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ORIGINAL ARTICLE

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Intrathecal 5-fluoro-2′-deoxyuridine (FdUrd) for the treatment of solid tumor neoplastic meningitis: an in vivo study

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Abstract To evaluate the possible intrathecal use of 5-fluoro-2′-deoxyuridine (FdUrd) for neoplastic meningitis, its antitumor activity and neurotoxicity in vivo were assessed. FdUrd at doses in the range 5–100 μg/animal was effective against meningeal carcinomatosis using Walker 256 carcinoma cells in rats and MM46 mammary cancer cells in mice and against meningeal gliomatosis using 203 glioma cells in mice. After four intrathecal injections, FdUrd at these doses also showed minimal neurotoxicity in the C57BL/6 mouse brain. To estimate the mechanism of FdUrd efficacy, thymidine phosphorylase (TPase) and thymidine kinase (TK), key enzymes in the metabolism of FdUrd, were measured in rat, mouse and normal human brain tissue, and in human brain tumor tissues and cerebrospinal fluid (CSF) from patients with malignant brain tumors including meningeal carcinomatosis. TPase levels were lower in brain and malignant brain tumors than in other organs and their tumors. Moreover, the activity of TPase in the gray matter of human brain, which faces the cerebrospinal fluid across the cortical surface and into which malignant cells invade in meningeal carcinomatosis, was lower than that in the white matter. TK was undetectable, and TPase was detected (at very low concentrations) in only 4 of 56 patients with brain tumors or meningeal carcinomatosis. These findings indicate that brain tissue and CSF are favorable sites for FdUrd chemotherapy because the rate of conversion of FdUrd to 5-FU would be minimal. In conclusion, FdUrd is potentially useful for intrathecal treatment of neoplastic meningitis from primary brain tumors and systemic cancer.

Key words Brain tumor · Fluorodeoxyuridine · Meningeal carcinomatosis · Intrathecal chemotherapy · Thymidine phosphorylase

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Introduction

Meningeal dissemination of malignant tumors is generally treated by intrathecal administration of anticancer agents, radiation therapy or a combination of both. However, few anticancer drugs can be injected intrathecally, with methotrexate (MTX), cytosine arabinoside (Ara-C), and thio-TEPA being the major examples [9, 11, 22, 29]. The intrathecal administration of these anticancer agents has a therapeutic efficacy of about 40 to 60%. Such treatment regimens may produce clinical stability in some patients for a short time, but rarely result in reversal of established deficits. Acute neurotoxicity and necrotizing encephalopathy, side effects of MTX, impose limitations on the achievement of a dose sufficient to produce a satisfactory therapeutic effect, with the result that the effect achieved still remains to be improved [2, 23].

5-Fluoro-2′-deoxyuridine (FdUrd) is an active metabolite of 5-fluorouracil (5-FU) and is a compound known to have very high antitumor activity against liver metastases of colon cancer [1, 12–14, 25]. Although the compound has been used clinically to treat metastatic brain tumors by continuous intravenous administration in one case, the results achieved have yet to be established as standard therapeutic effects [8]. FdUrd is metabolized in vivo by thymidine kinase (TK) (EC 2.7.1.21) and converted to 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which inhibits thymidylate

synthase (EC 2.1.1.45), a key enzyme in DNA synthesis, and thus exhibits a high degree of cytotoxicity. On the other hand, FdUrd is anabolized to 5-FU by thymidine phosphorylase (TPase) (EC 2.4.2.4) and thereby its antitumor effect is diminished [3, 6, 24]. In using FdUrd, therefore, it is critical to consider the balance between the activities of these enzymes in tumor tissues and in normal tissue.

Previously, we have shown good antitumor activity of FdUrd compared with 5-FU and minimal neurotoxicity using mouse fetal brain (ED14) in vitro [32]. In this study, we evaluated the potential intrathecal use of FdUrd by examining its antitumor effect and neurotoxicity in vivo and by measuring TPase and TK activities in normal human brain tissue, brain tumors and cerebrospinal fluid (CSF).

Materials and methods

In vivo therapeutic experiments

Female Wistar rats at 8 weeks of age weighing about 200 g, C3H/He mice at 6 weeks of age weighing about 18 g and C57BL/6 mice at 7 weeks of age weighing about 19 g were used for evaluation of the antitumor efficacy of intrathecal FdUrd. The Wister rats were divided into groups of 10 and the C3H/He and C57BL/6 mice into groups of 20–23. The model of rat meningeal carcinomatosis was established by puncturing the rat cisterna magna with a 26-gauge needle under anesthesia with ethyl ether, and injecting 0.1 ml of a suspension of Walker 256 carcinoma cells (5×10^5cells), which had been obtained from rats, minced with scissors, filtered with gauze and suspended in phosphate-buffered saline (PBS) [27]. In the model of meningeal gliomatosis and meningeal carcinomatosis, 0.05 ml of a suspension of cultured 203 glioma cells (5×10^5 cells) for C57BL/6 mice [21], and 0.05 ml of a suspension of transplantable ascitic cells (1×10^6 cells) of MM46 mammary cancer [26] (supplied by Cancer Cell Repository, Research Institute for Tuberculosis and Cancer, Tohoku University, Sendai, Japan) for C3H/He mice were injected into the cisterna magna. Various doses of FdUrd (5, 25, 50 and 100 μg/animal; 0.15 ml for rats and 0.05 ml for mice) were directly injected into the cisterna magna for 5 consecutive days, starting 2 days after tumor implantation. 1-day treatment regimen was also added to obtain better data regarding the effects of FdUrd, and a daily for 5 days regimen was used in the antitumor activity experiments because we observed few side effects. In control animals, PBS was injected intrathecally as a sham control. The antitumor effects of intrathecal FdUrd were evaluated in terms of the increased life span of drug-treated rats and mice compared with the controls.

