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DIAGNOSTIC AND THERAPEUTIC APPLICATIONS OF AUGER ELECTRON EMITTING 5-[^{123}I/^{125}I]IODO-2'-DEOXYURIDINE IN CANCER

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ABSTRACT

We have examined the diagnostic and therapeutic potential of the thymidine analog 5-iodo-2'-deoxyuridine (IUdR), radiolabeled with the Auger emitters ^{123}I and ^{125}I, in a variety of animal and human malignancies. The results indicate that intracerebral gliosarcoma in rats could be detected by scintigraphy after a single intratumoral injection of [^{123}I]IUdR and that [^{123}I/^{125}I]IUdR was specifically incorporated into tumor-cell DNA in high concentration and not into actively dividing epithelia of the normal tissues examined, or into bone marrow. We compared the intratumoral route of administration with the intraarterial injection of the radiopharmaceutical and demonstrated that only the intratumoral route achieved optimal target to background ratios. These data suggest that intratumoral administration of

this Auger-electron-emitting carrier may be useful for the diagnosis and treatment of human CNS malignancies, and a clinical trial is underway. In addition, the specificity of targeting of this radiopharmaceutical is being tested in patients with colon adenocarcinoma following direct intratumoral injection during colonoscopy. The results show that [^{125}I]IUdR can be safely administered in these patients and autoradiographic studies of the tumor after surgical resection indicate high tumor to nontumor ratios with [^{125}I]IUdR mainly incorporated into the DNA of tumor cell nuclei. Preliminary studies have also been carried out in animals bearing bladder cancer. Following the direct intravesical administration of [^{123}I/^{125}I]IUdR, highly favorable tumor to normal bladder ratios were demonstrated, and IUdR uptake in early neoplastic transformation of the bladder urothelium was observed. These data strongly suggest the potential for radiolabeled IUdR in the diagnosis and therapy of human tumors when direct intralesional or intracavitary administration of this radiopharmaceutical is feasible.

INTRODUCTION

Our continued interest in the potential diagnostic and therapeutic applications of Auger electron emitters has led us to examine the use of 5-iodo-2'-deoxyuridine (IUdR) radiolabeled with the Auger emitters ^{123}I and ^{125}I ([^{123}I/^{125}I]IUdR) in several animal and human malignancies including glial neoplasms (1), ovarian cancer (2-4), bladder cancer, and colon adenocarcinoma (5). This thymidine analog is specifically incorporated into DNA during the synthetic phase of the cell cycle (6,7) and during the repair of sublethal chromatin damage in the G_1 phase (8). Most of the DNA incorporated IUdR is retained for the life of the cell or its progeny (9-12). In contrast, the unincorporated IUdR is rapidly catabolized to iodouracil and/or dehalogenated (13,14) and its half-life in circulation is very short, less than five minutes in man and seven minutes in mice (11,13). Although this *in vivo* instability might appear to be a negative feature for any radiodiagnostic or radiotherapeutic agent, it may under certain circumstances (*i.e.* intralesional administration) prove to be a positive characteristic, since the rapid systemic degradation and subsequent elimination of the product would result in detoxification. Moreover, none of the major catabolic products are incorporated into nuclear DNA resulting, therefore, in minimal incorporation into the DNA of normal dividing cells distant from the administration site.

The radionuclides iodine-123 and iodine-125 are both prolific emitters of Auger electrons. While relatively innocuous when located outside the nucleus (15-17), they have considerable toxicity following intranuclear localization (12,15-22). They also have demonstrated therapeutic effectiveness *in vivo* in a murine ovarian tumor model following intracavitary administration (2-4). In addition, iodine-123 emits 159 keV γ rays that are favorable for external gamma camera imaging. Since unincorporated IUdR is rapidly catabolized and eliminated, the resulting background activity is low, an ideal situation for imaging purposes. Furthermore, the very high specific activity of this radionuclide (8,510 TBq/mmol (23,24)) will allow acquisition of images even if only a few molecules of IUdR become incorporated. Thus, the diagnostic potential of [^{123}I]IUdR in cancer can also be envisioned.

The rationale behind the potential use of radioiodinated IUdR in the diagnosis and therapy of cancer is based on the following arguments. Since neoplastic tissues are actively dividing, usually more so than normal tissues, the thymidine analog IUdR will become incorporated into these proliferating cells and, radiolabeled with an Auger emitter, it will also be lethal. Based on its short half-life in the circulation, the clinical application of radiolabeled IUdR in cancer requires tumors that are accessible to either intralesional, intracavitary or intraarterial administration in order to (i) bypass the rapid and extensive dehalogenation and degradation, (ii) maximize the uptake by the tumor and (iii) minimize the toxicity to normal dividing tissues.

