Positron Imaging Feasibility Studies. II: Characteristics of 2-Deoxyglucose Uptake in Rodent and Canine Neoplasms: Concise Communication

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Uptake of [^{3}H]2-deoxyglucose was studied in BALB/c mice with EMT-6 sarcoma, in Buffalo rats with Morris 7777 hepatoma, and in eight dogs with spontaneous neoplasms: five osteosarcomas and three diffuse lymphomas. High tumor-to-tissue ratios were observed for all tumor types studied. In rodents, peak levels of uptake occurred between 30 min and 1 hr, with a slow loss from the tumor of about 10% per hour thereafter. In dogs there was considerable variability in uptake, both between individuals and at different tumor sites within an individual. Necrotic tumor did not take up the radiotracer. Absolute uptakes, when normalized for body weight, were similar for spontaneous and transplanted neoplasms.

These studies provide additional support for the concept that positron emission tomography can be used to obtain functional images of important metabolic processes of tumors, including glycolysis.

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Positron emission tomography (PET) is a new imaging technique with the potential for noninvasive measurement of tissue metabolism in living patients. We are interested in the possible application of PET to oncology.

Som et al. at Brookhaven (*1*,*2*) studied several mouse tumors and a spontaneous seminoma in a dog and found active tumor uptake of 2-[^{18}F]fluoro-2-deoxyglucose, a tracer widely used for PET imaging studies of tissue glycolysis. In a preliminary communication (*3*), we extended these studies to the EMT-6 sarcoma of BALB/c mice and observed that the uptake of 2-deoxyglucose at one hour after intravenous injection was as high as the corresponding maximum Ga-67 citrate uptakes at 48 hr.

Because of the importance of obtaining broader experience with the time course of 2-deoxyglucose distribution, we performed further studies to assess the kinetics of uptake into two rodent tumors, EMT-6 sarcoma (BALB/c mice) and Morris 7777 hepatoma (Buffalo rat). Also, in order to determine tumor uptake of 2-deoxyglucose in comparison with metabolically active tissues such as brain and heart, in larger animals with spontaneous tumors, we studied outbred dogs (pets) with osteosarcomas or diffuse lymphomas.

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MATERIALS AND METHODS

Details of the biodistribution protocols and characteristics of the animals used are presented in detail in a companion article (*4*). Briefly, BALB/c mice bearing solid EMT-6 sarcoma tumors that ranged in weight between 50 and 200 mg received intravenous injection of 20 μCi (0.74 MBq) of 2-deoxy-D-glucose, 2-[G-^{3}H] (sp ac = 8.3 mCi/millimol = 307 MBq/mMol). Immediately before sacrifice, 100 μl of blood was removed from the retro-orbital plexus. Animals were killed by cervical dislocation at 0.5, 1, and 3 hr after administration of the radiotracer. Five animals were studied per group. Radioactivity content was assayed in the blood, liver, spleen, kidney, muscle, small intestine, brain, and

tumor, after the radioactive tissues were oxidized using an automatic sample oxidizer.* Samples were then counted in a liquid-scintillation counter.

Eight-week-old Buffalo rats (180 g) bearing Morris 7777 hepatomas of about 2 g were also randomly allocated to study groups of three animals each, and received 30 μCi (1.1 MBq) of 2DG(H-3) intravenously via tail vein. Immediately before sacrifice (at 0.5, 1, and 3 hr), the animals were anesthetized with ether, and 100 μl of blood was removed from the retro-orbital plexus. The animals were then placed in a bell jar containing dry ice as a source of CO_2, and allowed to die. Tissues were removed and assayed for radioactivity content as described above.

Dogs with spontaneous osteosarcomas and lymphomas were studied, with permission of the owners. Animals received 1-2 mCi of 2DG(H-3) intravenously, one hour before being killed with intravenous sodium pentobarbital. Tissues obtained at autopsy included brain, heart, tumor, kidney, lung, small intestine, liver, spleen, and muscle. Multiple samples of primary and metastatic lesions were taken and divided into two parts: one for histologic examination and the other for liquid-scintillation counting. Normal tissue and tumor samples were processed for liquid-scintillation counting and assayed as described above. Data were expressed as percent injected dose per kilogram of tissue, and tumor-to-tissue ratios were computed.

RESULTS

Tumor uptake of [^{3}H]2-deoxyglucose by EMT-6 sarcoma of BALB/c mice is shown in Fig. 1 (left). Data are presented as mean ± standard deviation for percent dose per gram, and as tumor-to-blood ratios. There was rapid uptake of the 2DG(H-3), with highest uptakes observed at the earliest sampling times. There was a trend towards loss of the radiotracer from the tumors at later times, although the differences were not statistically significant. In any event, most of the radioactivity (>90%) was retained even at 3 hr after intravenous injection. Tumor-to-blood ratios rapidly increased over the first hour, and leveled off thereafter.