In vivo side effects

In vivo systemic side effects and neurotoxicity of intrathecal FdUrd in normal C57BL/6 mice was evaluated during and after intrathecal administration. Intrathecal administration of a high dose of 4 mg/ml FdUrd five times (200 μg/0.05 ml per animal) caused ascites due to liver dysfunction in 20% of the mice. Intrathecal administration according to the same schedule but at a dose of 2 mg/ml (100 μg / 0.05 ml per animal) caused no ascites. Therefore, we chose concentrations of FdUrd up to 100 μg/0.05 ml per animal for this study. Normal C57BL/6 mice in groups of 20 were given FdUrd by intrathecal injection at specified doses (5, 25, 50 or 100 μg/0.05 ml per animal) on days 1, 3, 5 and 7. The body weight of the mice was measured and the neuronal manifestations were observed during and after the course of intrathecal chemotherapy. Two months after cessation of drug administration, the mice were sacrificed. The brain tissues were collected and stained with hematoxylin and eosin and Klüver Barrera stain for histological investigation. Two mice were also sacrificed 24 h after a single administration of FdUrd (100 μg/0.05 ml) and histologically examined for evaluation of acute changes.

Chemicals

Thymidine was purchased from Sigma Chemical Co. (St. Louis, Mo.) and [2-^{14}C]thymidine (2.2 GBq/mmol) from DuPont Co. (Wilmington, Mass.). Fetal calf serum and horse serum were obtained from GIBCO BRL (Tokyo, Japan). All other chemicals used were commercial products.

Samples of human brain and tumor tissues

Tissues from several different anatomical structures were sampled from six autopsied brains obtained within 2 h of death and confirmed to be histologically normal. Fresh gray and white matter were also sampled from patients with resected metastatic brain tumors when corticotomy was required to approach deeply located tumors, and brain tissues attached to the resected tumors were definitely separated from gray and white matter and defined as "normal brain" in the present study, that is normal brain with edema surrounding tumor tissue. Resected brain tissues were proven to be histologically normal. Other tumor tissues such as glioblastoma, malignant lymphoma and meningioma were sampled during surgery. Tissue samples were quickly frozen at −80 °C and stored until assay. This study was approved by the Ethics Committee of Osaka. Medical Center for Cancer & Cardiovascular Diseases.

Samples of animal brain tissues and other organs

Five rats and mice were sacrificed for tissue sampling. Female Wistar rats at 7 weeks of age and male C57BL/6 mice at 8 weeks of age were anesthetized with ethyl ether and perfused via the heart with cold saline until decoloration of the liver was observed after opening the abdominal cavity and cutting the large abdominal vein. They were decapitated, and the brain and other organs were quickly sampled and stored at −80 °C until assay.

Samples of CSF

CSF was obtained from 56 patients with malignant brain tumors before treatment by lumbar puncture or through an Ommaya reservoir placed in the lateral ventricle. Cell counts were normal in malignant brain tumors without meningeal dissemination, and 45 to 750 cells/mm^3 were observed in those with meningeal dissemination. Samples were quickly frozen at −80 °C and stored until assay.

Assay of thymidine kinase and thymidine phosphorylase

All procedures were carried out at 4 °C. Tissue samples were minced with scissors and homogenized in four volumes of 50 m*M* Tris-HCl (pH 8.0) containing 10 m*M* 2-mercaptoethanol, 25 m*M* KCl, and 5 m*M* $MgCl_2$. The homogenate was centrifuged at 105 000 *g* for 60 min, and the resultant supernatant was used for enzyme assays.

TK activity was measured by determining the conversion of labeled thymidine to labeled nucleotide employing the DEAE cellulose disc method [10, 17]. The reaction mixture, in a total volume of 0.25 ml, containing 50 m*M* Tris-HCl buffer (pH 8.0), 5 m*M* $MgCl_2$, 5 m*M* ATP, 6 m*M* α-glycerophosphate, 0.05 m*M* [2-^{14}C] thymidine (0.0625 μCi/tube) and 0.2 ml enzyme solution, was incubated at 37 °C for 30 min and the reaction was stopped by heating in a boiling waterbath for 3 min. The mixture was centrifuged at 3000 rpm for 10 min and 100 μl of the supernant was

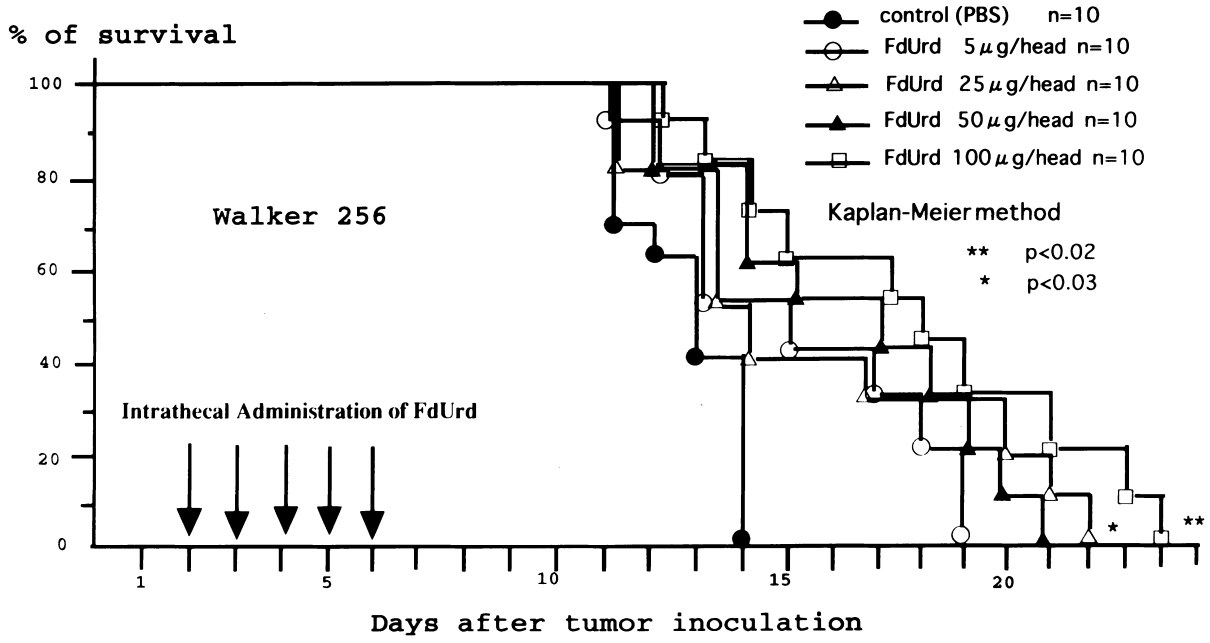


Fig. 1 Antitumor effects of FdUrd on rat meningeal carcinomatosis using Walker 256 carcinoma cells. Intrathecal administration of FdUrd through the cisterna magna was started 2 days after intrathecal inoculation of 5×10^5 Walker 256 carcinoma cells in suspension and repeated 5 times for 5 consecutive days, and animals were observed daily until death. Each group consisted of ten rats. The groups that received 50 and 100 μg/0.15 ml ×5 of FdUrd showed significantly prolonged survival (50 μg/0.15 ml, 128% of control, $P < 0.05$; 100 μg/0.15 ml, 139% of control, $P < 0.02$)