To test these hypotheses, we selected two animal tumor models and one human malignancy in which site-directed administration of the radiopharmaceutical was feasible. One animal tumor model consisted of intracerebral gliosarcoma in rats in which either the direct intratumoral or the intraarterial route of administration was tested. This model has the additional advantage of consisting of a neoplasm surrounded by essentially nonproliferating tissue. We also examined the potential of radiolabeled IUdR in rats bearing a chemically-induced bladder cancer following the direct intravesical administration of the radiopharmaceutical. Finally, we tested the specificity of targeting and metabolic fate of [^{125}I]IUdR in patients with colon adenocarcinoma after direct intratumoral injection during colonoscopy.

MATERIALS AND METHODS

1. 5-[^{123}I/^{125}I]iodo-2'-deoxyuridine

No carrier added [^{123}I/^{125}I]IUdR was synthesized by a recently developed, single-step process using a mercuro-derivative of 2'-deoxyuridine (23,24). ^{123}I and ^{125}I were supplied by Nordion International Inc. (Vancouver, British Columbia, Canada) and Amersham (Arlington Heights, IL), respectively. The compound was purified on a C18 reverse phase HPLC column.

2. Animal Tumor Models

a. Rat brain tumor model: Exponentially growing, cultured 9L gliosarcoma cells (2 X 10^4 cells in 10 μl normal saline, [NS]) were injected intracranially (right caudate nucleus) into anesthetized Fisher 344 rats (three-week-old males) using a stereotactic apparatus, as previously described (1). Tumors 0.5 to 5 mm in diameter developed within 2 weeks. Control animals were sham-operated (10 μl of NS). Fifteen to 17 days following tumor or NS inoculation, a mixture of [^{123}I/^{125}I]IUdR was administered either intratumorally (i.t.) or intraarterially (i.a.) and the results obtained by these two routes of administration were compared. The drinking water of all animals was supplemented with potassium iodide (0.1% KI) 48 to 72 h prior to the administration of the radiopharmaceutical. Sixteen tumor-bearing and 8 control animals received a single intracranial injection (10 μl) of a mixture of 5.55 to 14.8 MBq [^{123}I]IUdR (150-400 μCi) and 0.56 to 1.48 MBq [^{125}I]IUdR (15-40 μCi) through the same hole and with the same coordinates used to introduce the tumor or NS inoculum (1). Another group of 4 tumor-bearing and 4 control rats were injected i.a. (100 μl) with a mixture of 14.8 MBq [^{123}I]IUdR (400 μCi) and 2.96 MBq [^{125}I]IUdR (80 μCi). The right common carotid artery (CCA) was accessed using the method described by Bullard *et al.* (25). Briefly, following exposure of the CCA, separation from the vagus nerve, and dissection towards the bifurcation, the external carotid artery was ligated in order to increase drug delivery to the ipsilateral cerebral hemisphere. Radiolabeled IUdR was then administered in a single bolus injection, the opening in the arterial wall closed with fast drying glue (Quick Gel from Duro™), and the wound closed with surgical clips.

b. Rat bladder tumor model: The carcinogen N-methyl-N-nitrosourea (MNU) known to induce transitional cell carcinoma of the bladder (26,27) was instilled directly into the bladder lumen of 4-to-5-week old female Fisher 344 rats (4 animals) via bladder catheterization using a 22-gauge angiocatheter (1.5 mg/0.15 ml saline intravesically, every other week for a total of 3 doses). The drinking water was supplemented with a combination of trimethoprim-sulfamethoxasole, neomycin sulfate and polymixin B formulated as described previously (28). Twelve weeks after the last MNU infusion, the bladder was catheterized and emptied and [^{123}I/^{125}I]IUdR (22.75 MBq [^{123}I]IUdR [615 μCi] and 2.07 MBq [^{125}I]IUdR [56 μCi]) was administered through the catheter in a 200 μl volume and left in place for 2 h. The bladder contents were then withdrawn and the bladder rinsed several times with normal saline (300 μl wash). Nontumor-bearing control rats (4 animals) were injected by the same route with identical amounts of [^{123}I/^{125}I]IUdR. The drinking water of all animals was supplemented with potassium iodide (0.1% KI) from 48 to 72 h prior to the administration of the radiopharmaceutical up to the time of sacrifice.

