

methionine or leucine is more suitable for hyperglycemic patients.

ACKNOWLEDGMENTS

This work was supported by National Cancer Institute grants CA52880, CA53172 and CA56731.

REFERENCES

- Higashi K, Clavo AC, Wahl RL. In vitro assessment of 2-fluoro-2-deoxy-D-glucose, L-methionine and thymidine as agents to monitor the early response of a human adenocarcinoma cell line to radiotherapy. *Nuklearmedizin* 1993;34:773-779.
- Minn H, Clavo AC, Grenman R, Wahl RL. In vitro comparison of cell proliferation kinetics and uptake of tritiated 2-fluoro-2-deoxy-D-glucose and L-methionine in squamous cell carcinoma of the head and neck. *J Nucl Med* 1995;36:252-258.
- Kubota K, Ishiwata K, Kubota R, et al. Tracer feasibility for monitoring tumor radiotherapy: a quadruple tracer study with [¹⁸F]FDG or [¹⁸F]fluorine-18-fluorodeoxyuridine, L-[methyl-¹⁴C]methionine, [³H]thymidine and ⁶⁷Ga. *J Nucl Med* 1991;32:2118-2123.
- Ishiwata K, Kubota K, Murakami M, Kubota R, Senda M. A comparative study on protein incorporation of L-[methyl-³H]methionine, L-[^{1-¹⁴C]leucine and L-2-[¹⁸F]fluorotyrosine in tumor bearing mice. *Nucl Med Biol* 1993;20:895-899.}
- Miyazawa H, Arai T, Iio M, Hara T. PET imaging of non-small-cell lung carcinoma with carbon-11-methionine: relationship between radioactivity uptake and flow-cytometric parameters. *J Nucl Med* 1993;34:1886-1891.
- Koh WJ, Griffin TW, Rasey JS, Laramore GE. Positron emission tomography. A new tool for characterization of malignant disease and selection of therapy. *Acta Oncol* 1994;33:323-327.
- Vander Borgh T, Pauwels S, Lambotte L, et al. Brain tumor imaging with PET and 2-[carbon-11]thymidine. *J Nucl Med* 1994;35:974-982.
- Wahl RL, Quint LE, Greenough RL, Meyer CR, White RI, Orringer MB. Staging of mediastinal non-small cell lung cancer with FDG PET, CT and fusion images: preliminary prospective evaluation. *Radiology* 1994;191:371-377.
- Jansson T, Westlin JE, Ahlstrom H, Lilja A, Langstrom B, Bergh J. Positron emission tomography studies in patients with locally advanced and/or metastatic breast cancer: a method for early therapy evaluation? *J Clin Oncol* 1995;13:1470-1477.
- Torizuka T, Tamaki N, Inokuma T, et al. In vivo assessment of glucose metabolism in hepatocellular carcinoma with FDG-PET. *J Nucl Med* 1995;36:1811-1817.
- Wahl RL, Cody RL, Hutchins GD, Mudgett EE. Primary and metastatic breast carcinoma: initial clinical evaluation with PET with the radiolabeled glucose analogue 2-[¹⁸F]-fluoro-2-deoxy-D-glucose. *Radiology* 1991;179:765-770.
- Wahl RL, Henry CA, Ethier SP. Serum glucose: effects on tumor and normal tissue accumulation of 2-[¹⁸F]-fluoro-2-deoxy-D-glucose in rodents with mammary carcinoma. *Radiology* 1992;183:643-647.
- Langen KJ, Braun U, Rota Kops E, et al. The influence of plasma glucose levels on fluorine-18-fluorodeoxyglucose uptake in bronchial carcinomas. *J Nucl Med* 1993;34:355-359.
- Lindholm P, Minn H, Leskinen-Kallio S, Bergman J, Ruotsalainen U, Joensuu H. Influence of the blood glucose concentration on FDG uptake in cancer - a PET study. *J Nucl Med* 1993;34:1-6.
- Minn H, Zasadny KR, Quint LE, Wahl RL. Lung cancer: reproducibility of quantitative measurements for evaluating 2-[¹⁸F]-fluoro-2-deoxy-D-glucose uptake at PET. *Radiology* 1995;196:167-173.
- Clavo AC, Brown RS, Wahl RL. Fluorodeoxyglucose uptake in human cancer cell lines is increased by hypoxia. *J Nucl Med* 1995;36:1625-1632.
- Higashi K, Clavo AC, Wahl RL. Does FDG uptake measure proliferative activity of human cancer cells? In vitro comparison with DNA flow cytometry and tritiated thymidine uptake. *J Nucl Med* 1993;34:414-419.
- Freshney RI. Biology of the cultured cells. In: Freshney RI, ed. *Culture of animal cells - a manual of basic technique*, 2nd ed. New York: Alan R. Liss Inc.; 1987:7-13.
- Levin L, Gevers W. Metabolic alterations in cancer. I. Carbohydrate metabolism. *S Afr Med J* 1981;59:518-521.
- Shapot VS. Some biochemical aspects of the relationship between the tumor and the host. *Adv Cancer Res* 1972;15:253-286.
- Boado RJ, Pardridge WM. Glucose deprivation causes posttranscriptional enhancement of brain capillary endothelial glucose transporter gene expression via Glut-1 mRNA stabilization. *J Neurochem* 1993;60:2290-2296.
- Gould GW, Holman GD. The glucose transporter family: structure, function and tissue-specific expression. *Biochem J* 1993;295:329-341.
- Simmons RA, Flozak AS, Ogata ES. Glucose regulates glut 1 function and expression in fetal rat lung and muscle in vitro. *Endocrinology* 1993;132:2312-2318.
- Klip A, Tsakiridis T, Marette A, Ortiz PA. Regulation of expression of glucose transporters by glucose: a review of studies in vivo and in cell cultures. *FASEB J* 1994;8:43-53.
- Gullino PM, Grantham FH, Courtney AH. Glucose consumption by transplanted tumors in vivo. *Cancer Res* 1967;27:1031-1040.
- Haberkorn U, Morr I, Oberdorfer F, et al. Fluorodeoxyglucose uptake in vitro: aspects of method and effects of treatment with gemcitabine. *J Nucl Med* 1994;35:1842-1850.
- Goodgame J Jr, Lowry SF, Brennan MF. Nutritional manipulations and tumor growth. II. The effects of intravenous feeding. *Am J Clin Nutr* 1979;32:2285-2294.
- Asano T, Shibasaki Y, Lin JL, et al. Expression of the Glut-1 glucose transporter increases thymidine uptake in chinese hamster ovary cells at low glucose concentrations. *Cancer Res* 1991;51:4450-4454.
- Reilly JJ, Goodgame JT, Jones DC, Brennan MF. DNA synthesis in rat sarcoma and liver: the effect of starvation. *J Surg Res* 1977;22:281-286.
- Goodgame J Jr, Lowry SF, Reilly JJ, Jones DC, Brennan MF. Nutritional manipulations and tumor growth. I. The effects of starvation. *Am J Clin Nutr* 1979;32:2277-2284.
- Sauer LA, Nagel WO, Dauchy RT, Miceli LA, Austin JE. Stimulation of tumor growth in adult rats in vivo during an acute fast. *Cancer Res* 1986;46:3469-3475.