applied to a DEAE-cellulose disc, 3 cm in diameter. The disc was immersed in 1 m*M* ammonium formate for 20 min. The washing liquid was discarded, and the disc was then washed with distilled water. This procedure was repeated once more. Nucleotides were retained on the disc during this procedure. Next, the dried disc was placed in a vial containing 10 ml of ACS-II scintillation fluid (Amersham) and its radioactivity was determined.

TPase activity was measured as described previously [31]. Briefly, the reaction mixture consisting of 60 m*M* potassium phosphate buffer (pH 7.6), 0.6 m*M* [2-^{14}C]thymidine (0.025 μCi/tube), and 0.05 ml enzyme solution in a total volume of 0.125 ml, was incubated at 37 °C for 30 min. Perchloric acid (2*M*, 25 μl) was added to the reaction mixture, and the mixture was centrifuged at 3000 rpm for 10 min. Then 100 μl of the supernatant was added to 30 μl 2 *M* KOH solution. The resulting precipitate was removed by centrifugation (3000 rpm, 10 min) and 10 μl of the supernatant was applied to a silica gel TLC plate (precoated with silica gel 60 F254, 3 × 10 cm, thickness, 0.25 mm: Merck) and developed with a mixture of chloroform, methanol and acetic acid (17:3:1, v/v/v). Samples containing nonlabeled thymidine and thymine were applied to the plate before the test sample and were located under UV light (254 nm). The spots of radiolabeled thymine were scraped into vials and extracted with 50 μl 4 *N* HCl, and their radioactivity was measured as described above.

Statistical analyses

TPase and TK levels in gray and white matter in six autopsied brains were compared using a paired *t*-test. TPase and TK levels in the brain and those in other extracranial tissues as reported by Maehara et al. [17], which were measured by the same method as used in the present study, were compared using Welch's *t*-test. An unpaired *t*-test was also used in the other comparative study. In the animal study, the Kaplan-Meier method (log rank comparison) was used to analyze the survival data.

Results

Antitumor activity

In rat models of meningeal carcinomatosis using Walker 256 carcinoma cells, intrathecal FdUrd chemotherapy at doses of 50 μg/0.15 ml and 100 μg/0.15 ml, delivered via injection into the cisterna magna, showed antitumor effects (control, mean survival time ± SEM 12.7 ± 0.4 days, ten surviving rats; 5 μg/0.15 ml, 15.0 ± 0.9 days, ten rats; 25 μg/0.15 ml, 15.5 ± 1.3 days, ten rats; 50 μg/0.15 ml, 16.2 ± 1.0 days, ten rats; 100 μg/0.15 ml, 17.6 ± 1.0 days, ten rats; $P < 0.03$, < 0.02, respectively; Fig. 1). In mouse models of meningeal carcinomatosis using MM46 mammary cancer cells, the groups that received 5, 50 and 100 μg/0.05 ml of FdUrd showed significantly prolonged survival (control, mean survival time ± SEM 9.8 ± 0.3 days, 20 surviving mice; 5 μg/0.05 ml, 11.3 ± 0.5 days, 22 mice; 25 μg/0.05 ml, 10.6 ± 0.4 days, 21 mice; 50 μg/0.05 ml, 12.1 ± 0.5 days, 20 mice; 100 μg/0.05 ml, 13.8 ± 0.6 days, 21 mice; $P < 0.05$, < 0.01 and < 0.0001, respectively; Fig. 2). In the mouse models of meningeal gliomatosis using 203 glioma cells, all groups that received 25 to 100 μg/0.05 ml of FdUrd showed significantly prolonged survival (control, mean survival time ± SEM 11.0 ± 0.3, 20 surviving mice; 5 μg/0.05 ml, 12.2 ± 0.4 days, 20 mice; 25 μg/0.05 ml, 13.8 ± 0.5 days, 20 mice; 50 μg/0.05 ml, 15.2 ± 0.5 days, 20 mice; 100 μg/0.05 ml, 16.8 ± 0.8 days, 20 mice; $P < 0.001$, < 0.0001, < 0.0001, respectively; Fig. 3).