3. Human Studies

Following approval by the Committee for the Protection of Human Subjects in Research and after informed consent forms were signed, 9 patients with colon adenocarcinoma were injected intratumorally with [^{125}I]IUdR (7-8 MBq [190-216 μCi] in 0.5 ml NS) during endoscopy 24 to 72 h prior to ablative surgery. All patients were asked to take potassium iodide supplements (10 drops of saturated KI solution 3 times a day) for 10 days beginning 48 h prior to the injection of radiolabeled IUdR.

4. Scintigraphy

a. Rat brain tumor model: Planar scintigraphic images were obtained at 1, 14, 24 and 38 h postinjection of [^{123}I/^{125}I]IUdR (GE Starcam gamma camera equipped with a medium energy collimator, anterior views, 128 X 128 matrix, 10 min acquisition, 2.67 magnification). With the intraarterial injection, in addition to the planar images, single photon emission computed tomographic (SPECT) images were also acquired using the ASPECT (annular SPECT) camera (128 X 128 matrix, 120 projections with a 360° rotation of the collimators and 15 s data collection per projection [total acquisition time of 30 min], with coronal, sagittal and rotating three-dimensional displays calculated from reconstructed slices (29)).

b. Rat bladder tumor model: Planar scintigraphic images were obtained with a GE Camstar gamma camera at 2 to 5 h and 14 to 16 h postinjection of [^{123}I/^{125}I]IUdR (gamma camera equipped with a LEAP collimator, anterior views, 128 X 128 matrix, 10 min acquisition, 2.67 and 4.0 magnification). ASPECT images were also acquired as described above.

5. Biodistribution of Radiolabeled IUdR

These studies were conducted in order to quantitate the *in vivo* distribution of the radiopharmaceutical (percent injected dose per gram of tissue, %ID/g) and to derive the tumor to normal tissue ratios.

a. Animal tumor models: In the rat brain tumor model, blood samples were collected from the time of injection up to the time of sacrifice and clearance curves were derived. Following the last imaging session (36-43 h postinjection) the animals were sacrificed and various organs and tissues - tumor and/or right brain, left brain, frontal lobes, bladder, urine, kidney, skin, muscle, stomach, small intestine, large intestine, spleen, liver, heart, lung, right skull, left skull, femoral bone, and thyroid - were dissected, rinsed, blotted dry, weighed, and their radioactive content was determined in a gamma counter (Packard Auto-Gamma 500) along with that of stomach contents, blood, and bone marrow.

In the rat bladder tumor model, biodistribution of radiolabeled IUdR in tumor- and nontumor-bearing rats was determined 18 h after intravesical administration of the radiopharmaceutical. Various organs and tissues of interest - bladder, urine, kidney, uterus, ovaries, skin, muscle, stomach, stomach contents, small intestine, large intestine, spleen, liver, heart, lung, bone, bone marrow, blood and thyroid - were obtained, and processed as described above.

b. Human studies: Following the intratumoral injection of [^{125}I]IUdR, blood and urine samples were collected up to 72 h after injection, and clearance curves were derived. At the time of surgery (24-72 h postinjection), in addition to the tumor, biopsy samples of the following normal tissues were obtained, weighed and their radioactive content determined: colon (both within 1 cm and at 15 cm from the injection site, n=9), abdominal muscle (n=9), omentum (n=4), abdominal fat (n=4), lymph nodes (n=4), liver (n=2), gallbladder (n=2), bile (n=2), and iliac crest (n=1).

Urine samples (pooled from 0-24 h and 24-48 h; n=6) were filtered through 0.2 μm Millipore filters and analyzed on a C18 reverse phase HPLC column. Samples with high radioactivity content (usually the earlier time points) were injected directly on the column after filtration since a small volume (approximately 50 μl) attained the limits of detection. For samples with less radioactivity, the following procedure was applied: filtered urine samples were extracted with a chloroform/tetrahydrofuran (5/1) mixture (4 X 1 ml/ml urine) to remove any IUdR and metabolites soluble in organic solvents. Aliquots from organic and aqueous layers were counted in a gamma counter to follow the distribution of radioactivity. The organic layer was evaporated under a stream of N_2 to a volume of about 100 μl and analyzed on a C18 column. The aqueous layer was treated with chloramine-T (0.1 mg/ml urine) and extracted with methyl ethyl ketone or chloroform (4 X 0.5 ml) to collect oxidized ^{125}I. Aliquots of each fraction were counted. This second organic layer was treated with 10 μl (10 mg/ml) aqueous sodium metabisulfite solution, evaporated to about 100 μl and analyzed.

