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Brain tumor accumulation and plasma pharmacokinetic parameters of 2′-deoxy-5-^{18}F-fluorouridine

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Using positron emission tomography and radio-high performance liquid chromatography, the accumulation of 2′-deoxy-5-^{18}F-fluorouridine in the brain tumors and plasma pharmacokinetic parameters were investigated in 20 patients. High accumulation of the tracer in high grade gliomas and meningiomas and very rapid degradation of the tracer in the plasma were found. Very large variations were observed in both tumor accumulation and pharmacokinetic data. The tumor accumulation, however, did not correlate with any of the plasma pharmacokinetic parameters: area under the plasma concentration-time curve, mean residence time, total body clearance and steady-state volume of distribution. The results suggest that the accumulation of the tracer reflects the metabolic activity of the brain tumor tissues and that the effect of the rapid metabolic change in the tracer in the plasma on the tumor accumulation may be minor.

Key words: brain tumor, 2′-deoxy-5-^{18}F-fluorouridine, positron emission tomography, nucleic acid metabolism, pharmacokinetics

INTRODUCTION

FLUORINATED PYRIMIDINE derivatives are antineoplastic agents widely used in the treatment of gastrointestinal and breast cancers.[1] The cytotoxicity is most likely mediated by the formation of an irreversible complex with thymidylate synthase and in part by incorporation into RNA via fluorinated nucleotides. Because the fluorinated pyrimidines are converted to nucleotide forms by several enzymes in nucleic acid metabolism, we have proposed ^{18}F-labeled 2′-deoxy-5-fluorouridine (^{18}F-FdUrd) as a radiopharmaceutical for the nucleic acid metabolism to assess cancer viability *in vivo* by positron emission tomography (PET).[2–13] High grade gliomas were clearly visualized and the accumulation of ^{18}F-FdUrd was higher than that in the low grade glioma or surrounding normal brain tissues.[7,8] However, a large variation in the accumulation in high grade gliomas was found. Several reasons are considered to explain the variation; 1) the heterogeneity of high grade gliomas and the difference in proliferative potential in the same grade gliomas, 2) the effect of treatment on the tumor metabolism and the drug metabolism in the whole body, 3) limitations in reliable biopsies to assess the histological grade and 4) the individual difference in the catabolism of the tracer. In a preliminary metabolic study of human plasma, degradation of the ^{18}F-FdUrd; ^{18}F-FdUrd → 5-^{18}F-fluorouracil (^{18}F-FUra) → 5,6-dihydro-5-^{18}F-fluorouracil (^{18}F-DHFU) → α-^{18}F-fluoro-β-ureidopropionic acid (^{18}F-FUPA) → α-^{18}F-fluoro-β-alanine (^{18}F-FBAL), was very rapid and seemed to be dose-dependent.[10] The rapid degradation is probably controlled by the individual catabolic activity in the whole body, especially in the liver. The plasma pharmacokinetic parameters of ^{18}F-FdUrd may affect the accumulation of the tracer in the high grade gliomas in which integrity of the blood-brain barrier cannot be preserved. This study investigated whether the different

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Table 1 Clinical data and PET studies

No.	Age	Sex	Body Weight (kg)	Histology	Dose			Uptake*	
					MBq	mg	μg/kg	DAR-PET	ng/ml
Patients before radio-chemotherapy									
1	60	M	50	glioma, grade 3	189	3.83	76.6	0.72	55.4
2	56	F	55	glioma, grade 3	218	3.64	66.2	0.29	19.2
3	67	F	60	glioma, grade 3	366	3.97	66.2	1.20	79.4
4	69	F	61	glioma, grade 3	281	3.05	49.6	0.92	45.7
5	49	F	50	glioma, grade 3	353	1.86	37.2	0.63	23.4
6	56	M	62	glioma, grade 3	407	2.29	36.9	0.65	24.0
7	68	M	61	glioma, grade 3	292	2.59	32.8	1.01	33.1
8	48	M	65	glioma, grade 3	259	2.12	32.6	1.23	40.2
9	35	M	55	glioma, grade 3	153	1.70	30.9	0.38	11.6
10	68	F	55	meningioma	159	1.51	27.5	0.76	20.9
11	67	F	48	meningioma	122	0.55	11.5	0.77	8.8
Patients during and after radio-chemotherapy									
12	66	M	62	glioma, grade 4	340	2.72	41.8	1.28	53.3
13	61	M	70	glioma, grade 4	130	0.95	14.6	0.68	9.2
14	49	M	64	glioma, grade 3–4	174	1.60	25.0	0.83	20.6
15	6	M	27	glioma, grade 3	315	1.85	68.5	0.67	45.9
16	44	M	58	glioma, grade 3	363	2.45	42.2	0.57	24.2
17	43	M	64	glioma, grade 3	185	1.61	37.5	0.39	14.6
18	34	F	52	glioma, grade 3	133	1.68	33.6	0.26	8.9
19	38	M	81	glioma, grade 3	259	2.54	31.4	0.26	8.0
20	63	F	51	glioma, grade 3	130	0.90	17.6	0.65	11.5