Intrathecal 5-[¹²⁵I]Iodo-2'-Deoxyuridine in a Rat Model of Leptomeningeal Metastases

Shailendra K. Sahu, Patrick Y.C. Wen, Catherine F. Foulon, James S. Nagel, Peter McL. Black, S. James Adelstein and Amin I. Kassir

Departments of Radiology, Neurology and Surgery, Harvard Medical School, Boston, Massachusetts

The antitumor effect of 5-[¹²⁵I]iodo-2'-deoxyuridine (¹²⁵IUdR) was examined in a rat model of leptomeningeal metastases. In this model, 50% of rats develop paralysis of hind limbs in 9.20 ± 0.02 days and die in 12.1 ± 2.1 days after intrathecal (i.t.) implantation of 5 × 10⁵ 9L rat gliosarcoma cells. **Methods:** Three days after implantation of 9L gliosarcoma cells, ¹²⁵IUdR was administered intrathecally to rats as: (a) a single injection (500 μCi/rat), (b) five daily injections (100 μCi/day) or (c) a continuous 5-day infusion (0.5 μl/hr, total of 500 μCi), and the animals were monitored for the onset of paralysis. Control groups received physiologic saline. For biodistribution studies, rats received a bolus injection of ¹²⁵IUdR (10 μCi) 5 days after tumor-cell implantation and were killed 1, 8, 24, and 48 hr later. Tissues and organs, including the spinal cord, were isolated and their radioactive content determined. The results were expressed as percent injected dose per gram of wet tissue. Histological sections of the spinal cord were also prepared and used for autoradiographic detection of DNA-incorporated ¹²⁵IUdR. **Results:**

Treatment with i.t. administered ¹²⁵IUdR (500 μCi/rat) significantly (p ≤ 0.005) prolonged the median time of paralysis to 11.2 ± 0.1, 12.3 ± 0.1 and 15.2 ± 0.4 days for the single-dose, five daily injections and continuous infusion groups, respectively. Radioactivity cleared rapidly from all tissues except the thyroid and tumor cells growing within the spinal cord. Autoradiography demonstrated that normal cells in the tumor-bearing spinal cord were void of radioactivity. **Conclusion:** The results suggest that a selective antitumor effect could be achieved in treating leptomeningeal metastases with i.t. administered ¹²⁵IUdR.

Key Words: leptomeningeal metastases; intrathecal tumor; iodine-125-IUdR; gliosarcoma

J Nucl Med 1997; 38:386-390

Leptomeningeal metastases are a serious complication of cancer characterized by neurologic dysfunction at multiple levels of the neuraxis. This disease develops in 5%-8% of patients with solid tumors, in 5%-29% of patients with non-Hodgkin's lymphoma and in 11%-70% of patients with leukemia (1,2). The prognosis of patients who develop leptomeningeal

Received Feb. 22, 1996; revision accepted Jul 1, 1996.
For correspondence or reprints contact: Amin I. Kassir, PhD, Shields Warren Radiation Laboratory, 50 Binney St., Boston, MA 02115.

geal metastases is poor. Without therapy, the median survival is 4–6 wk. With current treatment regimens, the median survival is 3–6 mo (1–3).

Iodine-125 is a prolific emitter of low-energy (<1 keV) electrons (~20 electrons per decay) that dissipate their energy typically within nanometer distances from the decay site (4,5). Consequently, the biologic toxicity of this Auger electron emitter resembles that of high-LET radiations (exponential decrease in survival of mammalian cells) when ^{125}I decays in close proximity to DNA (5,6–8). The thymidine analog 5-iodo-2'-deoxyuridine radiolabeled with ^{125}I is readily incorporated into DNA of proliferating cells during DNA synthesis. The DNA-incorporated $^{125}\text{IUdR}$ is retained in the cells and their progeny and has been shown to be extremely radiotoxic to cells (5–7). Intravenously administered $^{125}\text{IUdR}$, however, is unlikely to be useful as an antitumor agent because of its nonspecific uptake by all proliferating cells (9,10) and its rapid dehalogenation ($T_{1/2} = 5\text{--}7$ min) in the liver (9,11,12). On the other hand, locoregional administration of IUdR radiolabeled with the Auger-electron-emitting radionuclide ^{125}I or ^{123}I has been shown to be therapeutically effective in mice with intraperitoneal ovarian tumors (13,14) and in rats with solid brain tumors (15). In this investigation, we demonstrate the therapeutic effectiveness of intrathecally administered $^{125}\text{IUdR}$ in a rat model of leptomeningeal metastases.