Tumor uptake of [^{3}H]2-deoxyglucose by Morris 7777 hepatoma is shown in Fig. 1 (right). In this tumor model there was also rapid uptake into the tumor, so that high levels were observed at one-half hour after injection. Here again, there was a trend toward slight loss of radioactivity with time, but by 3 hr, over 90% of peak levels were still retained in the tumor. The tumor-to-blood ratios increased over the 1.5 hr, and seemed to reach a plateau by 2-3 hr after injection of the radiotracer.

In both mice and rats, the percent dose per gram of a variety of other tissues was available for comparison; the most glucose-avid of these were the brain and the heart. For both rodents, tumor uptake was about 50% greater than corresponding brain concentration. Heart uptake was highly variable, ranging from one quarter to three times the tumor concentration. Such variability most likely depends on the nutritional state of the animal, since the heart uses glucose as an energy source predominantly after food intake (5). Neither tumor nor any other tissue showed such marked variation in 2DG(H-3) uptake.

The uptake of [^{3}H]2-deoxyglucose in osteosarcomas and lymphoma of the dog is shown in Table 1. Data are expressed as percent dose/kilogram tumor as an indicator of absolute concentration, and as tumor-to-tissue ratios, to obtain a relative assessment of concentration compared with other tissues. Two glucose-avid tissues, heart muscle and brain, were included for comparison. Blood and skeletal muscle were included as more passive tissues in the resting animal. For the osteosarcoma dogs, tumor-to-blood ratios ranged from 1.2 to 6.3, with tissue-to-muscle ratios from 1.0 to 18.9. There was no clear-cut difference in 2DG uptake between primary and metastatic lesions. Totally necrotic lesions did not take up the radiotracer. The untreated subjects had higher uptakes in terms of tumor-to-blood and tumor-to-muscle

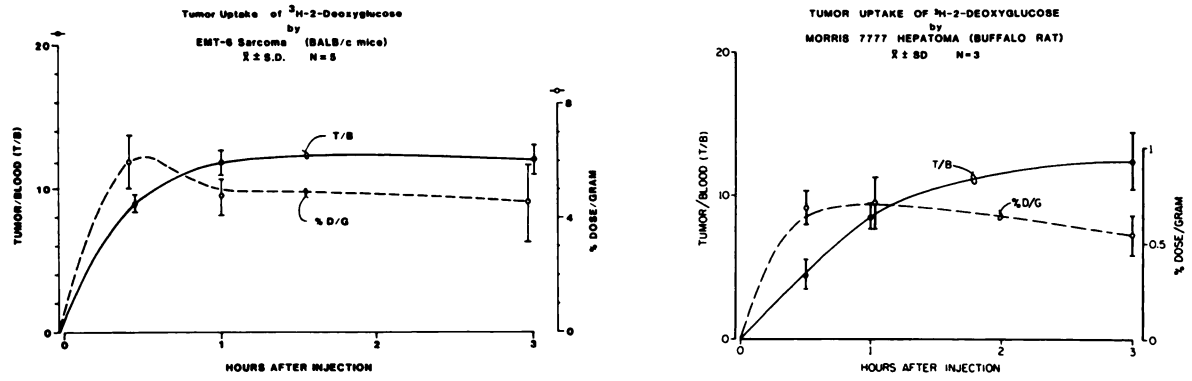

FIG. 1. Uptake of 2-deoxyglucose(H-3) by transplanted rodent tumors: uptake (% dose/g, dashed line) and tumor-to-blood ratios (solid line). Left: EMT-6 sarcoma in BALB/c mice. Uptake reaches early plateau, then declines gradually; T/B levels off at a later time. Right: Morris 7777 hepatoma in Buffalo rat. Uptake reaches early plateau, then declines slowly; T/B rises more slowly than in left figure and levels off at a later time.

ratios. For the more 2DG-avid tumors, tumor concentration exceeded brain concentration by 10–20%. Body weight was not a major factor in determining uptake, and even some of the larger dogs (40–50 kg) had very avid uptake. For the dogs with lymphoma, tumor concentration was even more marked, with tumor-to-blood

