INVESTIGATIVE NUCLEAR MEDICINE

Short-Term Thymidine Uptake in Normal and Neoplastic Tissues: Studies for Pet

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Uptake of H-3 thymidine was studied in mice, both normal and with spontaneous lymphoma, and in the organs and tumors of dogs with spontaneous tumors. Uptake was compared with relative blood flow as measured by the distribution of C-14 iodoantipyrine. Initial distribution of thymidine in normal mice measured 20 sec after injection, correlated with the relative perfusion measurements; however, all measurements of thymidine uptake made between 1 and 60 min after injection showed no correlation with perfusion. This indicates that the distribution more than 1 min after injection is primarily dependent on subsequent redistribution and/or metabolism of thymidine. A time-course study demonstrated that normal mouse organs with high rates of proliferation retained all the labeled thymidine initially taken up. Organs with low rates of proliferation lost their label in a nearly exponential washout. These studies provide further evidence of the feasibility of using C-11 thymidine for positron emission tomography (PET).

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Positron emission tomography (PET) offers the opportunity to study noninvasively the in vivo metabolism of tissues. We are interested in applying this technique to the measurement of tumor metabolism. One potential imaging agent is C-11 thymidine (*1*). Thymidine is taken up primarily by replicating cells and used in DNA synthesis (*2*). The relative rate of uptake of thymidine correlates with tumor growth rate, as demonstrated in a series of Morris rat hepatomas (*3*). Given the short half-life of C-11 (20 min), the uptake of C-11 thymidine by tumors must be rapid to allow for imaging within about 5 half-lives. In previous studies we have shown that thymidine is taken up fast enough to make imaging feasible by 30 min after injection, and that the label is retained in tissue for at least 3 hr (*4*). Furthermore, in mice, rats, dogs, and humans with tumors, the relative thymidine uptake of tumors compared with normal tissues was high enough to allow for detection of lesions and quantification of uptake.

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When given intravenously, most thymidine is cleared from the circulation very rapidly in humans, rats, and mice (*5–7*). This had led to concern that the distribution of thymidine in vivo might predominantly reflect blood flow rather than cellular metabolism. We have examined this question directly by measuring both thymidine distribution and relative blood flow in mice and dogs. This comparison was done both in several normal tissues and in spontaneous tumors of both species.

MATERIALS AND METHODS

A colony of inbred AKR mice from commercial breeders* is maintained at our research center. For the tumor studies, mice were held until signs of spontaneous tumors developed (ruffled fur, humped back), generally 6 to 14 mo. For the control time-course experiments, AKR mice 5 to 8 wk of age were used. Mice received 2 μCi of [H-3 methyl]thymidine (sp. act. = 60–77 Ci/m*M*) by tail vein. We initially assessed the reproducibility of our measurements in mice with metastatic lymphoma, by comparing the 1-hr uptake, by extraabdominal lymph nodes, of i.v. [H-3]thymidine with that

of [C-14 methyl]thymidine (sp. act. 53.4 Ci/m*M*) given by intraperitoneal injection. The techniques were found to give comparable results (r = .98, p = 0.0001, data not shown). For the initial time-course experiments, animals were killed 1, 3, 5, 10, 15, 30, and 60 min later by cervical dislocation and decapitation, and were promptly dissected. In the other experiments, unless stated otherwise, mice were killed 60 min after thymidine injection. In experiments measuring blood flow, the mice received 1 to 2.5 μCi of C-14 iodoantipyrine (IAP) (sp. act. = 49.8 mCi/m*M*) by tail vein at 15 to 20 sec before death (*8*). At autopsy we removed and weighed lymph nodes in tumor-bearing mice, also brain, thymus, lungs, heart, liver, spleen, kidneys, and duodenum in all mice. The tissues were then airdried, oxidized with a sample oxidizer, and counted in a liquid scintillation spectrometer.

Dogs with spontaneous tumors, no longer amenable to further treatment, were referred by veterinarians to our center, with the permission of the owner. Further details regarding the dog referral program are described elsewhere (*9*). While awake the animals received 500 μCi of H-3 thymidine i.v. 60 min before sacrifice. In blood-flow studies, during the last minute before sacrifice the animals received an increasing infusion of C-14 IAP in three steps, with a total of 50 to 100 μCi. The measurement of blood flow by IAP distribution has been shown to correlate closely with microsphere measurements in the dog kidney (*10*). At 55 sec into the IAP infusion, the dog was given a bolus of saturated KCl and pentobarbital. Cardiac arrest occurred in 10 to 20 sec. At autopsy multiple samples of the primary and metastatic tumors were removed and weighed. Samples of lung, heart, renal cortex and medulla, spleen, liver, small intestine, colon, pancreas, bladder, bone and muscle were also taken. Samples were processed for liquid scintillation counting as before.