MATERIALS AND METHODS

A modification of the leptomeningeal metastases model developed by Kooistra et al. (16) was used. Male CDF (Fischer 344) rats, weighing about 300 g, were anesthetized with an intraperitoneal injection of ketamine–xylazine–acepromazine maleate (75 mg–3.9 mg–0.75 mg per kg body weight). The anesthetized rats were secured on a special stand with their heads elevated and the long-axis of the body at a 90° angle. The atlanto-occipital membrane caudal to the external occipital protuberance in the neck region was surgically exposed and punctured using a 20-G needle. Approximately 8 cm of a polyethylene catheter (PE-10 tubing prethinned by stretching) was inserted through the puncture in the atlanto-occipital membrane into the subarachnoid space dorsal to the spinal cord. The external end of the catheter was sealed and tied under the skin before closing the wound in three layers. Rats were observed for 1 wk and only those rats free of any signs of paralysis were used in the study.

Monolayers of exponentially growing 9L gliosarcoma cells (17) were trypsinized, washed and suspended in phosphate-buffered saline, pH 7.2, and 5×10^5 cells were implanted intrathecally in rats ($n = 17$) in a bolus of 5- μl volume through the catheter already in place, which was then flushed with 10 μl 0.9% saline. The cell suspension was replaced with phosphate-buffered saline, pH 7.2, in the nontumor-bearing rats ($n = 9$). The rats were observed daily for symptoms of paralysis, defined as the inability to walk and to stand on all four limbs. All rats that developed paralysis of the hind limbs were killed except in one experiment where death caused by the growth of intrathecal tumor was recorded. In this experiment, food and water were lowered into the cage so they could be easily accessed by the paralyzed rats. Routine laboratory techniques were used to prepare histology slides of tumor-bearing spinal cords.

Carrier-free $^{125}\text{IUdR}$ was prepared by a method developed in this laboratory (18), solubilized in 0.9% saline and sterilized by 0.22- μm Millipore filtration before use.

Biodistribution studies using $^{125}\text{IUdR}$ were performed in tumor-bearing ($n = 9$, injected i.t. with 5×10^5 9L gliosarcoma cells) and in nontumor-bearing control ($n = 10$, injected i.t. with 5 μl PBS) rats. In these experiments, each rat received 10 μCi of $^{125}\text{IUdR}$ (a bolus of 5- μl volume, i.t. injection) five days after i.t. administra-

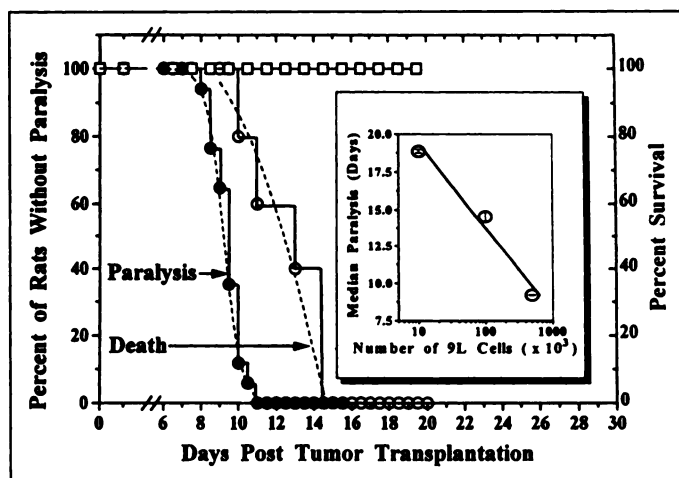


FIGURE 1. Paralysis (●) and death (○) of rats ($n = 17$) injected intrathecally with 5×10^5 9L gliosarcoma cells. Control rats ($n = 9$) injected intrathecally with saline (□). Insert contains data previously obtained indicating relationship between number of tumor cells injected and onset of paralysis.

tion of the 9L tumor cells or PBS. The rats were killed at 1, 8, 24 and 48 hr after $^{125}\text{IUdR}$ injection. Various tissues and organs were isolated, blotted and weighed; their radioactive content was determined in a gamma counter, and the results were expressed as percent injected dose per gram of wet tissue. The entire spinal column of each rat was also excised and fixed in 10% buffered formalin before isolating the spinal cord from the surrounding bones and measuring the radioactive content in 1-cm slices. Forty-eight hours after the $^{125}\text{IUdR}$ i.t. injection, histological sections (6- μm thick) of fixed spinal cords from tumor-bearing rats were also prepared and used for the autoradiographic detection of DNA-incorporated $^{125}\text{IUdR}$. To this end, the slides were washed in methanol at -20°C , coated in NTB emulsion (Kodak), and stored at 4°C . After 14 days of storage in total darkness, the slides were developed with Kodak developer D19 for 3 min at 15°C , fixed with Kodak fixer for 5 min, and washed and stained with hematoxylin-eosin. Finally, the slides were dehydrated and mounted in Permount.