* Uptake was measured from a 30 to 40 min acquisition, and includes all ^{18}F-FdUrd and its metabolites. ng/ml was calculated from the specific activity of the compound and the radioactivity concentration, and was expressed to be equivalent to ^{18}F-FdUrd.

potential of drug metabolism affects on the accumulation of ^{18}F-FdUrd in brain tumors. The pharmacokinetic parameters were measured by means of a radio-high performance liquid chromatographic (radio-HPLC) technique.

MATERIALS AND METHODS

Chemicals

Uracil, FUra and FdUrd were purchased from Wako Pure Chemical Industry (Tokyo), 5-fluorouridine from Sigma Chemical Company (St. Louis), FBPA from Tokyo Kasei Kogyo (Tokyo), and 2-deoxyuridine from Seikagaku Kogyo (Tokyo). DHFU was generously supplied by Hoffmann La Roche, Basel, Switzerland. All other reagents were of the highest grade available.

Preparation of ^{18}F-FdUrd

^{18}F-FdUrd was prepared by a previously described method,[14] or the following modified method. Acetyl ^{18}F-hypofluorite was bubbled into 6 mL of acetic acid containing 15 mg of 2-deoxyuridine at room temperature. After drying the solution by evaporation, the residue was dissolved in 2 ml of triethylamine and heated at 90°C for 5 min. After drying the solution, the residue was dissolved in 1.0 ml of 0.1% acetic acid and applied to HPLC with a Delta-Pak C18 cartridge (25 mm i.d. × 100 mm length) with a pre-column cartridge (25 mm i.d. × 10 mm) equipped with an RCM 25 × 10 compression module (Waters). The mobile phase was 0.1% acetic acid containing 2% ethanol and the flow rate was 20 ml/min. ^{18}F-FdUrd eluted 8.5 to 10.5 min was collected and evaporated to dryness. The residue was dissolved in physiological saline, and the solution was passed through a 0.22 μm membrane filter for injection. The specific activity was 26–45 GBq/mmol.

PET studies

Twenty patients with brain tumors listed in Table 1, were examined by PET. Eleven patients were studied before radio-chemotherapy, and the remaining 9 patients were studied during or after the therapy. All of the patients showed normal liver functions which were investigated by routine blood biochemical examinations. Some patients were treated with radial surgery after the PET examination. Histological diagnoses were made from CT-guided stereotaxic biopsies or operative specimens.

Before the PET study, CT scanning was carried out. The PET scanner used was a PT-931 model with four detector rings and a spatial resolution of 8 mm full width at half maximum (CTI, Knoxville, Ten-

nessee). The patients were injected intravenously with 120 to 410 MBq (0.9 to 4.0 mg) of ^{18}F-FdUrd as a bolus, and sequential images with 5 min data acquisition were obtained over a period of 40 to 60 min. Before the emission scan, a transmission scan with a ^{68}Ge/^{68}Ga external ring source was performed. The emission data were corrected for attenuation by means of the transmission data. Each pixel count was converted to a radioactivity concentration (nCi/m*l*) by applying the cross-calibration factor for the day between the well scintillation counter and the positron camera. The radioactivity in the tumor was measured in the region of interest (volume: 2.2 m*l*), including the highest radioactivity point, and was expressed as the differential absorption ratio {DAR-PET, [tissue radioactivity/total injected radioactivity]/[tissue volume (m*l*)/body weight (g)]}. The mass concentrations of the tracer (equivalent to FdUrd) in the tumor were also represented as ng/m*l* tissue, calculated from the uptake of radioactivity and specific activity of ^{18}F-FdUrd injected. This project was approved by the Committee for Clinical PET Study of Tohoku University and informed consent was obtained from every patient.