All scintillation counts were corrected for quenching by the external standard method. Absolute blood flow was not calculated, but relative blood flow was obtained by dividing tissue IAP activity by the wet weight of the sample and by the total injected dose. Normalized thymidine uptake was calculated in the same way. Thus all data are expressed as percent injected dose per gram of tissue. Linear correlation analysis and step-wise multiple regression analysis were done on the PROPHET statistical package sponsored by the National Institutes of Health. To take into account the organ specificity of label uptake, "dummy" variables were used to represent the different organs in multiple regression analysis.

RESULTS

In initial experiments in normal AKR mice, the uptake of H-3 thymidine was compared with the distribution of blood-flow tracer, C-14 iodoantipyrine (IAP),

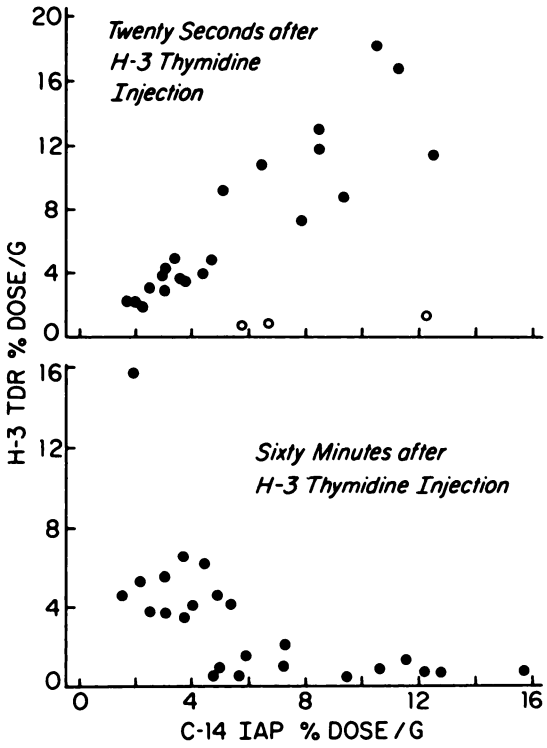

FIG. 1. Relationship between H-3 thymidine uptake and blood flow in organs of normal AKR mice. Blood-flow tracer C-14 iodoantipyrine was given by tail vein 20 sec before sacrifice. H-3 thymidine was given 20 sec or 60 min before sacrifice, as indicated. Data are from three mice at each time point, with each data point representing one organ (see Table 1 for list of organs). Samples of brain tissue taken at 20 sec are indicated (O).

after simultaneous i.v. injection and sacrifice 20 sec later. Tissue samples were dried to remove tritiated water, before oxidation and scintillation spectrometry. This experiment demonstrated a significant correlation between thymidine uptake and relative perfusion (r = 0.64, p = 0.001 with brain tissue included, and r = 0.90 without brain) (Fig. 1). The brain tissue took up significantly less thymidine ($p < 0.005$) than expected from its relative perfusion (based on a comparison of slopes by multiple regression analysis). This is consistent with previous studies in rats demonstrating that thymidine crosses the blood-brain barrier poorly (*11*).

In other experiments we studied the time course of thymidine uptake. A summary of these uptake results at the 1-, 5-, and 60-min time points is given in Table 1. Although thymidine uptake correlated with relative perfusion 20 sec after injection, by 1 min later this correlation was lost (r = 0.06). At 1 hr after thymidine injection there was an inverse correlation between thymidine uptake and the relative blood-flow measurement (r = −0.60, p = 0.002) (Fig. 1). Although there is an apparent inverse correlation, multiple regression analysis showed that this correlation could be accounted for by the characteristics of the organs. As expected, brain, lung, and heart had a high blood flow but low thymidine