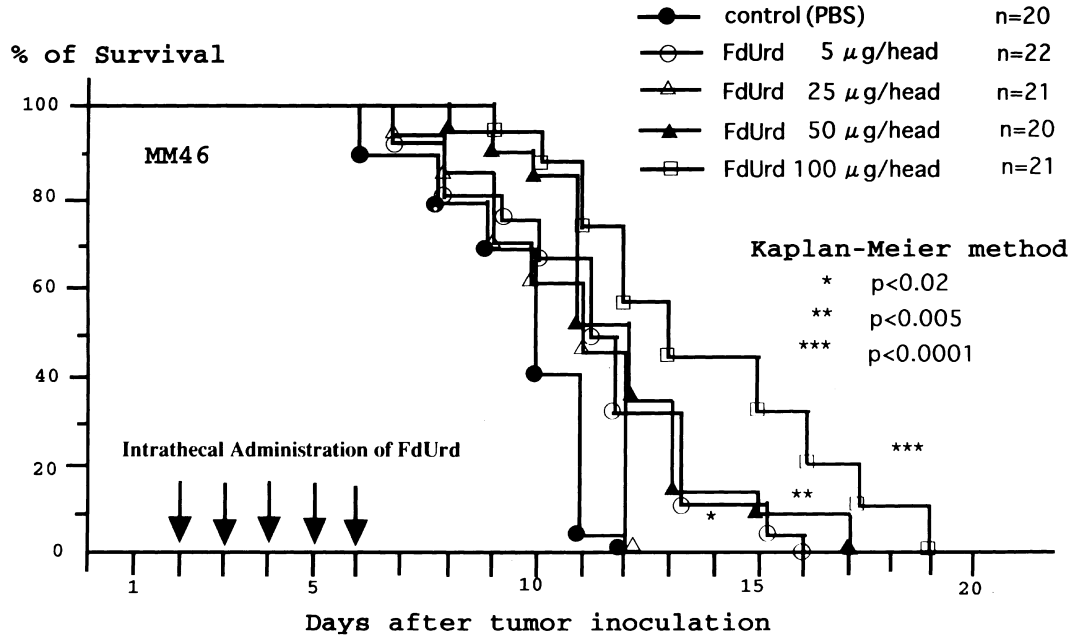
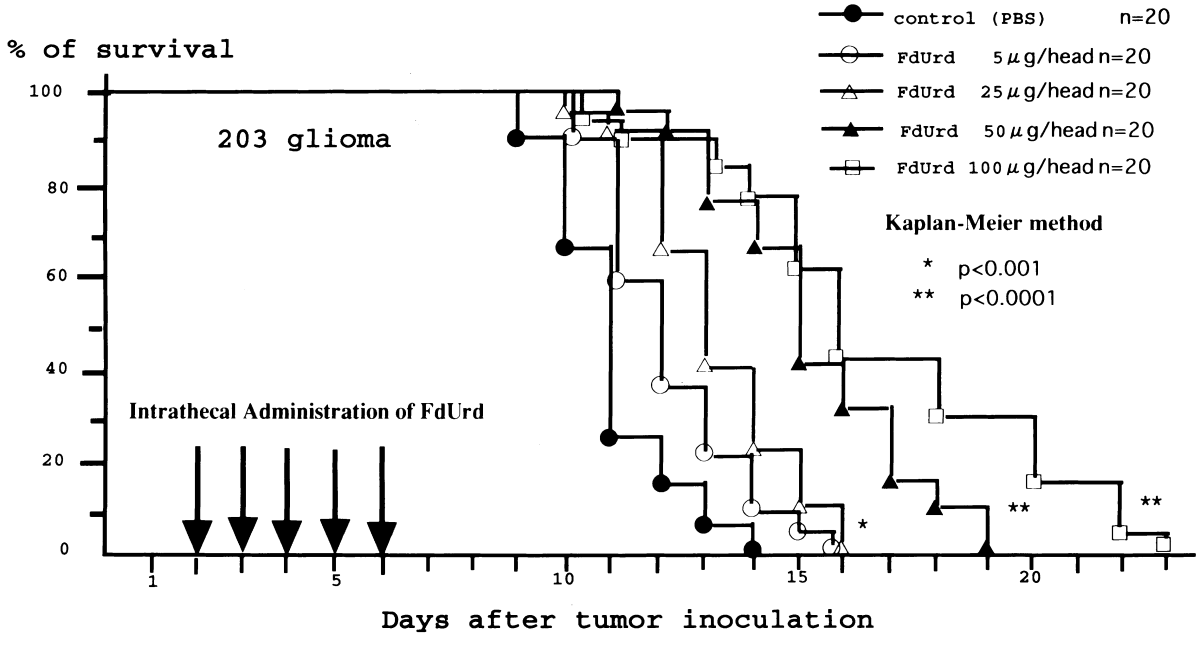


Fig. 2 Antitumor effects of FdUrd on mouse meningeal carcinomatosis using MM46 mammary cancer cells. Intrathecal injection of FdUrd was started 2 days after intrathecal inoculation of MM46 transplantable ascitic mammary cancer cells (10^6 cells) and continued every day for 5 days. Mice were observed daily until death. Each group consisted of 20–22 mice. The groups that received 5, 50 and 100 μg/0.05 ml ×5 of FdUrd showed significantly prolonged survival (115, 123 and 141% of control, respectively; $P < 0.02$, < 0.005 and < 0.0001, respectively)

Neurotoxicity of FdUrd in vivo

In C57BL/6 mice, body weight as an indicator of neurotoxicity and systemic side effects showed a transient decrease at a dose of 100 μg/0.05 ml of FdUrd after four intrathecal injections within 1 week. However, none of the mice died within the 2-month observation period (Fig. 4). The medium-dose group (50 μg/0.05 ml) and low-dose groups (25 and 10 μg/0.05 ml) exhibited no weight decrease. Furthermore, none of the mice developed neuronal manifestations during or after the period of administration of FdUrd. At the end of this period,

Fig. 3 Antitumor effects of FdUrd on mouse meningeal gliomatosis using 203 glioma cells. FdUrd was given intrathecally five times starting 2 days after intrathecal inoculation of 5×10^5 203 glioma cells in suspension, and mice were observed daily until death. Each group consisted of 20 mice. The groups that receved 25, 50 and 100 μg/0.05 ml ×5 of FdUrd showed significantly prolonged survival in a dose-dependent manner (125, 138 and 153% of control; $P < 0.001$, < 0.0001 and < 0.0001, respectively)



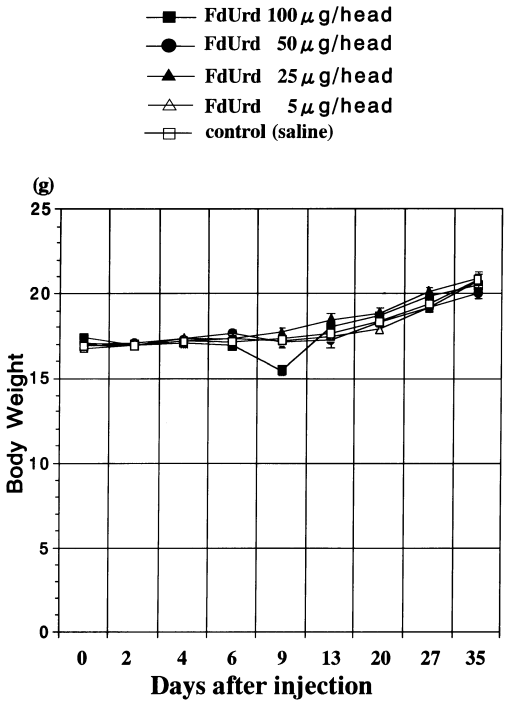


Fig. 4 In vivo drug toxicity after intrathecal injection of FdUrd. The effect of repeated intrathecal treatment with FdUrd on body weight of C57BL/6 mice was determined. FdUrd was injected into the cisterna magna at various doses (5–100 μg/0.05 ml) once daily on days 1, 3, 5 and 7. Each group consisted of 20 mice. A transient decrease in body weight was observed in the group receiving the highest dose of FdUrd (100 μg/0.05 ml). However, none of the mice died during the observation period