Treatments of i.t. tumors with $^{125}\text{IUdR}$ were performed in four groups of 11–12 rats with a total dose of 500 μCi of $^{125}\text{IUdR}$ /rat administered i.t. as: (a) a single injection (20 μl), (b) five daily injections (100 $\mu\text{Ci}/20 \mu\text{l/day}$) or (c) a continuous 5-day infusion (0.5 $\mu\text{l/hr}$, 20 μl) using micro-osmotic pumps. Rats in the control group received 0.9% saline i.t. The treatments were started 3 days after 5×10^5 9L gliosarcoma tumor cells were injected i.t. into each rat. All i.t. injections or infusions were performed through the i.t. catheter already in place. Rats were monitored daily for symptoms of paralysis. The median duration for the onset of paralysis in each group of rats was statistically compared using the Mantel-Haenszel (log-rank) test for comparison of survival curves.

RESULTS

Approximately 95% of rats implanted i.t. with catheters into the subarachnoid space remained free of any sign of paralysis in the immediate period after the surgical procedure. The onset of paralysis in the hind limbs of these rats was caused by i.t. growth of tumor cells and was predictable (Fig. 1). For example, 50% of the rats that received 5×10^5 tumor cells i.t. were paralyzed in 9.20 ± 0.02 days and died in 12.1 ± 2.1 days, whereas none of the nontumor-bearing control animals with i.t. catheter and injected i.t. with saline were paralyzed or died during 20 days of observation. Furthermore, paralysis occurred at an earlier time when the number of transplanted tumor cells was increased (insert, Fig. 1).

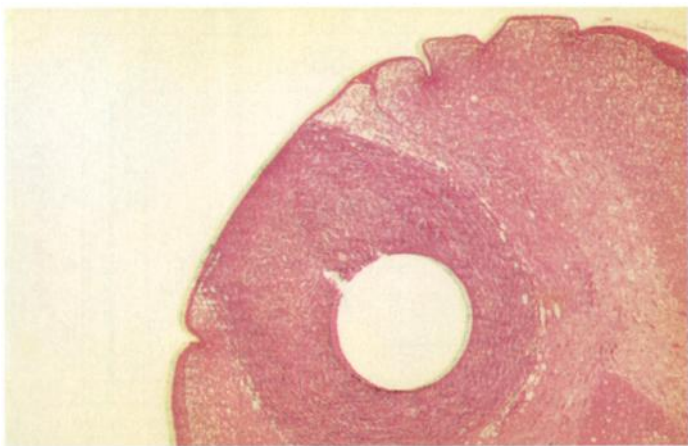


FIGURE 2. Photomicrograph of spinal cord of 9L-gliosarcoma-bearing rat showing catheter and surrounding tumor.

Histological examination of spinal cords revealed the bulk of the tumor mass was around the tip of the catheter. The tumor was confined to the subarachnoid space around the catheter and in various places encircled the spinal cord and compressed it (Fig. 2). In a few instances, tumor cells infiltrated the white matter and grew around the blood vessels (data not shown).

In both tumor- and nontumor-bearing rats, the radioactivity from i.t.-administered ^{125}I UdR was cleared over time from all normal tissues with the exception of the thyroid (Figs. 3 and 4). The percentage of the injected dose in the urine, stomach, and blood of tumor-bearing rats appeared to be higher than that in the controls, possibly reflecting increased leakage of radioactivity into the systemic circulation due to the presence of tumor within the intrathecal space. In tumor-bearing animals, 3%–4% of the injected radioactive dose remained associated with the spinal cord. At 48 hr, the percent injected dose per gram of the spinal cords from tumor-bearing (2.94 ± 0.48) and nontumor-bearing (0.23 ± 0.06) rats was significantly different ($p \leq 10^{-6}$, Student's paired t-test). When the radioactive content of 1-cm consecutive sections of spinal cord was plotted as a function of distance from the cranial apex of the excised spinal cord, the radioactivity (area under the curve) in tumor-bearing rats was ~17-fold greater than that of nontumor-bearing rats

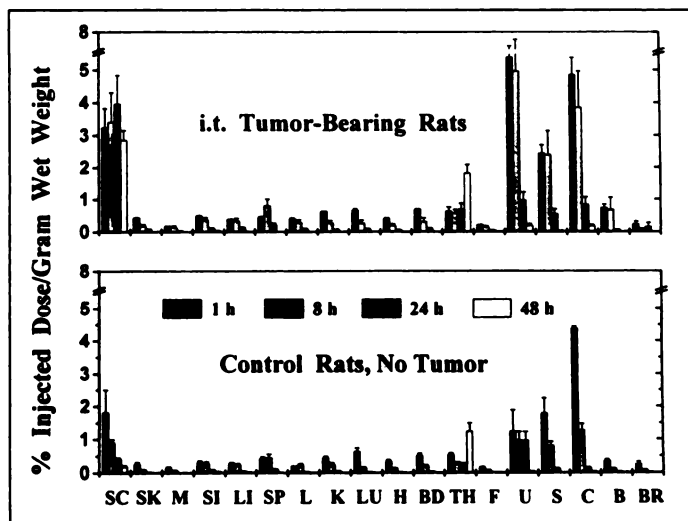


FIGURE 3. Biodistribution of ^{125}I in 9L-gliosarcoma-bearing and control rats after intrathecal administration of $10 \mu\text{Ci } ^{125}\text{I}$ UdR. SC = spinal cord; SK = skin, M = muscle, SI = small intestine, LI = large intestine, SP = spleen, L = liver, K = kidney, LU = lung, H = heart, BD = bladder, TH = thyroid, F = femur, U = urine, S = stomach, C = stomach contents, B = blood, BR = brain.