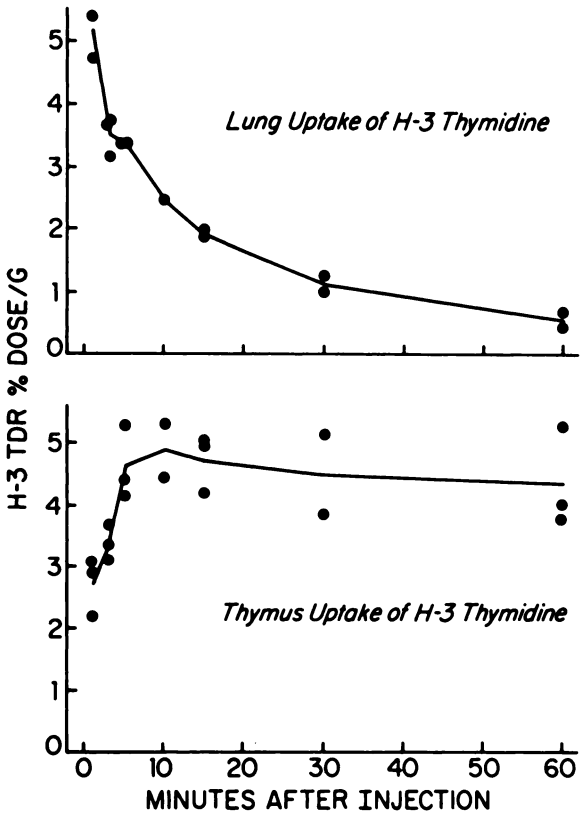

FIG. 2. Time course of H-3 thymidine uptake in normal thymus and lung. Each data point represents organ from one animal. Line connects means.

uptake. Spleen and duodenum had a relatively low blood flow but high thymidine uptake. Blood flow as measured by C-14 IAP generally gave results consistent with published data for mice and rats using highly extracted tracers such as rubidium and microspheres (*12*, *13*)—except for the kidney, which had less C-14 IAP uptake than expected (data not shown). Under these circumstances, the relationship between actual blood flow and IAP distribution becomes nonlinear at high flow rates because of washout (*14*). The time to sacrifice was chosen to minimize washout, but cannot eliminate the problem, thus the relatively low uptake in the kidneys. Our results demonstrate that thymidine uptake between 1 and 60 min after injection is not distributed proportional to relative blood flow.

The time course of thymidine uptake by each organ was analyzed to examine the kinetics of metabolism. Graphs of thymidine uptake against time for two representative organs (lung and thymus) demonstrated different patterns of metabolism (Fig. 2). The lung, which has a low growth rate and high blood flow, had its greatest level of thymidine uptake at the first time point (1 min after injection), then rapidly lost activity in a nearly exponential washout. A similar pattern of washout was seen in kidney, heart, liver, and brain (Table 1). On the other hand, the thymus has a high growth rate in these young animals but relatively low blood flow; it reached its maximum H-3 level at 5 min and then had no measurable loss of label (Fig. 2). The other organs with high growth rates (spleen and duodenum) show a pattern similar to that of the thymus in that there was insigificant loss of label over the hour (Table 1). The retention of the H-3 in the rapidly growing tissues is presumably the result of phosphorylation of H-3 thymidine and subsequent use in DNA synthesis (*2*). In other tissues, such as the lung, thymidine is rapidly cleared from the organ, but it is not known whether this reflects redistribution of the intact molecule or of its degradation products.

Metastatic tumors in a given individual might show less variability in relative blood flow and thymidine uptake than do normal organs, with their widely varying rates of blood flow and tissue proliferation. In the metastatic situation thymidine uptake might correlate more strongly with blood flow because the tissue samples are more homogeneous. We tested this hypothesis in mice with spontaneous, metastatic AKR lymphoma. The use of animals with multiple lesions allowed us to discount confounding variables between animals, such as differences in the serum levels of thymidine, general physical condition, and age and size of the animal. Two

TABLE 1. UPTAKE OF H-3 THYMIDINE BY MOUSE ORGANS

Organ*	Time after injection (min)		
	1	5	60
Lung	5.18 (5.41–4.74)	3.40 (3.41–3.38)	0.62 (0.72–0.45)
Kidney	8.51 (8.87–8.33)	7.13 (7.38–6.78)	1.60 (2.02–1.28)
Heart	5.34 (5.93–4.78)	3.37 (3.57–3.21)	0.84 (0.86–0.81)
Brain	0.86 (0.90–0.84)	1.28 (1.30–1.25)	0.51 (0.61–0.45)
Liver	9.44 (10.27–8.92)	12.86 (13.58–11.78)	4.05 (4.53–3.46)
Thymus	2.72 (3.08–2.18)	4.62 (5.29–4.16)	4.37 (5.30–3.80)
Duodenum	5.67 (6.02–5.11)	7.52 (8.21–6.62)	6.09 (6.53–5.54)
Spleen	4.86 (5.53–4.21)	5.69 (6.81–4.69)	7.99 (15.70–3.72)

* % dose/g as mean (range), n = 3.

