
5-[125]IODO-2'-DEOXYURIDINE IN THE RADIOThERAPY OF SOLID CNS TUMORS IN RATS

AMIN I. KASSIS and S. JAMES ADELSTEIN

We have been investigating the therapeutic efficacy of the thymidine analog 5-iodo-2'-deoxyuridine (IUdR) when radiolabeled with the Auger electron emitter 125I in rats bearing intrathecal (i.t.) or intracerebral (i.c.) 9L gliosarcoma solid tumors. [125]IUdR was infused i.t. (via subarachnoid catheters) or intracerebrally over a 5- or 2-day period; equimolar concentrations of [125]IUdR were infused into control animals. Hind-leg paralysis and/or survival were followed over time. The results indicate that compared with [125]IUdR, rats bearing intrathecal tumors and infused i.t. with [125]IUdR showed significant prolongation of the onset of median paralysis (15.2 versus 9 days). Similarly, the median survival of rats bearing intracerebral tumors and infused i.c. with [125]IUdR was significantly increased (24 versus 17 days). The data substantiate the antineoplastic potential of [125]IUdR and indicate a promising role for this radiopharmaceutical in the treatment of CNS cancers.

Gliomas comprise approximately 60% of all primary CNS tumors and constitute the bulk of the intrinsic intraparenchymal tumors of both brain and spinal cord. Regardless of the location of the malignant glioma, the prognosis for patients has not changed greatly in the last 20 years. Following treatment, recurrence is usually observed within 6 months, and 80% of these patients die within 6 to 12 months. Progress in the therapy of high-grade brain tumors has been modest at best. The fundamental problem lies in the impossibility of total removal or effective sterilization (radiation, chemotherapy, etc.) of the tumor. This impasse motivates the search for alternate treatments modalities that will show preferential uptake and selective killing of tumors.

Iodine-125 (125I) is a radionuclide that decays by electron capture and internal conversion. The primary vacancies in the inner electronic shells of the daughter atoms lead to the emission of low energy (<1 keV), short-range electrons. These electrons dissipate their energy in the immediate vicinity of the decaying atom and deposit extremely high doses (107 to 108 cGy/decay) within a few nanometers (1–6). The thymidine analog 5-iodo-2'-deoxyuridine (IUdR) radiolabeled with 125I ([125]IUdR) has been utilized by many investigators to incorporate this radionuclide into the DNA of dividing mammalian cells. Previous studies have demonstrated:

- After DNA incorporation, 125I is highly toxic to mammalian cells in vitro (3–5, 7–9). The decay of this radionuclide outside the mammalian cell nucleus produces no extraordinary lethal effects (3–5, 10, 11).
- The intratumoral injection of [125]IUdR in patients with colon, breast, and stomach cancers leads to its incorporation into the DNA of cancerous cells with extremely favorable tumor to normal tissue ratios (12, 13).
- IUdR, radiolabeled with 125I, a radionuclide with superb imaging characteristics, is a highly reliable radiopharmaceutical for the scintigraphic detection of brain tumors in rats (intratumoral administration) (14), ovarian tumors in mice (intraperitoneal injection) (15), bladder cancer in rats (intravesical administration) (16), and liver metastases from colorectal cancer in man (intrahepatic artery infusion) (17).
cells were trypsinized, suspended in phosphate-buffered saline, and stereotactically implanted into the right caudate nucleus of three-week-old CDF (Fisher) rats. Tumor cells (2 x 10^6) were injected slowly (within 30 sec) at a depth of 8 mm from the lateral epiphysis and then slowly withdrawn. The hole was plugged with bone wax and the incision closed.

We have examined the potential of [125I]IUdR in the treatment of a solid CNS malignancy. The results substantiate its therapeutic potential following direct intracerebral/intrathecal infusion into an intracranial/intrathecal 9L gliosarcoma in rats and indicate that it may be a useful agent for the therapy of solid tumors that are accessible to direct radiopharmaceutical administration.