Measurement of labeled metabolites in plasma

During the PET scanning, arterial blood samples were taken at 0.33, 0.67, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.5, 10, 15, 20, 30, 40, 50 and 60 min after the i.v. administration. The blood volumes were 3 m*l* at 1, 3, 5, 10, 15, 30 and 60 min, and 1 m*l* at other times. About 0.5 m*l* of plasma was weighed and measured for radioactivity with an NaI(Tl) gamma scintillation counter. The radioactivity level was expressed as the differential absorption ratio {DAR, [plasma radioactivity/total injected radioactivity]/[plasma weight (g)/body weight (g)]}.

Plasma samples taken at 1, 3, 5, 10, 15, 30 and 60 min were assayed for measurement of radioactive metabolites by a previously described method[10] with slight modification. Briefly, 0.5 to 1.5 m*l* of the plasma was treated with 1 M $HClO_4$. After centrifugation, the acid-soluble supernatant was neutralized with 1 M KOH and the precipitated $KClO_4$ was removed by centrifugation. The supernatant was applied on a reverse phase column (ERC-ODS-2121, 8 mm i.d. $\times$250 mm length, Erma Optical Industry, Tokyo). The column was eluted with an ion pair solution with a gradient modifier at room temperature at a constant flow rate of 1.5 m*l*/min. For the first 10 min the initial solvent (buffer A: 5 mM sodium phosphate buffer, pH 6.8, containing 10 mM tetrabutylammonium hydrogensulfate) was eluted. For the next 10 min, the column was eluted with a solvent with a liner gradient of the following composition: 0% to 100% of the stronger elution solvent {buffer B: a mixture of methanol and 10 mM sodium phosphate, pH 6.8, containing 10 mM tetrabutylammonium hydrogensulfate (50: 50, v/v)}. For the last 7.5 min, the buffer B was eluted, and then the column was reequilibrated with the initial buffer A. The elution profile was detected with a radioactivity monitor (Ramona-D equipped with an IM-2020X flow cell for ^{3}H/^{14}C measurement, Raytest), and the radioactivity in each 1.0 m*l* was measured with an auto-gamma scintillation counter. The radioactivity was corrected for the half-life of ^{18}F. A portion of the sample applied was also measured to calculate the total applied radioactivity, and the percentage of radioactivity in each peak of the total applied radioactivity was calculated. Recovery of the radioactivity was essentially quantitative.

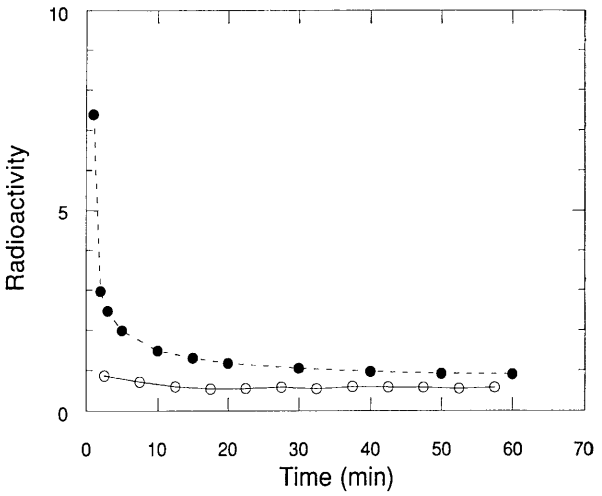

Fig. 1 Time-radioactivity curves of the glioma and plasma after i.v. injection of ^{18}F-FdUrd into a patient. Radioactivity level was expressed as DAR for total plasma (●) and DAR-PET for tumor (○).