the mice were decapitated, and their brains were sectioned and stained with hematoxylin and eosin and by the Klüver Barrera method. No abnormal findings, i.e. demyelination, decreases in the number of neuronal cells, or rupture of the lining of ventricular ependymal cells in the vicinity of the site of administration, were observed in any mice treated with FdUrd (Fig. 5). In the two mice which received a single administration of 100 μg/0.05 ml and were decapitated 24 h after intrathecal administration, histological examinations showed no apparent acute changes such as arachnoiditis.

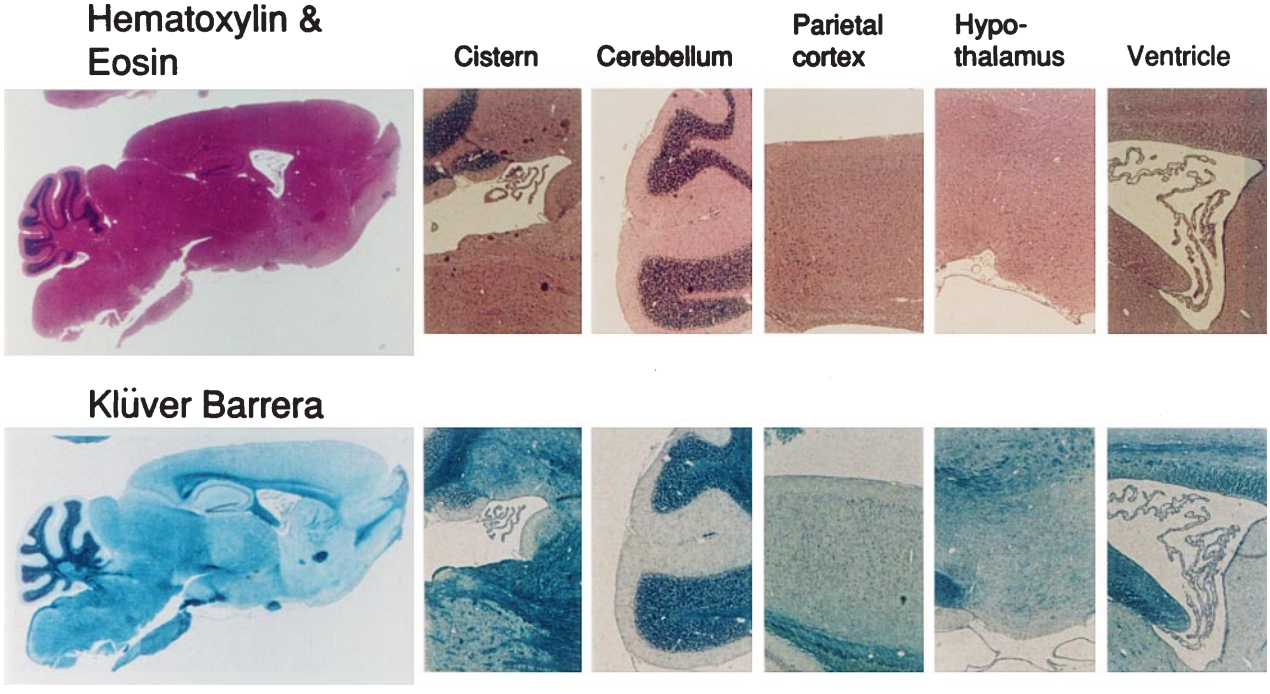
Activities of thymidine phosphorylase and thymidine kinase

Normal mouse, rat and human brain tissues

In the human brain obtained at autopsy, the levels of TPase (nanomoles per milligram protein per 30 min) were slightly lower in the parietal lobe and cerebellum than in the frontal, temporal and occipital lobes and brain stem. The levels of TK (nanomoles per milligram protein per 30 min) in the frontal, temporal, parietal and occipital lobes and cerebellum were similar and there was a significant difference only between the parietal lobe and brain stem ($P < 0.05$, unpaired t-test; Fig. 6).

The levels of TPase in the human brain obtained at both autopsy (Fig. 6) and surgery (Fig. 7) were significantly lower ($P < 0.01$, Welch's t-test), while the level of TK in the human brain obtained at autopsy was sig-

Fig. 5 Histological confirmation of neurotoxicity of FdUrd in mouse (C57BL/6) brain two months after the fourth intrathecal injection. Hematoxylin and eosin and Klüver Barrera staining were focused in the area of the cisterna magna, cerebellum, hypothalamus and parietal cortex. No abnormal histological abnormalities were observed even after four injections of FdUrd at 100 μg/0.05 ml



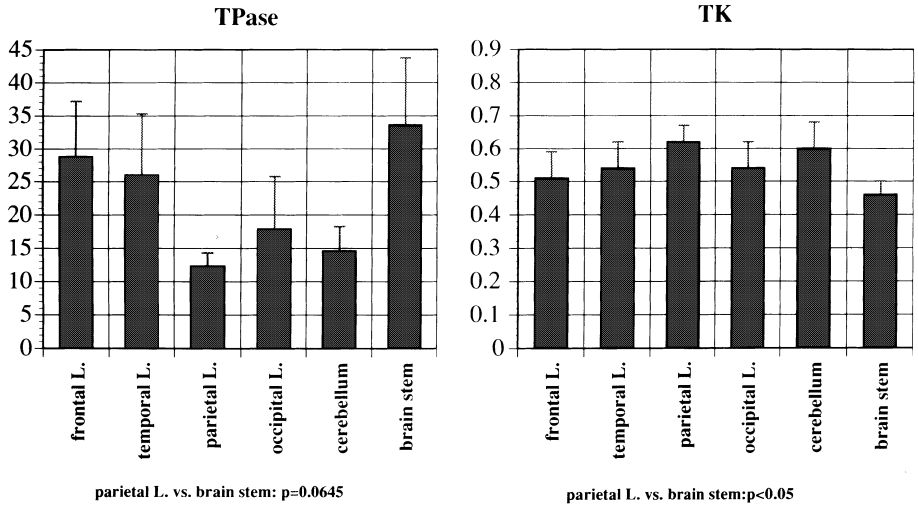


Fig. 6 Activities of TPase and TK in pyrimidine nucleotide and DNA synthesis in normal tissues of six human autopsied brains. The activities are expressed as nanomoles per milligram protein per 30 min (mean ± SE)