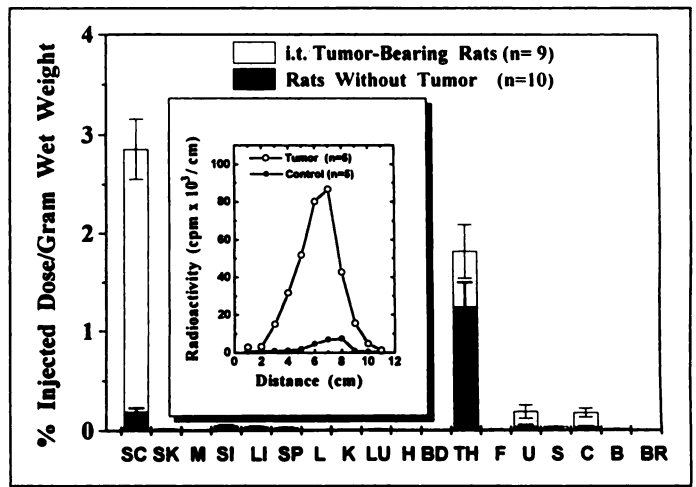


FIGURE 4. Comparison of biodistribution of ^{125}I in 9L-gliosarcoma-bearing and control rats 48 hr after intrathecal administration of $10 \mu\text{Ci } ^{125}\text{I}$ UdR. Insert indicates distribution of ^{125}I along spinal cord (0 cm = neck region) of 9L-gliosarcoma-bearing (○) and control (●) rats at 48 hr. See Figure 3 for abbreviations.

(insert, Fig. 4). The peak activity seemed to correspond to the position of the catheter tip where the bulk of the tumor mass was located. Autoradiography of the tissue sections from tumor-bearing spinal cords showed no radioactivity associated with normal cells of the spinal cord: silver grains were associated only with tumor cells (Fig. 5). Since any unbound radioactivity was washed away during processing of tissue

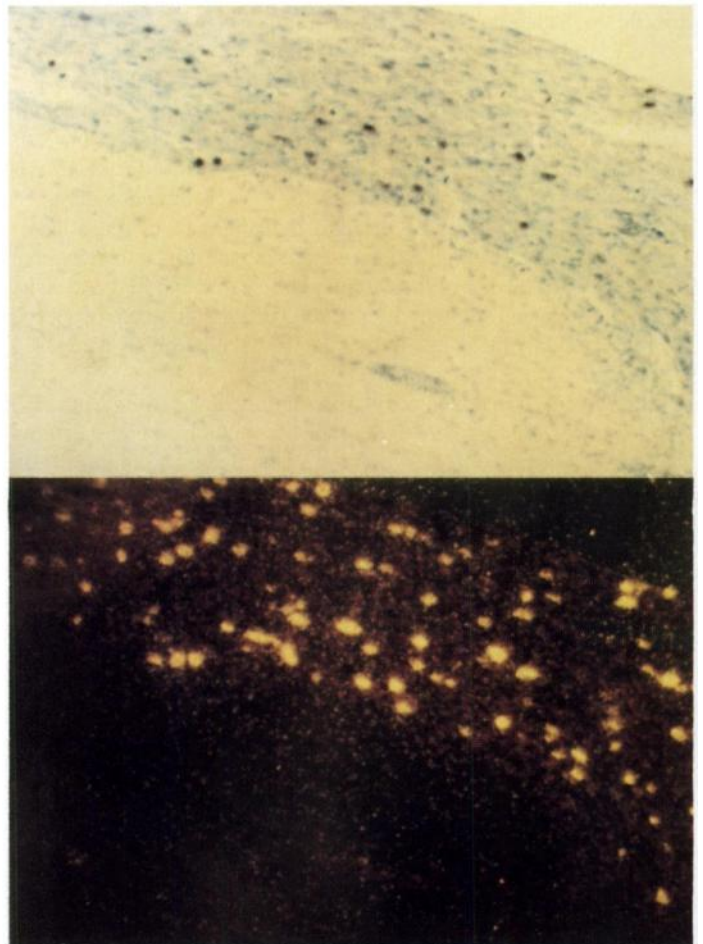


FIGURE 5. Autoradiograph of thin section from spinal cord of 9L-gliosarcoma-bearing rat 48 hr after intrathecal administration of $10 \mu\text{Ci } ^{125}\text{I}$ UdR, light (top) and dark (bottom) fields.

TABLE 1
Induction of Paralysis (days) in Rats Bearing Intrathecal Tumors After Intrathecal Administration of 500 μCi ^{125}I UdR*

Treatment	No. of animals	Median of paralysis \pm s.e.m.	Range	% Increase in median paralysis	p value [†]
Saline	11	9.0 \pm 0.1	4	0.0	—
^{125}I UdR					
single injection	12	11.2 \pm 0.1	3	24.4	\leq 0.005
five daily injections	12	12.3 \pm 0.1	6	36.7	\leq 0.005
continuous five-day infusion	11	15.2 \pm 0.4	11	68.9	\leq 0.005

*Rats were treated three days after i.t. injection of 5×10^5 tumor cells. Control rats received i.t. saline.

[†]Values were determined by Mantel-Haenszel (log-rank) test for comparison of survival curves.

sections for autoradiography, these grains reflect DNA-incorporated radionuclide.