Pharmacokinetic calculations

The area under the plasma concentration-time curve (AUC) or ^{18}F-FdUrd for the interval 0–60 min and the mean residence time (MRT), the steady-state volume of distribution (Vd) and the total body clearance (CL) were calculated according to the method of Yamaoka et al.[15] The AUC for ^{18}F-FdUrd was written as the AUC_1, and AUC_2 and AUC_3 were also calculated for ^{18}F-FUra and ^{18}F-DHFU.

RESULTS AND DISCUSSION

By PET with ^{18}F-FdUrd, all brain tumors investigated were clearly visualized as a positive spot in the region which was shown as the high density by post contrast CT. Time-radioactivity curves of the tumor and plasma are shown in Fig. 1. Radioactivity in the

Table 2 Labeled metabolites in plasma of 20 patients given ^{18}F-FdUrd

	% of total plasma radioactivity*					
	1 min	3 min	5 min	10 min	15 min	30 min
FdUrd	87.1±7.1	40.2±7.1	23.8±6.4	10.9±3.4	6.8±3.2	2.8±1.5
FUra	9.4±4.7	20.8±5.5	16.9±8.0	9.1±5.1	5.4±3.1	3.3±2.1
DHFU	4.9±3.3	24.0±9.0	35.5±10.6	34.2±10.9	28.5±10.3	16.7±8.1
Peaks 2+3**	1.1±1.7	2.7±2.2	4.2±3.2	4.6±3.7	5.0±2.4	4.8±2.3
Peak 1	0.3±0.4	6.1±3.2	15.0±6.3	35.8±10.7	48.0±16.1	69.0±9.1

* Mean±s.d. (n=20)