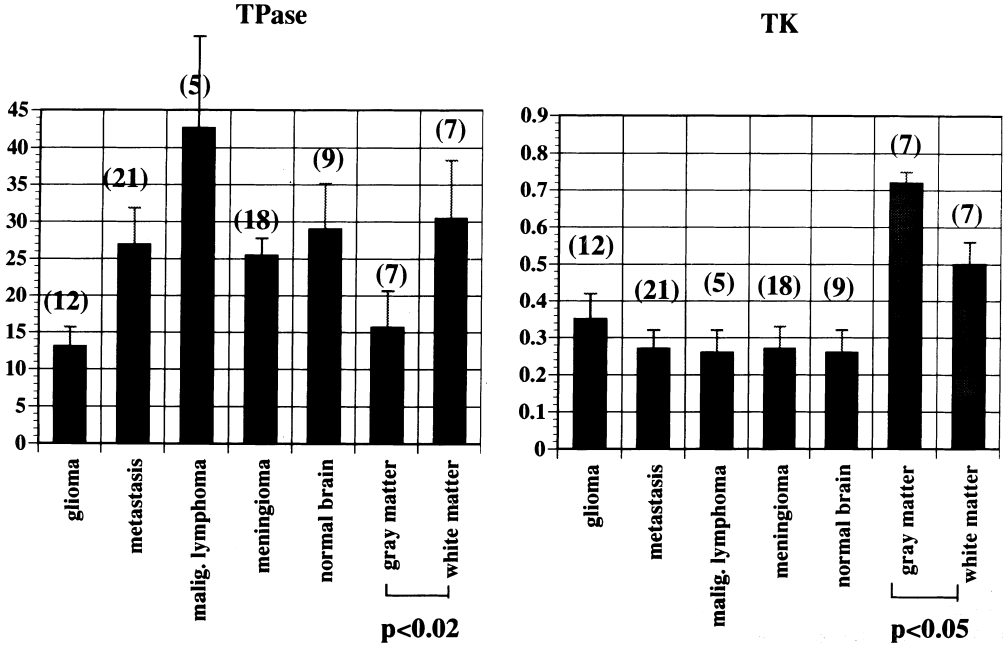


Fig. 7 Activities of TPase and TK in pyrimidine nucleotide and DNA synthesis in normal and tumor tissues of human brain obtained during surgery. The activities are expressed as nanomoles per milligram protein per 30 min (mean ± SE). Normal brain was brain tissue attached to metastatic brain tumor, and gray and white matter was resected when corticotomy was needed to approach a deeply located metastatic brain tumor

nificantly higher ($P < 0.01$, Welch's t-test) than the respective levels in the stomach (TPase, 58.31 ± 12.23 nmol/mg protein per 30 min; TK, 0.28 ± 0.16 nmol/mg protein per 30 min; $n = 9$), colon (TPase, 66.86 ± 20.61; TK, 0.28 ± 0.21; $n = 6$), liver (TPase, 113.1 ± 31.87; TK, 0.39 ± 0.08; $n = 6$) and lung (TPase, 138.2 ± 20.5; TK, 0.19 ± 0.04; $n = 3$) which were measured by the same method as used in the present study and reported by Maehara et al. [17] (values are means ± SE). Normal brain tissue attached to metastatic brain tumor (surrounding brain) showed lower levels of TK than gray and white matter sampled during surgery.

There were significant differences between the levels of TPase in the gray and white matter of the cerebral hemisphere ($P < 0.0005$, paired t-test) in the autopsied brain: the level of TPase in the gray matter was lower than that in the white matter. On the other hand, there was no significant difference in the level of TK between gray and white matter (Fig. 8). In human gray and white matter obtained at surgery, there were significant differences in both TPase and TK between the two tissue specimens: the level of TPase in gray matter was lower than that in the white matter ($P < 0.02$) and the level of TK in gray matter was higher than that in white matter ($P < 0.05$; Fig. 7). Thus, there were some differences in TPase and TK in gray and white matter between brain tissue obtained at autopsy and that obtained at surgical resection. There were marked differences in the levels of

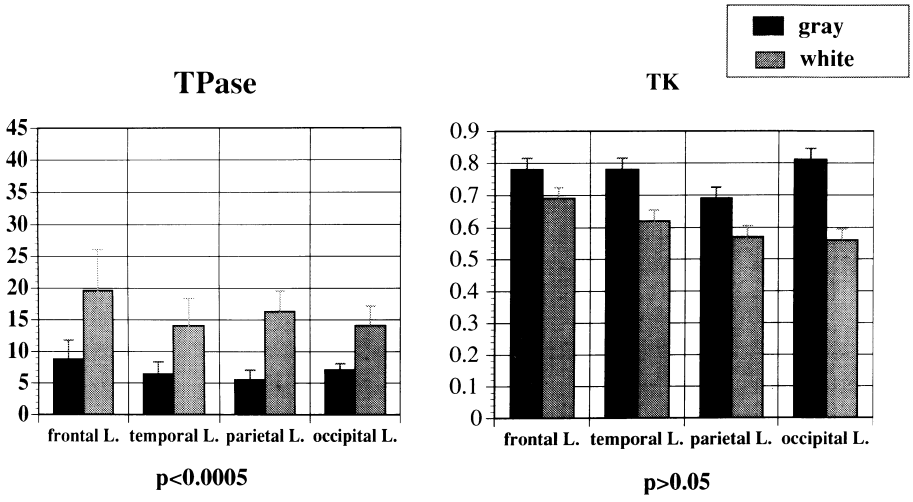


Fig. 8 Differences in activity of Tpase and TK between gray and white matter in six human autopsied brains. The activities are expressed as nanomoles per milligram protein per 30 min (mean ± SE)

TPase and TK in different organs between human, rat and mouse (Figs. 6–9), although in general the level of TPase in brain tissue was less than those in other organs.