Plasma samples (1 h, 2 h, 3 h; n=6) were treated in a similar manner. To samples with a high radioactivity content (usually earlier time points postinjection), an equal volume of ethanol was added to precipitate proteins (10 min on ice). Samples were centrifuged at 6000 rpm for 20 min, supernatant aliquots and pellets were counted, and approximately 100 μl of supernatant was injected on the C18 column. In samples with low radioactivity, proteins were precipitated with an equal volume of 20% TCA on ice (20 min), centrifuged as above, and counted. The supernatants were neutralized with either solid $NaHCO_3$ or its saturated solution and then treated as the urine samples described above. HPLC fractions were counted to determine ratios of degradation products. Each sample and each fraction were also run on silica TLC plates using dichloromethane/tetrahydrofuran (4/1) as solvent.

6. Autoradiography

The actual specificity of targeting and the microdistribution of DNA-incorporated [$^{123}I/^{125}I$]IUdR were determined by microautoradiography. The distribution and frequency of grains were assessed over the entire section and compared to the histopathological findings. As a result of the short range of the emitted electrons, the autoradiographic resolution of Auger-electron-emitting isotopes within thin-tissue sections is excellent.

a. Rat brain tumor: At the time of the biodistribution studies, the following tissues were quickly frozen in isopentane and liquid nitrogen: brain, tongue, skin, muscle, esophagus, small intestine, large intestine, spleen, liver, kidney, heart, lung, bladder, stomach, testes, eyes, and lymph node. Bone marrow smears were also obtained. Tissues were sectioned (6 μm sections), transferred onto glass slides and fixed with methanol (-20°C, brain sections; room temperature, bone marrow smears) or Bouin's solution (room temperature, all other tissues). The sections fixed in Bouin's solution were rinsed in ethanol (50% and 70%) and allowed to dry. The tissue sections and bone marrow slides were then coated with NTB2 emulsion (Kodak) and stored desiccated at 4°C in light-tight boxes. After various times of emulsion exposure (up to 7 months), the autoradiographic slides were developed for 3 min in D-19 developer (Kodak) and fixed for 5 min in D-11 fixer (Kodak). Finally, the tissue sections were washed in distilled water, stained with hematoxylin/eosin, dehydrated, cleared, and mounted in Permount. Bone marrow slides were stained with Giemsa stain. Tissue and bone marrow slides were then examined under light microscopy.

b. Rat bladder tumor: After rinsing the bladder with NS, 300 μl of 10% buffered formaldehyde was injected into the bladder and a suture was placed at the neck of the organ. The bladder was excised and placed in a vial containing 10% buffered formalin and subsequently embedded in paraffin. The bladder and the frozen tissues obtained from the biodistribution studies (skin, stomach, small and large intestine, kidney, ureters, uterus and ovaries) were then sectioned (5-7 μm thickness), fixed (except for the bladder sections already fixed *in vivo*) and processed as described above as were the bone marrow smears obtained in these animals.

c. Human studies: To determine the specificity of targeting of [^{125}I]IUdR, microautoradiography was performed at 1 and 2 h postinjection on blood smears and at the time of surgery on semi-thin tissue sections of the tumor and the following normal tissues: colonic mucosa, liver, lymph nodes, fat, and abdominal wall.

RESULTS

1. The Radiopharmaceutical

The radiochemical purity of the product was greater than 99% as determined by TLC and reverse phase HPLC. The specific activities for [^{123}I]IUdR and [^{125}I]IUdR were 8,510 and 81.4 TBq/mmol, respectively. Since the syntheses were carried out under no-carrier-added conditions, *i.e.* without the addition of Na^{127}I, the resulting specific activities are expected to be identical to those of ^{123}I and ^{125}I, respectively.

2. Rat Brain Tumor

a. Intratumoral injection: Scintigraphy was performed 1 to 38 h after [^{123}I]IUdR injection. In the nontumor-bearing rats, activity seen over the brain at 1 h had cleared by 14 h and was not seen at 24 h or 38 h (Fig. 1A). Other areas of intense activity appeared over the stomach and the bladder suggesting excretion of free iodine. Because the drinking water was supplemented with KI, there was no activity in the thyroid. The images obtained in tumor-bearing animals also showed activity in the brain at 1 h; however, as opposed to the controls, activity remained localized in a specific area of the brain in all the tumor-bearing rats at 14 h and at later time points (Fig. 1B). Tumors as small as 0.5 mm in diameter (measured at the time of dissection) were visualized.