Therapy with ^{125}I UdR in tumor-bearing rats was started 3 days after i.t. inoculation of 5×10^5 tumor cells. The results of these experiments are summarized in Table 1 and illustrated in Figure 6. In the experiment shown, 50% of the control rats (with i.t. tumor and treated with i.t. saline injection) developed paralysis on 9.0 ± 0.1 days. In comparison, the onset of paralysis in tumor-bearing rats treated with ^{125}I UdR (single i.t. administration—500 μCi , five daily i.t. injections—100 $\mu\text{Ci}/\text{day}$ or 5-day i.t. infusion—500 μCi) was significantly prolonged. In this study, the time to median paralysis was 11.2 ± 0.1 , 12.3 ± 0.1 and 15.2 ± 0.4 days, respectively, for the three experimental groups. The paralysis curves for all three treatments were statistically significant ($p \leq 0.005$) according to the Mantel-Haenszel test. However, all of the tumor-bearing rats eventually developed paralysis and were killed.

DISCUSSION

In the past two decades, efforts to develop therapy for leptomeningeal metastases have continued to be unsuccessful. The difficulty lies in the need to treat the entire neuraxis, since tumor cells are disseminated throughout the subarachnoid space in close proximity to neural structures. Consequently, various intrathecal therapies are being explored, including radioiodi-

nated monoclonal antibodies directed against tumor-associated antigens (19), gene therapy (20) and chemotherapy (3,21–23). All these attempts have been only partially effective. Although treatment often provides good local control, leptomeningeal metastases usually occur in the setting of systemic relapse, and patients eventually die of their systemic disease. Parallel efforts are needed to find therapies for systemic disease as well as for leptomeningeal metastases.

The radionuclide ^{125}I causes predominantly double-strand breaks (dsb) in DNA when ^{125}I decays in close proximity to DNA (24) or when it is incorporated into nuclear DNA (25–30). While most of these dsb seem to be repaired at the same rate as those observed with gamma rays (31), the decay of this radionuclide after DNA incorporation in the form of ^{125}I UdR is extremely toxic to cultured mammalian cells (5–7). In comparison, when ^{125}I decays outside the cell nucleus, it is quite innocuous (5,32,33). In fact, in cultured cells, the relative biological effectiveness of ^{125}I UdR compared with that of conventional gamma irradiation is ~ 8 -fold greater when ^{125}I UdR is DNA-incorporated and less than twofold greater when it decays outside the cell nucleus (5,7,33,34). While these biophysical characteristics demonstrate the high toxicity of ^{125}I UdR and therefore its suitability as a radiotherapeutic agent, the nonspecificity of this cycle-dependent agent (i.e., taken up by all dividing cells) may limit its utility to those cancers where local or regional administration is feasible.

In this report, 3%–4% of the administered radioactivity was associated with the spinal cords of tumor-bearing rats after 48 hr. It is important to note that these percent injected dose per gram values greatly underestimate the actual tumor uptake of ^{125}I UdR because the tumor mass constitutes only a small fraction of the weighed spinal cord. Moreover, the DNA-incorporated activity can be enhanced by the administration of various thymidylate synthetase antimetabolites. For example, both 5-fluorodeoxyurine (35) and methotrexate (Kassis AI, Adelstein SJ, unpublished results) have been shown to increase the DNA incorporation of ^{125}I UdR in mammalian cells. Since methotrexate is currently used in the clinic for therapy of tumors within the spinal cord (3,21), the potential of this combined therapy should be explored.

The data suggest the therapeutic effectiveness of intrathecally administered ^{125}I UdR against intrathecal gliosarcoma tumors in a rat model. However, while the total dose of ^{125}I UdR was equal in all cases, a direct comparison of the treatments cannot be made. Nevertheless, continuous infusion for 5 days was superior to single or five daily intrathecal injections of ^{125}I UdR (Fig. 6), an observation that is consistent with the effects of cell-cycle-specific agents. Furthermore, five daily injections of ^{125}I UdR had greater effect than the single injection ($p \leq 0.05$). However, in the present experiments, there were no long-term survivors. The failure of ^{125}I UdR to cure these rats may be due to the low growth fraction of intrathecally growing 9L tumor cells. DNA-incorporated ^{125}I UdR activity was observed throughout the tumor mass (Fig. 5), clearly demonstrating that the low molecular weight IudR molecule can diffuse throughout the cellular layers of the tumor and be incorporated into the DNA of tumor cells undergoing DNA synthesis. Similar problems may be encountered in patients with leptomeningeal metastases. In contrast, we have observed the survival of $\sim 20\%$ of rats bearing intracranial 9L gliosarcoma solid tumors and mice with ascites ovarian tumors treated with locoregionally administered ^{125}I UdR or ^{123}I UdR (13–15). In the studies with rats bearing brain tumors (15), this may have been due to the fact that 2×10^4 9L gliosarcoma cells were transplanted only 24 hr before ^{125}I UdR intratumoral injection, while the intrathe-

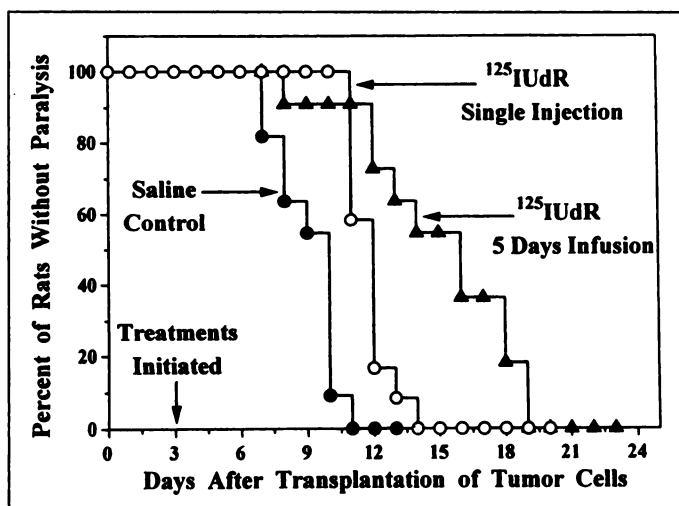


FIGURE 6. Induction of hind-leg paralysis after intrathecal administration of 500 μCi ^{125}I UdR in rats bearing intrathecal 9L gliosarcoma. Data from group having five daily injections not shown.

