeled thymidine tracer to the imaging of tumors by positron emission tomography. We have found this to be true in a variety of transplanted and spontaneous neoplasms in small and larger animals.

ACKNOWLEDGMENTS

This work was supported in part by the Medical Research Service of the Veterans Administration and by Grant No. CA18105 awarded by the National Cancer Institute, DHHS, and an institutional grant awarded by the University of Washington for support of positron emission tomography.

FOOTNOTES

* Tri-Carb sample oxidizer, Model #306, Packard Instrument Company.

† Kodak NTB-2.

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