The blood clearance curves demonstrated a rapid decrease of ^{123}I activity in both tumor-bearing and control animals with a $T_{1/2}$ of 7 h. As reported previously (1), the biodistribution data indicated that $0.36 \pm 0.14\%$ of the injected dose per gram was seen in the right brain compared to $0.09 \pm 0.015\%$ in the control animals. In some experiments, the tumors were invisible macroscopically and a large proportion of the weighed tumor sample was in fact normal brain tissue. Therefore this %ID/g underestimated the actual tumor uptake. The activity in all other organs and normal tissues was low, as suggested by the scintigraphic studies, with the exception of the bladder, urine, stomach and stomach contents, a reflection of the metabolism of IUdR. The tumor to normal tissue ratios derived from this typical experiment ranged from 8 to 190.

The autoradiographic studies demonstrated that all tumor-bearing animals had significant uptake of radiolabeled IUdR in the tumor bed but not in the surrounding normal brain (Fig. 2A); the brain sections of sham-operated control animals were free of silver grains. Only at late time points (> 5 month exposure) did we observe the presence of silver grains in one or two cuboidal epithelial cells lining the ventricular system (results not shown) of injected animals. Autoradiography of normal tissue sections did not show the presence of silver grains associated with actively dividing normal epithelia such as skin, tongue, small and large intestine. Of particular interest for therapeutic purposes, bone marrow smears were also free of cell-associated silver grains (Fig. 2B).

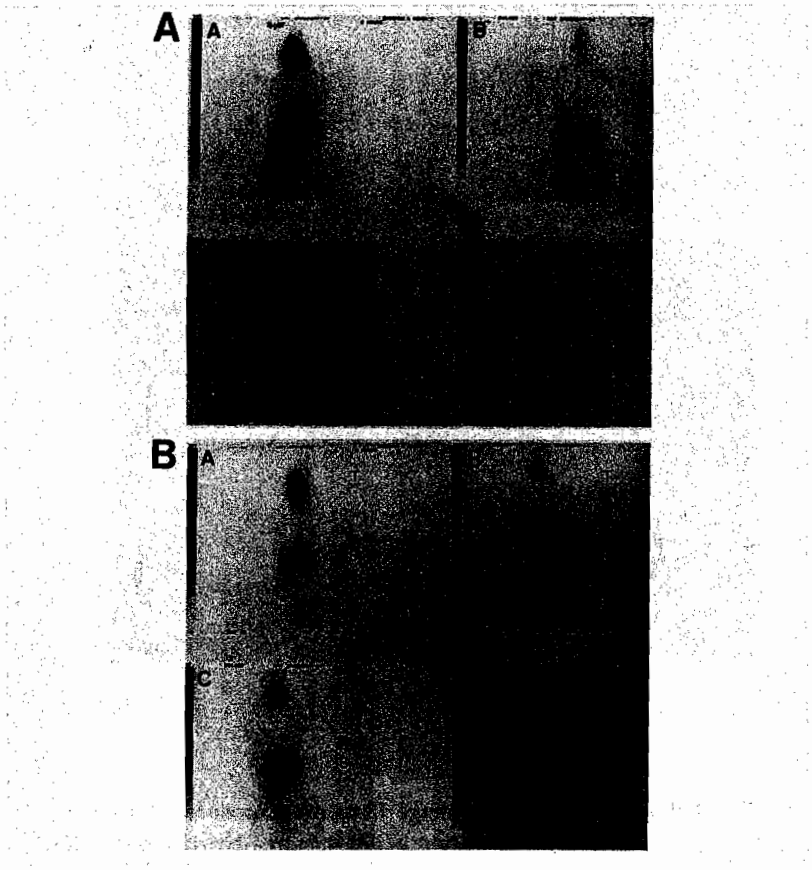

FIG. 1. Scintigraphic images obtained in **(A)** nontumor-bearing rat (sham-operated control) and **(B)** rat bearing 9L gliosarcoma intracranially, 1 h (A), 14 h (B), 24 h (C), and 38 h (D) following intratumoral injection of [^{123}I/^{125}I]IUdR. T, tumor; S, stomach; B, bladder.