TABLE 1. UPTAKE OF [^{3}H]2-DEOXYGLUCOSE BY SPONTANEOUS CANINE NEOPLASMS

Dog no. (weight)	Diagnosis	Rx	Tumor site	% Dose/ kg*	Tumor to tissue ratios*			
					T/Blood	T/Muscle	T/Heart	T/Brain (grey matter)
T-982 (40 kg)	Osteosarcoma	†	*Primary:* humerus. 2 viable sites	12.3 (12.1–12.5)	5.3 (5.2–5.3)	18.6 (18.3–18.9)	6.9 (6.8–7.0)	1.2 (1.1–1.2)
			1 necrotic site	0.05	0.02	0.1	0.03	0.005
			Metastases:					
			lung 50% viable	8.01	3.4	5.8	4.5	0.75
			lung 100% viable	14.75	6.3	22.4	8.2	1.4
T-984 (50 kg)	Osteosarcoma	†	*Primary:* leg, unspecified. 2 viable sites	5.4 (5.2–5.6)	4.4 (4.2–4.6)	5.9 (5.7–6.1)	0.4 (0.4–0.4)	1.0 (0.9–1.1)
			1 necrotic site	1.3	1.1	1.4	0.1	0.24
			Metastases: lung (miliary spread)	3.0	2.4	3.3	0.2	0.5
T-980 (50 kg)	Osteosarcoma	†	*Primary:* humerus (4 sites)	8.5 (3.4–12.4)	3.9 (1.6–5.6)	12.0 (4.8–17.4)	1.7 (0.7–2.5)	0.9 (0.4–1.4)
T-938 (42 kg)	Osteosarcoma	§,△	*Primary:* humerus (2 sites)	3.9 (3.1–4.6)	2.8 (2.2–3.4)	2.3 (1.8–2.8)	0.17 (0.14–0.2)	0.52 (0.42–0.64)
			Metastasis: lung	1.75 (1.6–1.9)	1.2 (1.1–1.3)	1.0 (0.7–1.1)	0.07 (0.05–0.08)	0.23 (0.2–0.26)
T-616 (24 kg)	Osteosarcoma	§	*Primary:* removed 1977	6.2 (3.4–10.0)	1.2 (0.7–2.0)	2.7 (1.5–4.4)	1.5 (0.8–2.4)	0.5 (0.3–0.9)
			Metastases: skin (3 sites); lung (3 sites)					
T-953 (26 kg)	Lymphoma	Φ	*Nodes:* Axillary, cervical, popliteal, and inguinal	7.0 (6.6–7.6)	18.3 (14.0–16.0)	4.4 (3.4–3.9)	3.6 (2.7–3.1)	0.58 (0.44–0.50)
			Organs: liver, spleen	8.7 (6.9–10.5)	18.3 (14.5–22.1)	4.4 (3.5–5.4)	3.6 (2.8–4.3)	0.58 (0.46–0.70)
T-988 (36 kg)	Lymphoma	†	*Nodes:* prescapular, inguinal, popliteal, axillary	12.1 (8.6–15.3)	9.5 (6.8–12.1)	16.0 (11.4–20.2)	2.15 (1.5–2.7)	1.9 (1.3–2.4)
			Organs: spleen, liver	8.0 (4.1–12.1)	6.3 (3.2–9.5)	10.6 (5.4–16.0)	1.4 (0.7–2.2)	1.2 (0.6–1.9)
T-963 (19 kg)	Lymphoma	†	*Nodes:* cervical, prescapular, axillary, popliteal, mediastinal, mesenteric	5.8 (4.6–10.4)	3.6 (2.8–6.4)	4.5 (3.6–8.1)	3.0 (2.4–5.3)	0.5 (0.4–0.9)
			Organs: lung	18.7	11.5	14.5	9.6	1.6

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

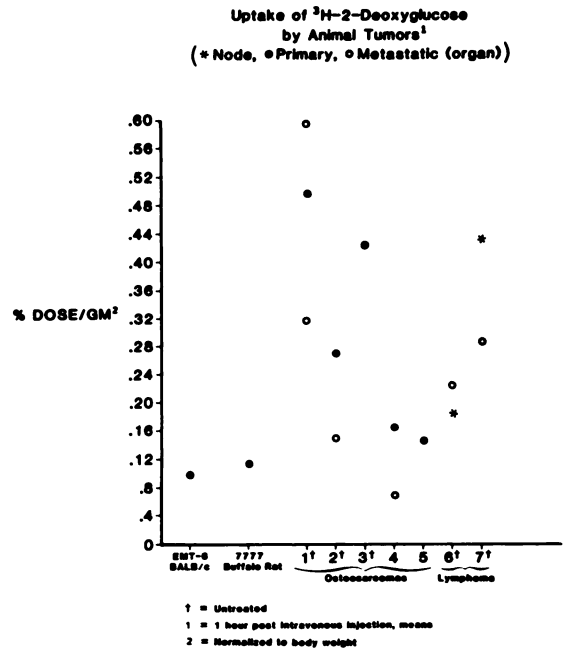

FIG. 2. 2-deoxyglucose(H-3) uptake in animal neoplasms. Data are normalized by multiplying percent dose per gram by kilogram body weight. Mean values for tumor uptake in Table 1, and 1-hr values from Figs. 1, left and 1, right, were used to compute normalized uptakes. For rodent tumors and spontaneous dog tumors, fractional uptake seems to be in the same range. No significant difference is seen between primary and metastatic lesions, but treated lesions tend to have lower uptakes.

ratios for individual tumors ranging from 2.8 to 22.1. Corresponding to these high uptakes were relatively higher average tumor-to-tissue ratios. Within an individual there was considerable variability of uptake from site to site. In most instances this variability was without obvious histologic cause.