** Peaks 2 and 3 overlapped when ratios were high. Data in two patients who showed very high ratios were removed.

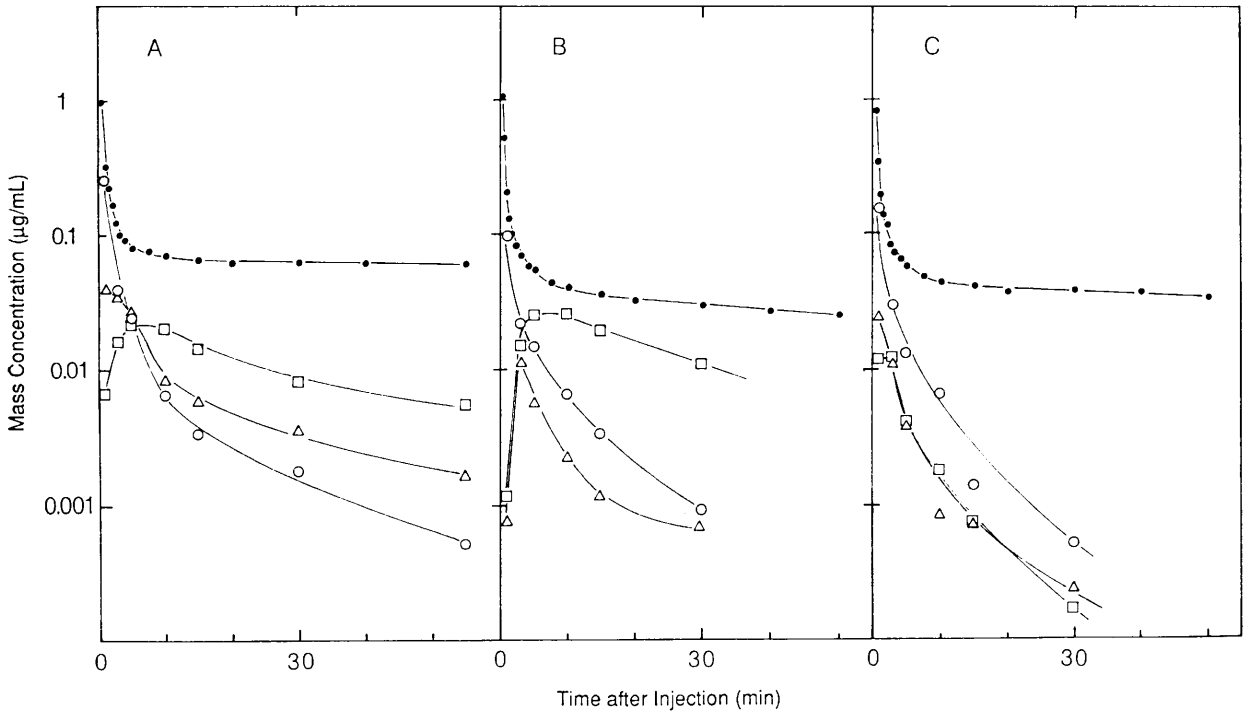

Fig. 2 Plasma mass concentrations of total FdUrd and its metabolites, FdUrd, FUra and DHFU in three patients received ^{18}F-FdUrd. Three patients were No. 5 (A), No. 10 (B) and No. 19 (C) described in Table 1. Total FdUrd and its metabolites (equivalent to FdUrd (●); FdUrd (○); FUra (△); and DHFU (□).

plasma was rapidly cleared for the first 20 min, while it decreased slightly in the tumor. After this time, both radioactivity levels remained constant. These patterns were similar in all 20 patients, and DAR-PET values at 30 to 40 min after the injection are summarized in Table 1. The mean DAR-PET values in patients before radiochemotherapy and in patients during and after treatment were 0.78±0.29 and 0.62 ±0.30, respectively, but the difference did not reach statistical significance. The DAR-PET values in the contralateral brain regions were 0.17±0.05. Although the radioactivity represents ^{18}F-FdUrd and its metabolites, the mass concentration in the tumors was also expressed as the mass equivalent of FdUrd (ng)/tissue volume (m*l*) (Table 1).

Radioactive metabolites in the plasma were analyzed by radio-HPLC. In a typical analysis, six radioactive peaks were detected: their retention times were 6.1, 7.8, 9.0, 11.8, 13.8 and 21.2 min. As previously described,[10] metabolites were identified as follows. Radioactive peaks 4, 5 and 6 were ^{18}F-DHFU, ^{18}F-FUra and ^{18}F-FdUrd, respectively, compared with retention times for the authentic samples. When peak 1 in the elution front was collected and analyzed by means of a cation-exchange column,[3] over 90% of the radioactivity was eluted as ^{18}F-FBPA. Peak 2 was not identified. Peak 3 was assumed to be ^{18}F-FUPA, because β-ureidopropionic acid showed a corresponding retention time. Peaks 2 and 3 overlapped when their ratios were high. 5-Fluorouridine was eluted in the retention time of 19.5 min, where the corresponding radioactivity was

Table 3 Pharmacokinetic data of plasma in 20 patients given ^{18}F-FdUrd

No.	FdUrd				FUra		DHFU		
	AUC_1 ($\mu g \cdot min/ml$)	MRT (min)	CL (ml/min)	Vd (ml)	AUC_2 ($\mu g \cdot min/ml$)	$\frac{AUC_2}{AUC_1}$	AUC_3 ($\mu g \cdot min/ml$)	$\frac{AUC_3}{AUC_1}$	$\frac{AUC_3}{AUC_2}$
Patients before radio-chemotherapy									
1	1.43	2.8	2.7	7.4	0.36	0.25	0.88	0.62	2.5
2	1.67	4.9	2.2	10.8	0.54	0.32	1.73	1.04	3.2
3	2.82	2.6	1.4	3.6	0.84	0.30	1.42	0.50	1.7
4	1.57	2.5	1.9	4.8	0.16	0.10	0.96	0.61	6.1
5	0.72	2.4	2.5	6.3	0.39	0.54	0.43	0.60	1.1
6	0.68	2.9	3.3	9.6	0.18	0.26	0.46	0.67	2.6
7	1.08	5.1	2.4	12.1	0.59	0.54	0.62	0.57	1.1
8	0.91	4.0	2.3	9.2	0.20	0.22	0.60	0.55	3.0
9	0.95	4.2	1.8	7.6	0.31	0.33	0.33	0.35	1.1
10	0.75	2.6	2.0	5.2	0.09	0.11	0.51	0.68	5.9
11	0.34	1.9	1.6	3.1	0.05	0.15	0.17	0.49	3.4
Patients during and after radio-chemotherapy									
12	0.80	2.6	3.4	9.3	0.16	0.19	0.56	0.70	3.6
13	0.26	2.1	2.5	5.1	0.04	0.15	0.13	0.50	3.3
14	0.50	4.3	3.3	13.8	0.07	0.14	0.40	0.81	5.7
15	1.08	2.0	1.7	3.4	0.20	0.19	0.90	0.84	4.4
16	1.30	4.6	1.9	8.7	0.36	0.27	0.63	0.49	1.8
17	0.76	3.9	2.1	8.3	0.18	0.24	0.27	0.36	1.5
18	0.97	2.8	1.7	4.8	0.10	0.10	0.67	0.70	6.7
19	0.74	2.4	3.4	8.3	0.09	0.12	0.03	0.04	0.4
20	0.31	4.5	2.9	13.1	0.10	0.32	0.20	0.64	2.0