Material and Methods

Rat brain tumor model

Monolayers of exponentially growing 9L gliosarcoma cells were trypsinized, suspended in phosphate-buffered saline (PBS), pH 7.3, and kept on ice. The cells were stereotactically implanted into the right caudate nucleus of three-week-old CDF (Fish 344) rats. The rats were anesthetized via an intraperitoneal (i.p.) injection of ketamine (40 mg/kg) and xylazine (10 mg/kg) and placed in a small-animal stereotactic frame (Kopf Instruments). A sagittal incision through the scalp exposed the skull and a small burr hole was made 1.3 mm posterior and 4 mm to the right of the bregma. Tumor cells (2 x 10^6) were then injected slowly (within 30 sec) at a depth of 4 mm using a 701 Hamilton syringe. The needle was left in place for 1 min and then slowly withdrawn. The hole was plugged with bone wax and the incision closed.

Autoradiography (ARG) studies

The actual specificity of targeting and the microdistribution of DNA-incorporated [125I]IUdR were determined by ARG in 6-μm tissue sections. The sections were fixed in methanol at -20°C, coated in NTB2 emulsion (Kodak), and stored at 4°C. Following exposure, the emulsions were developed (Kodak developer D-19) for 3 min and fixed (Kodak fixer) for 5 min. Finally, the slides were washed, stained with hematoxylin-eosin, dehydrated and mounted in Permount.

In-vivo therapy studies

5-Iodo-2'-deoxyuridine was radiolabeled with sodium [125I]iodide (19, 20). Following HPLC purification (C18 column), [125I]IUdR was resuspended in isotonic saline (9.35 mCi/ml). Rats bearing intracranial, nine-day-old 9L gliosarcoma were infused intracerebrally (i.c.) over a 2-day period via an Alzet pump (0.5 μl/h, total volume 20 μl, Alza Corporation) using the same coordinates as for tumor implantation. Control animals were infused i.c. with equimolar concentrations of [125I]IUdR. Two days prior to the start of the [125I]IUdR infusion and continuing through the first 10 days, the drinking water of all rats was supplemented with 0.1% solution of potassium iodide to prevent thyroidal accumulation of radioiodine.

The therapeutic efficacy of the [125I]IUdR intracerebral infusion into the 9L-gliosarcoma-bearing rats was evaluated by determining the prolongation of the median and absolute survival of animals.

Rat meningeal neoplasia model

A modification of the neoplastic meningitis model developed by Kooistra et al. (21) was used. Male CDF rats, weighing about 300 g, were anesthetized with an i.p. injection of ketamine-xylazine-acepromazine maleate. The anesthetized rats were secured in 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 pre-thinned by stretching, Clay Adams) were inserted through the puncture in the atlanto-occipital membrane. The catheter was led to the posterior region of the spinal cord in the subarachnoid space. The external end of the catheter was sealed and tied under the skin prior to closing the wound in three layers. Rats were observed for one week and only those rats free of any signs of paralysis were used. All injections were performed intrathecally (i.t.) in a bolus of 5-μl volume through the catheter already in place which was then flushed with 10 μl of 0.9% saline. The rats were observed daily for symptoms of paralysis, defined as the inability to walk. All rats that developed paralysis of the hind limbs were killed except in one experiment where the time of death caused by the growth of intrathecal tumor was recorded.

Biodistribution studies

Biodistribution studies of [125I]IUdR were performed in rats bearing tumor cells i.t. (5 x 10^5 9L gliosarcoma cells). Each animal received 10 μCi of [125I]IUdR i.t. 5 days after administration of the tumor cells. The rats were sacrificed at various times after [125I]IUdR injection. The radioactivity in the various tissues was determined and tumor to non-tumor ratios were calculated.

[125I]IUdR treatment of intrathecal tumors

Four groups of 11 to 12 rats were treated with a total dose of 500 μCi of [125I]IUdR/rat administered i.t. in a continuous 5-day infusion using micro-osmotic pumps (Alza Corporation). Rats in the control group received i.t. an infusion of 0.9% saline. The treatments were started 3 days after 5 x 10^5 9L gliosarcoma tumor cells were injected i.t. into each rat.
Results

Brain tumor studies

Autoradiography of sections obtained from the brains of rats bearing intracerebral tumors and injected with $^{[125]}	ext{I}UdR$ demonstrated that no radioactivity was associated with any normal tissues and that silver grains were associated only with tumor cells (Fig. 1).