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 uptake varied over a similar range. The highest uptakes were observed in dogs with spontaneous tumors, and prior treatment history did not appear to be a factor in determining uptake. Uptake of 2-deoxyglucose was roughly comparable in primary and metastatic lesions (Fig. 2).

DISCUSSION

Accelerated aerobic glycolysis is perhaps the most widely accepted biochemical signature of the malignant state (*6*), and is accompanied by accelerated activities of the key rate-controlling enzymes for glycolysis, including hexokinase, phosphofructokinase, and pyruvate kinase. Furthermore, there is a general correspondence between the rate of growth of the tumor and the magnitude of the increase in glycolysis (*7*). Compounds that inhibit glycolysis have been suggested for use as antitumor agents, and 2-deoxyglucose in particular has been extensively used for this purpose. This compound inhibits the activity of the hexokinase for conversion of glucose to glucose 6-P, and is highly cytotoxic to tumor cells at millimolar concentrations (*8*). At tracer concentrations the uptake of 2-deoxyglucose appears to be a valid measure of ATP formation via glycolysis, at least in the transplanted rat hepatoma (*9*).

A positron-emitting analog of 2-deoxyglucose has been synthesized, 2-[^{18}F]fluoro-2-deoxyglucose (*10*), and this compound has been widely used as a radiotracer for positron emission tomography of the brain (*11,12*) and the heart (*5*). In these glucose-avid tissues, the 2-deoxyglucose derivative is avidly concentrated and retained long enough to permit PET imaging. In these tissues, FDG(F-18) is readily converted to the 2-deoxy-glucose-6-phosphate (2DG-6P), but this is not a substrate for further conversion along the glycolytic chain. Because 2DG-6P does not diffuse readily across the cell membrane, it is "metabolically trapped" in the tissue. This metabolic pattern has been used by several investigators as a basis for mathematical models that calculate the metabolic rates of glucose utilization in brain tissue from tomographic sections obtained non-invasively by PET imaging (*13,14*). An analysis of the time course of uptake of [^{3}H]2-deoxyglucose by tumors demonstrates that there is a rapid uptake phase followed by a plateau in uptake and, still later, an actual decline in tumor concentration of the glucose analog. This occurs despite the fact that glucose is still being supplied to the tissue from the blood. This must mean that tracer quantities of 2DG-6P are gradually being hydrolyzed to compounds that can leave the cell. In kinetic terms, a model useful in computing glucose metabolic rates from the trapping of 2-deoxyglucose by tumors must take this hydrolysis rate into account. The half-time for the clearance of radioactivity from tumor is on the order of 9 hr, a value similar to the clearance reported from human brain. The enzyme that hydrolyzes DG-6P to DG is glucose-6-phosphate, which has low activity in brain (*13*). Interestingly, this enzyme appears to be important in controlling the rate of gluconeogenesis (essentially the reverse reaction sequence of glycolysis) and the activity of this enzyme is correspondingly decreased in tumors in a manner that is inversely proportional to the growth rate (*7*). Thus, the more rapidly growing the tumor, the more depressed will be the clearance rate of glucose in the malignant tissue. Some normal tissues, such as liver, have high glucose-6-phosphatase activity, and in the Morris 7777 hepatoma model we have observed significantly faster clearance of retained 2-deoxyglucose activity from liver, with a progressive increase in tumor-to-liver ratios with time (from 8:1 at 1 hr, to 12:1 at 3 hr). Since neoplastic lesions in the liver would normally have low activity of the glucose-6-phosphatase compared with normal liver tissue, the data suggest that increasingly

higher ratios of tumor to liver should be observed with time. Unfortunately, in the spontaneous dog tumors, we did not have sufficient numbers of neoplasms in liver to verify this point with confidence.

This study demonstrates that both the ratios of tumor to normal tissue and the kinetics of 2-deoxyglucose uptake are suitable for applying positron-labeled deoxyglucose tracers to positron emission tomography of tumors. We have found this to be true in a variety of transplanted and spontaneous neoplasms in small and larger animals.

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FOOTNOTE

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

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