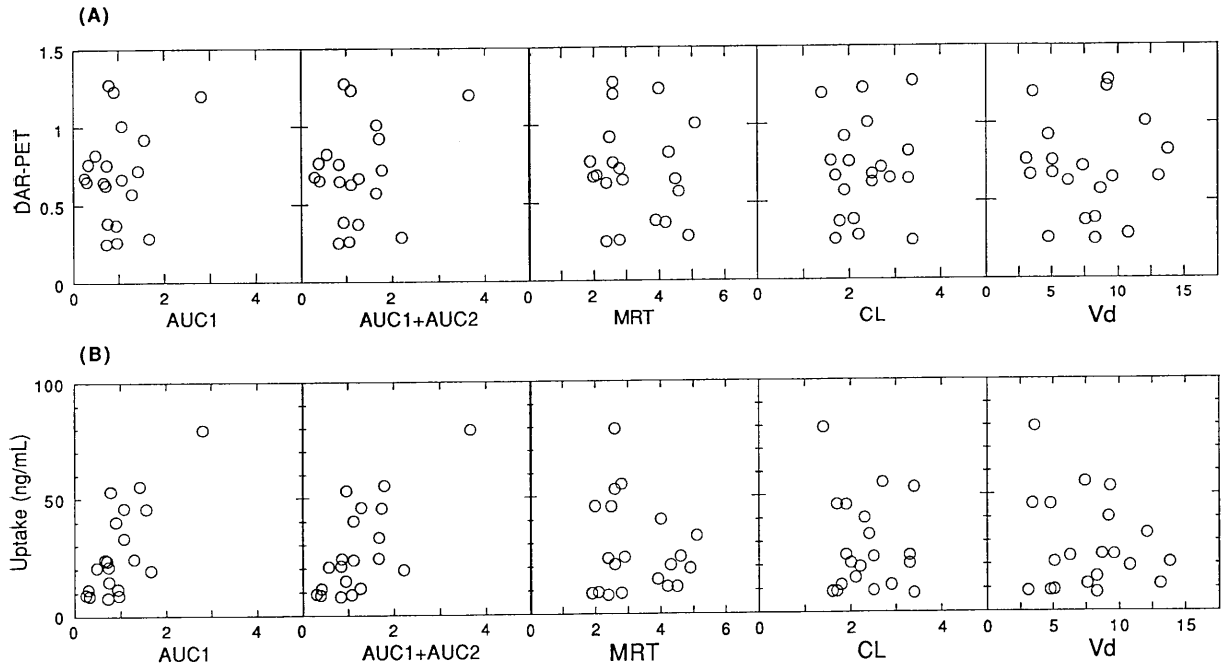

Fig. 3 Relationship between the tracer accumulation in tumors measured by PET and plasma pharmacokinetic parameters. The accumulation was expressed as the DAR-PET (A) and ng/ml tissue (B).

detected in very small amounts (<1%) in some cases. Radioactive nucleotides were negligible. Average percentages for the labeled metabolites in 20 patients are summarized in Table 2. Very rapid degradation of ^{18}F-FdUrd occurred. Subsequently, proportions of ^{18}F-FUra and ^{18}F-DHFU initially increased with time and then decreased. The proportion of peak 1 was predominant later. In 60 min-samples, the proportions of ^{18}F-FdUrd and ^{18}F-FUra were lower than 1%. In three typical cases, plasma clearance of ^{18}F-FdUrd and its metabolites expressed as mass concentrations (μg/m*l*) is shown in Fig. 2 (A, No. 5; B, No. 10; and C, No. 19).