The levels of TPase in the brain were lower than those in the stomach, colon, lung and liver in both rats and mice. The levels of TK in the brain were higher than those in stomach, colon, lung, liver and kidney in mice, and they were higher than those in the liver, kidney and spleen although they were almost equivalent to those in the colon and lung in rat. Thus, activities of TPase and TK showed some differences between mice and rats (Fig. 9). The levels of TPase in rat and mouse brains were markedly lower than those in human brain (Figs. 6–8); the levels of TPase in mouse brain were particularly low. On the other hand, the levels of TK in the brains of rats and mice were much higher than those in human brain.

Brain tumors

The levels of TPase in the malignant glioma were appreciably lower than those in brain metastases ($P < 0.05$, unpaired t-test), malignant lymphoma ($P < 0.005$, unpaired t-test), meningioma ($P < 0.002$, unpaired t-test) and normal brain ($P < 0.02$, unpaired t-test). The TPase levels in glioma, metastatic brain tumors and malignant lymphoma were significantly lower ($P < 0.01$, Welch's t-test; Fig. 7) than those in tumors in the stomach (135.6 ± 89.4 nmol/mg protein per 30 min, $n = 13$), colon (147.9 ± 68.6, $n = 12$), liver (204.1 ± 85.5, $n = 11$) and lung (319.3 ± 128.3, $n = 7$) as reported by Maehara et al. [17] (values are means ± SE, n = number of samples assayed).

The levels of TK in glioma, metastasis, malignant lymphoma, meningioma and normal brain were similar and almost equivalent to those in normal tissue in the

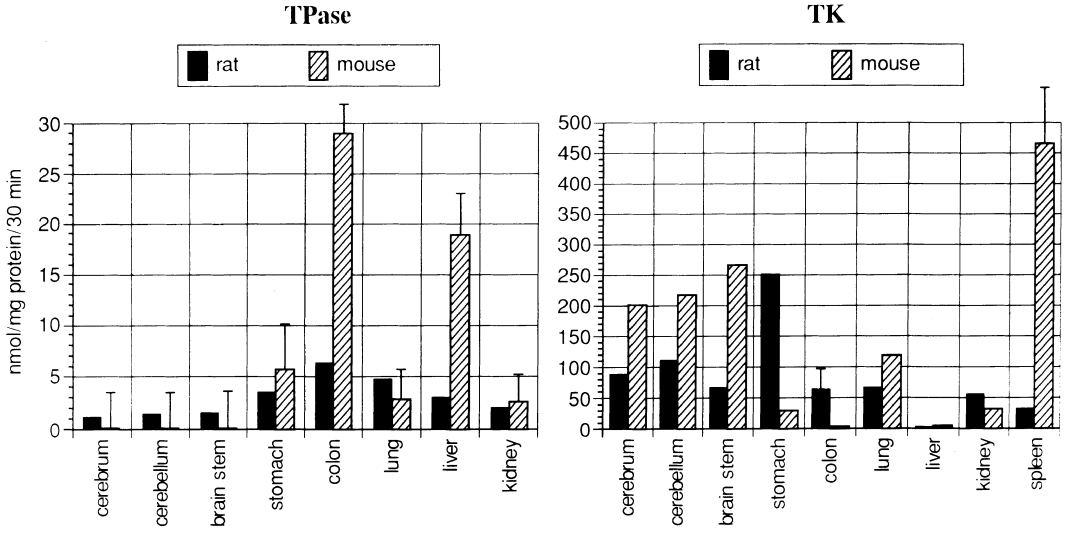


Fig. 9 Activities of TPase and TK in pyrimidine nucleotide and DNA synthesis in normal brain tissue and extracranial organs of rats (Wistar strain, female, 7 weeks old) and mice (C57BL/6, male, 8 weeks old). Five rats and mice were sacrificed and activity is expressed nanomoles per milligram protein per 30 min (mean ± SE)

Table 1 Activities of TPase and TK involving pyrimidine nucleotide and DNA synthesis in CSF from patients with brain tumors. Activity is expressed as nanomoles per milligram protein per 30 min (*ND*, not detectable)

	Number of patients	TPase	TK
Intraparenchymal brain metastasis	22	ND	ND
Single	10	ND	ND
Multiple	12	3.61	ND
Glioblastoma	9	8.63	ND
Malignant lymphoma	4	ND	ND
Meningeal dissemination			
Lung cancer	12	5.26	ND
Breast cancer	2	6.37	ND
Glioblastoma	5	ND	ND
Malignant lymphoma	2	ND	ND

stomach (0.28 ± 0.16 nmol/mg protein per 30 min, $n = 9$), colon (0.28 ± 0.21, $n = 6$) and liver (0.39 ± 0.08, $n = 6$). However, these levels were significantly lower ($P < 0.05$, Welch's t-test) than those in tumors in the stomach (1.49 ± 0.75 nmol/mg protein per 30 min, $n = 13$), colon (1.70 ± 0.80, $n = 12$), liver (1.84 ± 2.25, $n = 11$) and lung (3.06 ± 2.36, $n = 7$) as reported by Maehara et al. [17] (values are means ± SE, n = number of samples assayed).

Cerebrospinal fluid

TK was not detected in any CSF samples from patients with glioblastoma (14 patients, 5 with CSF tumor spread), brain metastases (36 patients, 14 with CSF tumor spread), or malignant lymphoma (6 patients, 2 with CSF tumor spread). TPase was also undetectable except at very low concentrations in four patients (one each with multiple brain metastases, meningeal carcinomatosis from lung cancer, breast cancer and glioblastoma; Table 1).