cal tumors treated in our current studies were initiated with 25-fold more cells than used in the brain tumor model and all treatments were begun after 3 days. Consequently, the tumor burden in the intrathecal studies may have been approximately 75-fold that in the brain tumor studies. Unless the growth fraction of intrathecal tumors is unity (which it certainly is not), more of the tumor cells will escape from the cell-cycle-dependent toxicity of ^{125}I UdR with an increase in tumor burden, leading to a diminished curability of the disease. Yet, ^{125}I UdR treatment increased time to paralysis by 69% in our intrathecal tumor model. This fact suggests the therapeutic effectiveness of ^{125}I UdR against intrathecal tumors that have advanced from the microscopic stage of the disease. Interestingly, the prolongation of survival in animal models observed using either intrathecally administered 4-hydroperoxycyclophosphamide or intrathecal gene therapy is similar in magnitude.

Being a cycle-dependent agent, ^{125}I UdR incorporates itself into the DNA of any dividing cell and as such does not specifically target tumor cells. However, since (a) very few noncancerous cells within the central nervous system are cycling at any one time period and (b) ^{125}I UdR is swiftly cleared and rapidly dehalogenated once it has entered the vascular space, the intrathecal administration of this radiopharmaceutical should lead to a high degree of specific uptake by intrathecally-growing tumor cells. Our biodistribution and autoradiography data corroborate these expectations; none of the normal proliferating cells in the tumor-bearing rats (i.e., skin, intestine, spleen, and bone marrow) incorporated ^{125}I UdR. These results are consistent with those reported earlier after the intracerebral injection of ^{125}I UdR in rats with brain tumors (36). The rapid accrual of large amounts of radioactivity in thyroid, urine, and stomach contents which is evident in the biodistribution data (Fig. 3) suggests the prompt release of ^{125}I UdR from the subarachnoid space, its rapid dehalogenation, and the excretion of free radioiodine through urine and stomach.

CONCLUSION

In the 9L gliosarcoma tumor rat model, intrathecal administration of the DNA precursor ^{125}I UdR directs the incorporation of the Auger-electron-emitting radionuclide ^{125}I selectively into dividing tumor cells, which are bathed by cerebrospinal fluid. This radiopharmaceutical is therapeutically effective against intrathecal tumor cells. Normal proliferating cells and tissues within the spinal cord and the remainder of the body escape the extreme toxicity of Auger electrons produced by the decay of ^{125}I incorporated into DNA.

ACKNOWLEDGMENTS

This study was supported in part by U.S. Public Health Service grant CA 15523.