Plasma pharmacokinetic data for 20 patients are summarized in Table 3. No clear relationship between the tumor uptake (DAR-PET values and ng/m*l*) and any plasma pharmacokinetic parameters was observed (Fig. 3). During the time-scale of PET scanning, very large individual variations and some interesting aspects were found in the pharmacokinetic parameters in plasma, although this study was carried out in patients with normal liver functions which were investigated by routine blood biochemical examinations. The following individual differences were found in the three catabolic reactions. The first reaction from FdUrd to FUra was catalyzed by thymidine phosphorylase, and the MRT values were in the 1.9 to 5.1 min range. Further degradation steps catalyzed by dihydrouracil dehydrogenase and dihydropyrimidinase did not necessarily parallel the MRT of ^{18}F-FdUrd. In two patients {Nos. 5 (Fig. 2A) and 7} who showed large AUC_2/AUC_1 ratios, the reaction from FUra to DHFU was slow. In another group {Nos. 4, 10 (Fig. 2B), 14 and 18}, the degradation of DHFU assessed by the AUC_3/AUC_2 ratios was slow. Among 20 patients patient No. 19 (Fig. 2C) showed signs of very rapid elimination of all FdUrd, FUra and DHFU. The lowest radioactivity accumulation in the tumor was observed in this case, whereas patient No. 12 who had similar MRT and AUC_1 values to No. 19 had the largest DAR-PET.

In experimental tumor tissues, a considerable amount of the total radioactivity was present as ^{18}F-FdUrd and ^{18}F-FUra as well as their biologically active forms which were activated by such enzymes as thymidine kinase, uridine phosphorylase, uridine kinase, thymidylate synthase and so on.[10] And catabolites of the ^{18}F-FdUrd were found in the tumor tissues.[10] Because AUC shows the bioavailability of drugs or input function in PET studies, AUC_1 and AUC_2 may be major parameters affecting the tumor uptake of the ^{18}F-FdUrd. DAR-PET values in the brain tumor, however, were not necessarily correlated with these parameters (Fig. 3). In animals[2–4] and humans,[5] most of the ^{18}F-FdUrd was degraded in the liver and/or excreted into urine through the kidneys, and total tumor uptake was low. MRT, CL and Vd values are controlled by the whole body metabolism. The effect of the catabolites of the ^{18}F-FdUrd on the tumor uptake may therefore be represented by these three parameters and total plasma radioactivity. And the DAR-PET values in the brain tumor were not correlated with these parameters (Fig. 3), with total radioactivity levels or with integrated plasma radioactivity in individual patients (data not shown). The sequential studies before and after radio-chemotherapy in the same patients may more clearly represent the relationship between the DAR-PET values and pharmacokinetic parameters. However, these studies were carried out in a limited number of patients, because some patients were treated with radical surgery after the first PET study and because metabolites analyses could not be carried out to obtain the pharmacokinetic parameters in some patients.

Several reasons were considered to explain a large variation in the DAR-PET values in previous and this studies. As discussed above, it was found that the individual difference in the catabolism of the tracer may be a minor contribution. In none of the patients investigated, was the blood-brain barrier in the tumor region reserved, because enhancement was observed in the region by CT scanning. Because the tracer does not essentially pass through the blood-brain barrier, the status of the barrier may be another factor. However, if the ^{18}F-FdUrd accumulation in the tumor is dependent on the breakdown of the barrier, it should have been decreased with time due to the concentration gradient between the plasma and brain tumor tissue. This was not true in our studies. On the other hand, the analysis of Patlak's plot[16] was done, and the active uptake pattern of ^{18}F-FdUrd was observed in high grade tumors (unpublished data). Radio-chemotherapy may also influence the ^{18}F-FdUrd accumulation in the tumor. In this study, no significant difference was found between the DAR-PET values for the patient during and after the radio-chemotherapy or those in patients before the treatment. However, the effect in the same patients in the process of treatment needs to be dealt with, and this work is in now progress. For the reasons given above and the experimental studies with tumors on the metabolic and autoradiographic comparison of ^{18}F-FdUrd and radio-thymidine[4,9,10] and radio- and chemotherapeutic effects on the ^{18}F-FdUrd accumulation,[11,13] we consider that the accumulation of ^{18}F-FdUrd in brain tumors is mainly dependent on the tumor metabolism and that the effect of rapid drug metabolism on tumor accumulaton may be only minor.