Rats inoculated with 9L gliosarcoma cells ($2 \times 10^4$) develop sizable tumors within two weeks and die by the 19th day (Fig. 2). The infusion of $^{[125]}	ext{I}UdR$ into animals had no effect on their survival. In contrast, the survival of rats infused with $^{[125]}	ext{I}UdR$ was substantially prolonged (Fig. 2).

Intrathecal tumor studies

The intrathecal placement of PE-10 pre-thinned catheters did not cause paralysis/death in $\geq 95\%$ of rats. The i.t. administration of $5 \times 10^3$ 9L gliosarcoma cells via these catheters led to predictable onset of hind-leg paralysis ($9.20 \pm 0.02$ days) and eventual death ($12.1 \pm 2.1$ days) of all animals.

Biodistribution studies showed the clearance of radioactivity over time in all tissues with the exception of thyroid and tumor and extremely favorable tumor to normal tissue ratios (Fig. 3). At 48 h, the percent injected dose per gram in spinal cords from tumor-bearing rats ($2.94 \pm 0.48$) was substantially higher than in those from nontumor-bearing animals ($0.23 \pm 0.06$).

Discussion

For a number of years, the scientific and medical communities have been exploring the possibility of using radionucleides for cancer therapy. The use of sealed radioactive sources (e.g. radium needles and capsules) is now commonplace. However, with the exception of a small number of applications (e.g. Na$^{31}$I for thyrotoxicosis), the hope of employing unsealed sources for the radiotherapy of human neoplastic disease remains largely unrealized. This failure is a consequence of the scarcity of carrier...
molecules that can bring the radionuclide into the vicinity of cancerous cells and achieve high therapeutic ratios between tumor cells and normal tissues.

To facilitate targeting to tumors, many investigators have relied on the direct introduction of the therapeutic/diagnostic agent into the target area. However, inherent to molecules that can bring the radionuclide into the vicinity of cells, and (c) is selectively taken up (passively/actively) and indefinitely retained by cancerous cells but not by noncancerous cells; 3) once the agent has diffused out of the target area, it must be either converted quickly into an inactive (i.e. nontoxic) form and/or excreted rapidly from the body; and 4) the biologic behavior of the agent is not altered by repeated injection, i.e. it lends itself to repeat/continuous injections.

5-Iodo-2-deoxyuridine is an agent that meets all of the above requirements when injected/infused intrathecally/intracerebrally:

- Being a low-molecular-weight molecule, IUdR diffuses readily within tissues (16).
- When radiolabeled with an Auger electron emitter (e.g. 125I), IUdR is innocuous outside the cell and rather ineffective at killing cells even when located within the cytoplasm (3, 5, 10, 11).
- IUdR is taken up selectively by dividing cancerous cells located within areas of nondividing cells (12–14, 16).
- The majority of the cells within the CNS are nondividing and will not incorporate IUdR into their DNA.
- For the most part, IUdR is indefinitely retained following DNA incorporation (4, 5, 22, 23).
- Most of the IUdR that will escape from the CNS is catabolized/dehalogenated rapidly (t1/2 of 5–7 min) and thus will not be incorporated into the DNA of distant noncancerous dividing cells (24, 25).
- Being a small molecule, IUdR will not induce an antibody response and as such will lend itself to repeated injection/continuous infusion.
- IUdR is a cycle-dependent agent. To be used as a cancer therapeutic agent, all cells must be exposed to the drug during their period of DNA replication. Considering that the DNA incorporation of IUdR is proportional to its extracellular concentration and exposure period (4, 5) and that IUdR is stable in the cerebrospinal fluid (26, 27), the intrathecal administration route should increase the absolute amount of IUdR that will be incorporated into the DNA of dividing tumor cells and enhance the tumor/nontumor ratios.

Based on the arguments described above as well as the data presented in this paper substantiating the antineoplastic potential of [125I]IUdR in the therapy of small, solid CNS tumors that are accessible to direct radiopharmaceutical administration, we propose to use 5-iodo-2-deoxyuridine, radiolabeled with the Auger electron emitter 125I, for the treatment of human CNS tumors.

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