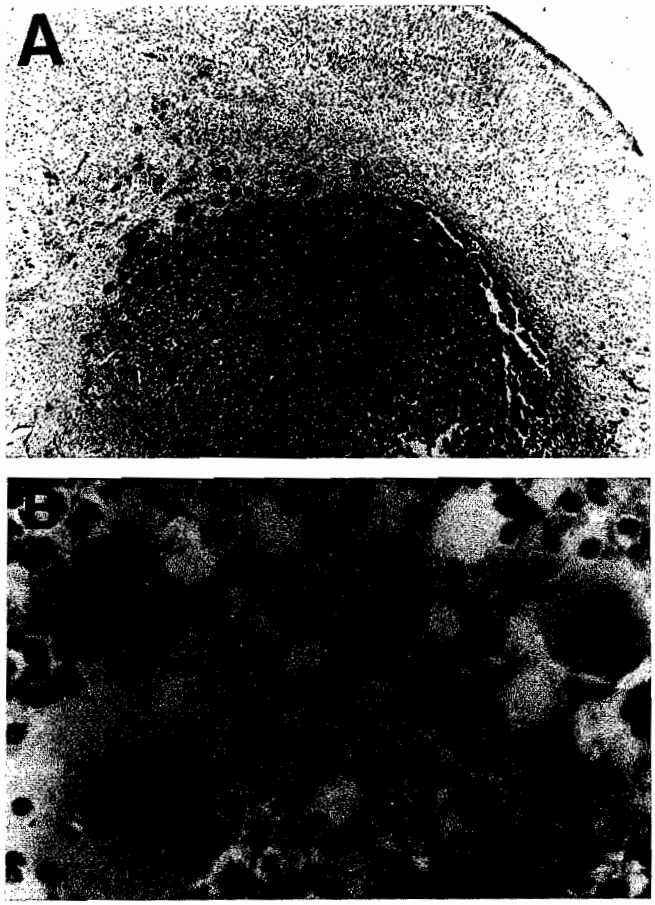

FIG. 2. (A) Autoradiographic image (5 month exposure) of thin (5-6 μm) section obtained from brain of gliosarcoma-bearing rat (X 30) following single intratumoral administration of [^{123}I/^{125}I]IUdR. **(B)** Autoradiographic image of bone marrow smear (X 600, 5 month exposure) of rat following single intratumoral administration of [^{123}I/^{125}I]IUdR.

b. Intraarterial injection: The intracerebral tumors in this group were large (> 0.5 cm), and these animals had signs of increased intracranial pressure, including lethargy and emesis. This circumstance may have compromised the distribution of the radiopharmaceutical and should be considered when interpreting the data.

The results of the imaging studies were similar in the control and experimental groups: (i) areas of intense activity included the right portion of the neck (*i.e.* the injection site), the nose/mouth area, the stomach and the bladder, and (ii) no specific uptake was observed in the brain.

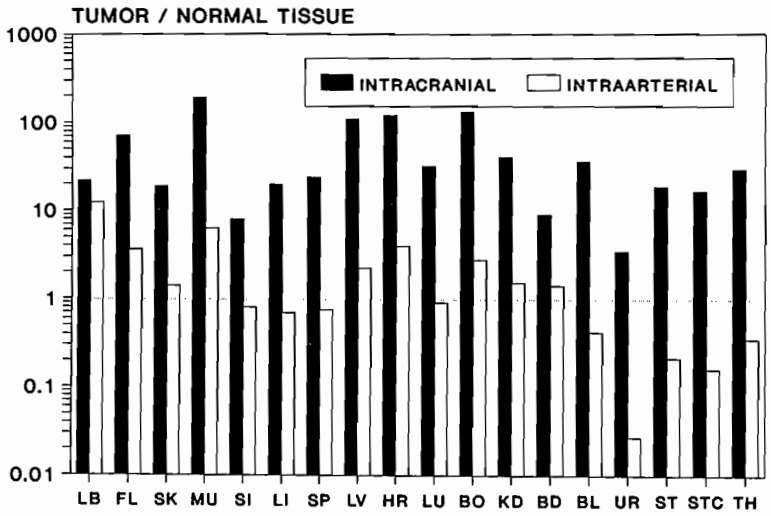

FIG. 3. Tumor to normal tissue ratios obtained from animals bearing intracerebral tumors and injected either intratumorally (closed bars) or intraarterially (open bars) with [^{123}I/^{125}I]IUdR approximately 40 h earlier. LB, left brain (uninjected side); FL, frontal lobes; SK, skin; MU, muscle; SI, small intestine; LI, large intestine; SP, spleen; LV, liver; HR, heart; LU, lung; BO, bone; KD, kidney; BD, blood; BL, bladder; UR, urine; ST, stomach; STC, stomach contents; TH, thyroid.