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REFERENCES

1. Heiderberger C, Danengerg PV, Moran R: Fluorinated pyrimidines and their nucleoside. *Adv Enzymol* 54: 57–119, 1983
2. Abe Y, Fukuda H, Ishiwata K, et al: Studies on ^{18}F-labeled pyrimidines. Tumor uptake of ^{18}F-5-fluorouracil, ^{18}F-5-fluorouridines and ^{18}F-5-fluorodeoxyuridine in animals. *Eur J Nucl Med* 8: 258–261, 1983
3. Ishiwata K, Ido T, Kawashima K, et al: Studies on ^{18}F-labeled pyrimidines II. Metabolic investigation of ^{18}F-5-fluorouracil, ^{18}F-5-fluoro-2′-deoxyuridine and ^{18}F-5-fluorouridine in animals. *Eur J Nucl Med* 9: 185–189, 1984
4. Ishiwata K, Ido T, Abe Y, et al: Studies on ^{18}F-labeled pyrimidines III. Biological investigation of ^{18}F-labeled pyrimidines and comparison with ^{3}H-deoxythimidine in tumor-bearing rats and mice. *Eur J Nucl Med* 10: 39–44, 1985
5. Abe Y, Matsuzawa T, Itoh M, et al: Cancer detection with ^{18}F-5-fluorodeoxyuridine—a new cancer diagnosis agent reflecting the nucleic acid metabolism. *Jpn J Nucl Med* 22: 583–586, 1985 (in Japanese)
6. Kiyosawa M, Mizumo K, Ishiwata K, et al: Orbital tumor diagnosis by positron emission tomography using ^{18}F-fluorodeoxyuridine. *Ophthalmic Res* 18: 292–298, 1986
7. Kameyama M, Tsurumi Y, Itoh J, et al: Biological malignancy and ^{18}FdUrd uptake in glioma patients —PET study of nucleic acid metabolism. *CYRIC Annual Report, Tohoku University, 1988*: 215–227, 1988
8. Kameyama M, Tsurumi Y, Shirane R, et al: Nucleic acid metabolism in glioma studied with ^{18}FdUrd and PET. *J Cereb Blood Flow Metab* 9 (suppl 1): S221, 1989
9. Tsurumi Y, Kameyama M, Ishiwata K, et al: ^{18}F-fluoro-2′-deoxyuridine as a tracer of nucleic acid metabolism in brain tumors. *J Neurosurg* 72: 110–113, 1990
10. Ishiwata K, Sato K, Kameyama M, et al: Metabolic fates of 2′-deoxy-5-[^{18}F]fluorouridine in tumor-bearing mice and human plasma. *Nucl Med Biol* 18: 539–545, 1991
11. Kubota K, Ishiwata K, Kubota R, et al: Tracer feasibility for monitoring tumor radiotherapy: A quadruple tracer study with fluorine-18-fluorodeoxyglucose or fluorine-18-fluorodeoxyuridine, L-[methyl-^{14}C]methionine, [6-^{3}H]thymidine, and gallium-67. *J Nucl Med* 32: 2118–2123, 1991
12. Ishiwata K, Takahashi T, Iwata R, et al: Tumor diagnosis by PET: Potential of seven tracers examined in five experimental tumors including an artificial metastasis model. *Nucl Med Biol* 19: 611–618, 1992
13. Sato K, Kameyama M, Ishiwata K, et al: Metabolic changes of glioma following chemotherapy: An experimental study using PET tracers. *J Neuro-Oncol* 14: 81–89, 1992
14. Ishiwata K, Monma M, Iwata R, et al: Automated synthesis of 5-[^{18}F]fluoro-2′-deoxyuridine. *Appl Radiat Isot* 38: 467–473, 1987
15. Yamaoka K, Tanigawara Y, Nakagawa T, et al: Capacity-limited elimination of cefmetazole in rat. *Int J Pharmaceu* 10: 291–300, 1978
16. Parlak CS, Blasberg RG, Fenstermacher JD: Graphical evaluation of blood-to-brain transfer constants from multi-time uptake data. *J Cereb Blood Flow Metab* 3: 1–7, 1983