REFERENCES

- Grossman SA, Moynihan TJ. Neoplastic meningitis. *Neurol Clin* 1991;9:843-856.
- Wen PYC, Fine HA. Leptomeningeal metastases. In: Black PMcL, Loeffler SS, eds. *Cancer in the nervous system*. Cambridge, MA: Blackwell Science Inc. 1997:288-309.
- Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients. *Cancer* 1982;49:759-772.
- Charlton DE, Booz J. A Monte Carlo treatment of the decay of ^{125}I . *Radiat Res* 1981;87:10-23.
- Kassis AI, Sastry KSR, Adelstein SJ. Kinetics of uptake, retention, and radiotoxicity of ^{125}I UdR in mammalian cells: implications of localized energy deposition by Auger processes. *Radiat Res* 1987;109:78-89.
- Hofer KG, Harris CR, Smith JM. Radiotoxicity of intracellular ^{67}Ga , ^{125}I and ^3H . Nuclear versus cytoplasmic radiation effects in murine L1210 leukaemia. *Int J Radiat Biol* 1975;28:225-241.
- Kassis AI, Howell RW, Sastry KSR, Adelstein SJ. Positional effects of Auger decays in mammalian cells in culture. In: Baverstock KF, Charlton DE, eds. *DNA damage by Auger emitters*. London: Taylor and Francis; 1988:1-13.
- Kassis AI, Fayad F, Kinsey BM, Sastry KSR, Adelstein SJ. Radiotoxicity of an ^{125}I -labeled DNA intercalator in mammalian cells. *Radiat Res* 1989;118:283-294.
- Calabresi P, Cardoso SS, Finch SC, et al. Initial clinical studies with 5-iodo-2'-deoxyuridine. *Cancer Res* 1961;21:550-559.
- Prusoff WH. A review of some aspects of 5-iododeoxyuridine and azauridine. *Cancer Res* 1963;23:1246-1259.
- Lee DJ, Prensley W, Krause G, Hughes WL. Blood thymidine level and iododeoxyuridine incorporation and reutilization in DNA in mice given long-acting thymidine pellets. *Cancer Res* 1976;36:4577-4583.
- Klecker RW Jr, Jenkins JF, Kinsella TJ, Fine RL, Strong JM, Collins JM. Clinical pharmacology of 5-iodo-2'-deoxyuridine and 5-iodouracil and endogenous pyrimidine modulation. *Clin Pharmacol Ther* 1985;38:45-51.
- Bloomer WD, Adelstein SJ. 5- ^{125}I -iododeoxyuridine as prototype for radionuclide therapy with Auger emitters. *Nature* 1977;265:620-621.
- Baranowska-Kortylewicz J, Makrigiorgos GM, Van den Abbeele AD, Berman RM, Adelstein SJ, Kassis AI. 5- ^{125}I iodo-2'-deoxyuridine in the radiotherapy of an early ascites tumor model. *Int J Radiat Oncol Biol Phys* 1991;21:1541-1551.
- Kassis AI, Van den Abbeele AD, Wen PYC, et al. 5- ^{125}I iodo-2'-deoxyuridine in the radiotherapy of solid brain tumors in rats [Abstract]. *J Nucl Med* 1993;34(suppl):241P-242P.
- Kooistra KL, Rodriguez M, Powis G, et al. Development of experimental models for meningeal neoplasia using intrathecal injection of 9L gliosarcoma and Walker 256 carcinosarcoma in the rat. *Cancer Res* 1986;46:317-323.
- Schmidk HH, Nielsen SL, Schiller AL, Messer J. Morphological studies of rat brain tumors induced by N-nitrosomethylurea. *J Neurosurg* 1971;34:335-340.
- Foulon CF, Zhang YZ, Adelstein SJ, Kassis AI. Instantaneous preparation of radiolabeled 5-iodo-2'-deoxyuridine. *Appl Radiat Isot* 1995;46:1039-1046.
- Lashford L, Moseley RP, Benjamin JC, et al. Antibody targeted irradiation for leptomeningeal neoplasia: current status. *J Neurooncol* 1989;7:S17.
- Ram Z, Walbridge S, Oshiro EM, et al. Intrathecal gene therapy for malignant leptomeningeal neoplasia. *Cancer Res* 1994;54:2141-2145.
- Grossman SA, Finkelstein DM, Ruckdeschel JC, Trump DL, Moynihan T, Ettinger DS, for the Eastern Cooperative Oncology Group. Randomized prospective comparison of intraventricular methotrexate and thiopeta in patients with previously untreated neoplastic meningitis. *J Clin Oncol* 1993;11:561-569.
- Berg SL, Balis FM, Zimm S, et al. Phase I/II trial and pharmacokinetics of intrathecal diaziquone in refractory meningeal malignancies. *J Clin Oncol* 1992;10:143-148.
- Friedman HS, Archer GE, McLendon RE, et al. Intrathecal melphalan therapy of human neoplastic meningitis in athymic nude rats. *Cancer Res* 1994;54:5118-5122.
- Martin RF, Bradley TR, Hodgson GS. Cytotoxicity of an ^{125}I -labeled DNA-binding compound that induces double-stranded DNA breaks. *Cancer Res* 1979;39:3244-3247.
- Schmidt A, Hotz G. The occurrence of double-strand breaks in coliphage T1-DNA by iodine-125 decay. *Int J Radiat Biol* 1973;24:307-313.
- Krisch RE, Ley RD. Induction of lethality and DNA breakage by the decay of iodine-125 in bacteriophage T₄. *Int J Radiat Biol* 1974;25:21-30.
- Krisch RE, Krasin F, Sauri CJ. DNA breakage, repair, and lethality accompanying ^{125}I decay in microorganisms. *Curr Top Radiat Res Q* 1978;12:355-368.
- Linz U, Stöcklin G. Chemical and biological consequences of the radioactive decay of iodine-125 in plasmid DNA. *Radiat Res* 1985;101:262-278.
- Krisch RE, Flick MB, Trumbore CN. Radiation chemical mechanisms of single- and double-strand break formation in irradiated SV40 DNA. *Radiat Res* 1991;126:251-259.
- Makrigiorgos GM, Berman RM, Baranowska-Kortylewicz J, et al. DNA damage produced in V79 cells by DNA-incorporated iodine-123: a comparison with iodine-125. *Radiat Res* 1992;129:309-314.
- Iliakis G, Pantelias GE, Okayasu R, Seane R. Iodine-123-IUdR-induced chromosome fragments, assayed by premature chromosome condensation, and DNA double-strand breaks have similar repair kinetics in G₁-phase CHO-cells. *Int J Radiat Biol* 1987;52:705-722.
- Warters RL, Hofer KG, Harris CR, Smith JM. Radionuclide toxicity in cultured mammalian cells: elucidation of the primary site of radiation damage. *Curr Top Radiat Res Q* 1977;12:389-407.
- Kassis AI, Fayad F, Kinsey BM, Sastry KSR, Taube RA, Adelstein SJ. Radiotoxicity of ^{125}I in mammalian cells. *Radiat Res* 1987;111:305-318.
- Kassis AI, Makrigiorgos GM, Adelstein SJ. Dosimetric considerations and therapeutic potential of Auger electron emitters. In: Adelstein SJ, Kassis AI, Burt RW, eds. *Frontiers in nuclear medicine: dosimetry of administered radionuclides*, proceedings of symposium, Washington, DC, 1989. Washington, DC: American College of Nuclear Physicians; 1990:257-274.
- Kassis AI, Guptill WE, Taube RA, Adelstein SJ. Radiotoxicity of 5- ^{125}I iodo-2'-deoxyuridine in mammalian cells following treatment with 5-fluoro-2'-deoxyuridine. *J Nucl Med* 1991;35:167-173.
- Kassis AI, Van den Abbeele AD, Wen PYC, et al. Specific uptake of the Auger electron-emitting thymidine analogue 5- ^{125}I iodo-2'-deoxyuridine in rat brain tumors: diagnostic and therapeutic implications in humans. *Cancer Res* 1990;50:5199-5203.