The biodistribution data indicated that 0.19 ± 0.19% of the %ID/g was seen in the right brain of the tumor-bearing animals compared to 0.0016 ± 0.0004 %ID/g in the sham-operated animals. The activity in all the normal organs or tissues was higher in the experimental group compared to the control and higher than the activity observed in the tissues of animals injected via the intracerebral route. This resulted in target to normal tissue ratios that were less favorable compared with the direct intracerebral injection (Fig. 3), ranging from 0.03 (urine) to 12 (left brain) for the intraarterial injection and 3.4 (urine) to 190 (muscle) for the intracerebral injection. In sharp contrast with what was observed following direct intracranial injection, microautoradiographic studies performed after intraarterial injection did not demonstrate any specificity of uptake by the intracerebral tumor. Furthermore, after intraarterial administration, uptake by normal dividing epithelia was observed in the skin, the tongue, the small and large intestine and the eye.

3. Rat Bladder Tumor

The scintigraphic images performed within 16 h following intravesical administration of the radiopharmaceutical were inconclusive because activity was observed in the bladder region in both tumor-bearing and control animals. This was most likely related to the presence of free iodine and other radiolabeled catabolites in the urine, which is a reflection of the metabolism of radiolabeled IUdR at these early time points after administration. The tumor-bearing animals retained a higher amount of radioactivity compared with the control animals, *i.e.* the systemic distribution of activity was higher in the experimental group.

The biodistribution data indicated, however, a significant difference ($p < 0.05$) in the percent injected dose per gram in the bladder of tumor-bearing animals compared to the control group (0.06 ± 0.03 %ID/g versus 0.0006 ± 0.0004 %ID/g, respectively). As suggested by the imaging studies, the overall distribution of activity among the normal tissues was higher in the tumor-bearing group compared with the control group as reflected by the following values (%ID/g) in various tissues such as blood (0.068 ± 0.044 versus 0.013 ± 0.011), liver (0.035 ± 0.029 versus 0.005 ± 0.005), spleen (0.042 ± 0.035 versus 0.006 ± 0.005), kidney (0.073 ± 0.039 versus 0.008 ± 0.008), small intestine (0.025 ± 0.01 versus 0.004 ± 0.003), and muscle (0.01 ± 0.007 versus 0.002 ± 0.002). At the time of the biodistribution studies it was noted that all the tumor-bearing animals had evidence of bilateral hydronephrosis

(with wide communication between the bladder and the ureters), urinary infection, and lithiasis. These complications, which seldom occur in bladder cancer patients, most probably contributed to the systemic distribution of the radiopharmaceutical. In the control group most of the intravesical inoculum remained within the bladder and permeation to the systemic circulation was very low, limited to normal diffusion (mediated by broad-specificity facilitated-diffusion nucleoside transporters (30)) and/or possible minimal trauma to the bladder wall secondary to the catheterization procedure. In order to obtain a better assessment of the expectations in humans where the delivery of the radiopharmaceutical would be well controlled and confined to the bladder, we expressed the target to nontarget ratios as the quotient of the activity observed in the bladder of the tumor-bearing animals to that of the normal tissues of the control animals (Fig. 4). These ratios are all above 1 ranging from 1.21 (stomach) to 91 (normal bladder) except for the urine and the stomach contents which are the sites of excretion of free iodine.

The autoradiographic studies confirmed the presence of infection/inflammation as well as stones in all the tumor-bearing animals. All animals had low grade papillary tumors with hyperplasia (stage Ta (31)) but no carcinoma *in situ* or squamous metaplasia or invasion. However, even at this early stage of tumor development, the autoradiographic studies demonstrated IUdR uptake in the areas of hyperplasia. Radiopharmaceutical uptake was also observed in normal inflammatory cells in the stroma (results not shown).

4. Human Studies

An average of $0.12 \pm 0.12\%$ of the injected dose was present per gram of tumor (range 0.02-0.38). The approximate amount of [^{125}I]IUdR effectively injected within the tumor was estimated by collecting and measuring the activity contained in the washing fluid used during the colonoscopy. Based on these measurements, the approximate injected dose ranged from 0.4 MBq to 7.4 MBq (11-200 μCi). The tumor to normal tissue (colon [within 1 cm and 15 cm from the tumor site], omentum, abdominal muscle, abdominal fat, liver, gallbladder, bile, lymph nodes, and iliac crest) ratios were very high (mean 873, range 122-2391). Microautoradiography confirmed these high tumor to nontumor ratios and localization of the IUdR mainly in the tumor cell nuclei (Fig. 5). No cell associated activity was observed in the blood smears.