Discussion

We have previously reported that the antitumor activity of FdUrd against mouse RSV-K glioma and 203 glioma, and T98G human glioblastoma cells is 20- to 500-fold greater than that of 5-FU on a microgram basis in vitro [32]. In vivo, FdUrd had an antitumor effect on mouse and rat meningeal carcinomatosis and mice meningeal gliomatosis. From these findings, the grade of the antitumor activity of FdUrd is considered to be good enough for chemotherapy.

The major mechanism of action of FdUrd is the inhibition of thymidylate synthase by its common metabolite FdUMP, thereby interfering with DNA synthesis [3, 6, 24]. TPase, which converts FdUrd to 5-FU, and TK, which converts FdUrd to FdUMP, are the two key enzymes involved in the metabolism of FdUrd. Theoretically, a lower level of TPase and a higher level of TK are considered better conditions for chemotherapy with FdUrd. TK and TPase are intracellular enzymes, and therefore no or very low levels of both enzymes were thought to be present in the CSF with meningeal dissemination. In fact, extremely low levels of TPase and TK in the CSF were confirmed in the present study, and the low levels of TPase and the high TK activity in tumor cells (which leads to high TK activity in disseminated cells) are conditions that favor intrathecal chemotherapy with FdUrd. In the present study, brain tissue showed low levels of TPase compared with levels in the stomach, colon, liver and lung as reported by Maehara et al. [17], and the levels of TPase in the cortex, where malignant disseminated cells invade, were lower than those in the white matter. These findings indicate that the enzymatic conditions in the CSF space are favorable for intrathecal administration of FdUrd.

The most favorable characteristic of FdUrd for intrathecal chemotherapy of central nervous system tumors is that it exhibits acceptable neurotoxicity. In this study, there were marked differences in TPase and TK activities in the brain between mouse, rat and human tissue. Accordingly, FdUrd could exhibit different degrees of neurotoxicity in different species. We have previously reported that the neurotoxic effect of FdUrd on cultured neurons is far less than those of 5-FU or 5-fluorouridine [32]. In the present in vivo study, none of the mice receiving four intrathecal injections of various doses of FdUrd (5, 25, 50, 100 μg/0.05 ml) showed abnormal histological findings in the brain. However, the possibility of neurotoxicity in mice was considered to be less than that in humans from the observation that the levels of TPase in mouse brain were much lower than those in human brain (Figs. 6–9).

Chemotherapy with FdUrd has been widely employed in patients with advanced colorectal cancer, in this application coinfused with leueovorin [7, 15, 16, 18]. In patients with liver metastasis of colorectal cancer, intra-arterial or intravenous infusion of FdUrd has been used [12, 13, 25]. Continuous intravenous or intra-arterial infusion of FdUrd has also been used for treatment of metastatic renal cell carcinoma [5, 28, 30]. However, to our knowledge, there have been no reports on the intrathecal use of FdUrd. We have found only one report on the penetration of FdUrd-2-^{14}C into the CSF [20]: nonradioactive FdUrd (30 mg/kg, to a total of 2 g) mixed with radioactive FdUrd-2-^{14}C was administered intravenously in a patient with an unknown cancer. Blood and CSF samples were withdrawn at various intervals, and the radioactivity in microgram drug equivalents per milliliter of CSF reached the plasma level in 1 h and remained at approximately the same level as in the plasma for up to 24 hours. Levels of 5 to 10 μg/ml FdUrd were maintained in the CSF for up to 4 hours. There have been other reports of FdUrd chemotherapy using intravenous bolus infusions of doses exceeding 30 mg/kg in which the above concentrations of FdUrd in CSF were estimated, and no severe side effects on the

central nervous system were seen [7, 15, 16, 18, 19]. From these findings, we considered that intrathecal administration of this drug is a safe procedure.

The optimal level of FdUrd required with intrathecal injection for safe and effective chemotherapy appears to be at least 1 ng/ml in the CSF based on the observation of 50% cell growth inhibition in RSV-K glioma, 203 glioma, Walker 256 carcinoma and T98G human glioblastoma cells in cell culture [32]. Consequently, a dose of 1 to 5 μg FdUrd may be recommended, taking the total volume of CSF to be approximately 150 ml, and that 1 to 5 μg FdUrd would be diluted to 6 to 30 ng/ml. However, assuming distribution only in the CSF may be erroneous. These concentrations may enable us to ignore the neurotoxicity from the findings of CSF penetration of FdUrd following intravenous injection at 30 mg/kg. A much higher dose of FdUrd, 1.5 mg for example, may be injected intrathecally, considering that a concentration of 10 μg/ml in CSF was obtained after intravenous injection of 30 μmg/kg FdUrd in the study cited above [19] and that the total volume of CSF is approximately 150 ml.

5-FU shows much greater neurotoxicity than FdUrd, as demonstrated in vitro [32]. 5-FU has been reported to penetrate the CSF space at the rate of a few percent of the plasma level following rapid intravenous injection in a patient without evidence of intracranial disease [4]. In this patient, 15 mg/kg 5-FU was injected and the maximum CSF concentration was 6.2 nmol/ml. This level is equivalent to 806.6 ng/ml. From this finding, the dose of FdUrd that we propose, 1–5 μg, can be considered to be safe. However, the changes in CSF FdUrd concentration over time following intrathecal administration may be more important than peak levels in determining tumor response and predicting neurotoxicity. Therefore, clinical studies should be performed as we do not currently have data regarding the changes in CSF FdUrd concentrations over time following intrathecal administration.

In conclusion, FdUrd shows good antitumor activity and minimal neurotoxicity both in vitro [32] and in vivo. We also showed that the levels of one of the two key enzymes involved in FdUrd metabolism, TPase, are low in brain tissue, malignant glioma and metastatic brain tumors compared with those in normal and tumor tissues of other organs. In particular, TPase activity in the gray matter was lower than that in the white matter. Moreover, TPase was not detected in 94% of patients with malignant brain tumors and TK was not detected in any of the patients with brain tumors in the CSF. These findings suggest that the central nervous system, particularly the intrathecal space, provides a favorable environment for FdUrd administration which would produce a good therapeutic effect on the meningeal dissemination of malignant tumor without significant toxicity. However, the response to intrathecal FdUrd, toxicity data and our dosing suggestions require confirmation. We also recommend that future studies be designed to examine biochemical markers (Tpase, TK, FdUMP, TS inhibition) as potential predictors of response.

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