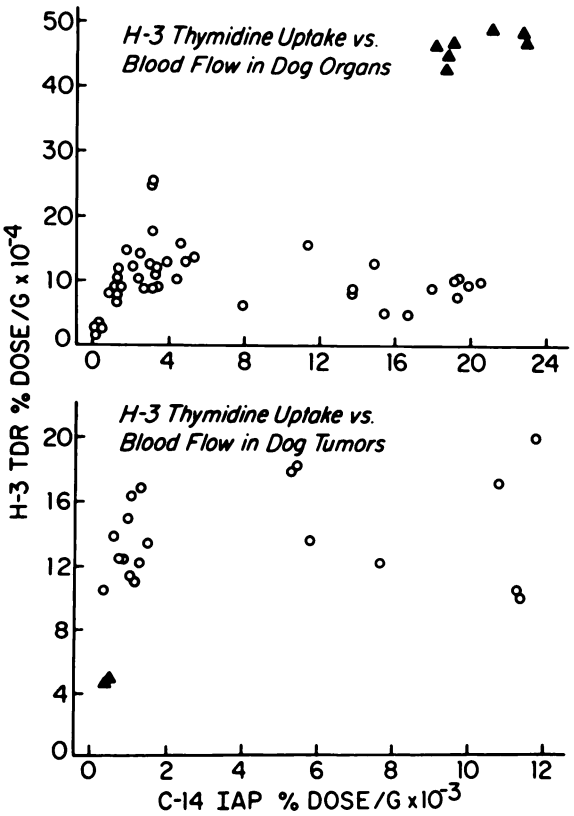

FIG. 3. Relationship between H-3 thymidine uptake and blood flow in normal organs and tumors of dog with metastatic osteosarcoma. Blood flow is measured by uptake of C-14 IAP. Each data point represents one tissue sample. Data points shown as ▲ represent renal cortex in normal organs (above) and in central tumor (below).

mice were given H-3 thymidine and killed 60 min later. Just before sacrifice the animals received C-14 IAP, and tissues were processed as usual. There was no significant correlation between blood flow and thymidine uptake in either of the animals studied ($r = 0.20$, $p = 0.46$ for mouse A; $r = 0.41$, $p = 0.12$ for mouse B). There was significant variability in the thymidine uptake from tumor to tumor, which did not correlate with tumor size or location in the animal.

To verify that the results in mice are applicable in other animals, we studied dogs with osteosarcoma metastatic to the lung, and with metastatic breast cancer. The animals received H-3 thymidine i.v., and 60 min later were killed. Blood flow was measured by an increasing infusion of C-14 IAP to minimize the effect of washout, a problem evident in the mouse kidney. This technique was effective, as shown by the relatively high IAP uptake seen in the dog's renal cortex (Fig. 3). The comparison of tumor blood flow and thymidine uptake in the metastatic and primary lesions from the dogs showed no significant correlation ($r = 0.32$, $p = 0.15$ for the dog with osteosarcoma; $r = 0.23$, $p = 0.38$ for the dog with breast carcinoma) (Fig. 3). As expected, the samples taken from the center of the large primary lesions demonstrated the lowest blood flow and thymidine uptake.

Analysis of C-14 IAP uptake relative to H-3 thymidine uptake in normal organs demonstrated that the samples taken from the renal cortex form a distinct group (Fig. 3). The uptake of H-3 and C-14 into normal organs was analyzed by multiple, step-wise regression analysis. The dependent variable was H-3 content, and the independent variables were C-14 content and type of tissue, whether from renal cortex or elsewhere. In the dog with osteosarcoma this analysis found a strong correlation between H-3 content and renal cortical tissue ($r = 0.93$, $p = 0.0001$). Once the contribution of the renal cortex was removed by multiple regression analysis, there was no significant contribution from relative blood flow to thymidine uptake ($r = 0.029$, $p = 0.84$). Similar results were found in the dog with breast carcinoma ($r = 0.89$, $p = 0.0001$ for renal cortex; $r = 0.06$, $p = 0.73$ without renal cortex contribution). The renal medulla had a relatively lower H-3 uptake. We have previously noted high renocortical uptake of H-3 thymidine in dogs, but not in mice or rats (*4*). Subsequent experiments demonstrated that most of the label taken up by the renal cortex was in small molecules, as determined by extractions (unpublished results). This indicates that the renal cortex is unique in the handling of the tritium tracer, although it may not be in the form of H-3 thymidine.

DISCUSSION

The relative rate of uptake of thymidine into transplantable rat hepatomas correlates with their growth rate (*3*), but this has not been clearly demonstrated in other tumors in vivo. However, in lung cancer the aggressiveness of the tumor correlated with the level of thymidine kinase in the tumor (*15*). The variability in tumor thymidine uptake that we observed in a previous study (*4*) suggests that this uptake might reflect biologic differences from tumor site to tumor site, which might be important to tumor responsiveness to therapy.

It is this background that has suggested the value of C-11 thymidine as an imaging agent for PET. In previous studies we have shown that thymidine is taken up with enough speed and specificity by tumors in mice, rats, and dogs to make imaging and quantification by PET feasible. In order for thymidine to be useful in imaging studies, however, its uptake by tumors in vivo must be separable from blood flow and must reflect the growth rate of the tumor cells.

Thymidine is primarily degraded through thymine, with the main end products being H_2O and CO_2. Our studies used [H-3 methyl]thymidine, which should have resulted in the degradation of most of the label to tritiated water. This ^3H_2O was not measured in these ex-

periments because we dried the tissue before oxidation and scintillation counting. Therefore, results we obtained should be equivalent to those that would be obtained in vivo with PET using [C-11 methyl]thymidine, since most of the degraded product would be rapidly cleared from the body as $^{11}CO_2$(2). In both situations, therefore, the ultimate labeled degradation product, either CO_2 or H_2O, is not measured. Aside from the H-3 in water, all label contained in DNA, as well as other large and small molecules, was considered together. This will not interfere with our conclusions regarding the influence of relative blood flow on the distribution of thymidine. Further experiments to measure the proportion of label in DNA will be needed to determine the accuracy of using thymidine uptake to measure tissue growth.

Previous studies have demonstrated that thymidine is cleared from the circulation very rapidly in humans, mice, and rats (5–7). This suggests that its distribution might be highly dependent on blood flow and therefore of little use in the metabolic imaging of DNA synthesis. We have tested this directly by comparing H-3 thymidine uptake to C-14 IAP distribution in mice and dogs. Although IAP, like all blood-flow tracers, has its practical and theoretical limitations, we think that it provides reasonable, qualitative data under conditions of use. This is corroborated by the analysis of the initial distribution of thymidine and IAP, which showed a strong perfusion dependence for thymidine. By 1 min later, however, and at other time points up to 1 hr, there was no positive correlation between thymidine uptake and relative blood flow. During this time, extensive metabolism and/or redistribution has overcome the initial blood-flow dependence. We are therefore encouraged to pursue the modeling of C-11 thymidine metabolism for the measurement of DNA synthesis.

The time-course experiments demonstrated that thymidine is handled differently in rapidly and slowly growing tissues. The rapidly growing organs in the mouse retained essentially all the label they took up. This indicated that the degradation pathway is relatively inactive compared with the DNA synthetic pathway in rapidly growing cells. If this proves to be true in tumors in vivo, it would simplify the modeling of the thymidine kinetics needed to interpret images with PET. The washout of H-3 thymidine by slowly growing tissues appears nearly exponential. In this situation, where there is no incorporation into DNA, the data support the model of thymidine metabolism proposed by Quastler and Cleaver, who assume that thymidine catabolism takes place by a first-order reaction (*2*).

This study demonstrates that there is little influence of normal blood flow on the ultimate distribution of labeled thymidine. Thymidine must of necessity get to the tissues through the circulation, as reflected by the initial distribution pattern. However, the activity of the cellular thymidine metabolic pathways appears to be relatively more important than blood flow. This is a necessary condition for thymidine to be useful as an imaging agent with PET. Subsequent studies will be directed at determining the fate of labeled thymidine in vivo in order to construct the kinetic models needed to interpret images with PET.

FOOTNOTE

* Jackson Laboratories.

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