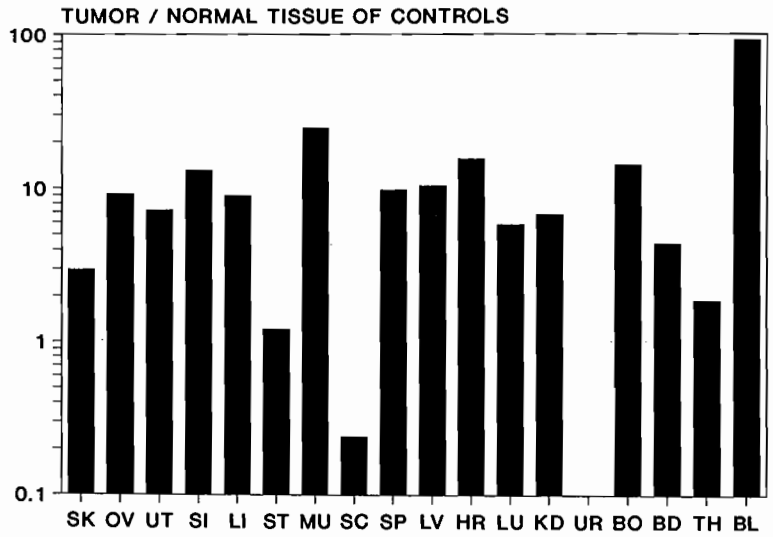

FIG. 4. Tumor to normal tissue ratios obtained from rats bearing bladder tumor and control animals. Results were expressed as the quotient of radioactivity associated with bladder of tumor-bearing animals divided by that associated with normal tissues of control animals. [^{123}I/^{125}I]IUdR had been administered 18 h earlier intravesically in all animals. SK, skin; OV, ovaries; UT, uterus; SI, small intestine; LI, large intestine; ST, stomach; MU, muscle; SC, stomach contents; SP, spleen; LV, liver; KD, kidney; UR, urine; BO, bone; BD, blood; TH, thyroid; BL, bladder.

Neither urine nor plasma samples had any radioactivity associated with the material retained on the filter or the protein precipitate, respectively. Most of the radioactivity injected was present in the urine. The 0-24 h pooled urine collection showed that on average 89 ± 9.9% was either $^{125}I^-$ or its oxidized form and 8.9 ± 8.8% was undegraded [^{125}I]IUdR (n=6). In 2 patients, 10.5% and 2.2% of the total activity was found associated with unknown species. In the 24-48 h pooled urine, 100% of the radioactivity was in the form of iodide. Activity in plasma peaked at 1 to 2 h following [^{125}I]IUdR injection and then decreased with a $T_{1/2}$ of 8 h. HPLC analysis indicated that at 1 to 2 h greater than 99.9% of the radioactivity was free iodide. After 3 h, 94.7 ± 6.7% was $^{125}I^-$ (range 82-100%) and 5.3 ± 6.7% (range 0-18%) was ^{125}IUdR (n=6).

FIG. 5. Autoradiographic image of thin (5-6 μm) section obtained at surgery from colon of patient with colon adenocarcinoma (300X : 30 d exposure) following single direct intratumoral injection of [^{125}I]IUdR 24 h earlier.

DISCUSSION

5-Iodo-2'-deoxyuridine is a thymidine (TdR) analog in which the 5-methyl group of TdR is replaced by iodine. The van der Waals radius of the 5-methyl group of thymidine is 2.0 Å, while that of the iodine atom is 2.15 Å. This similarity gives a compound that behaves remarkably like TdR (6,7,10,12), resulting in its efficient incorporation into the DNA of dividing cells with a thymidine replacement that can be as high as 50% (32). This substitution occurs only during the DNA synthetic (S) phase of the cell cycle (6,7) and during DNA repair of sublethal chromatin damage (G_1 phase) (8). Since at any particular time a certain fraction of the cells within a tumor undergo DNA synthesis, IUdR will be incorporated, and it could, therefore, become a suitable carrier of diagnostic and/or therapeutic radionuclides for

certain types of cancer. In the case of primary brain tumors, for example, high target to background ratios can be expected for this cell-cycle-phase-dependent agent since these tumors arise within a tissue that is essentially nondividing. This was confirmed in our previous studies when we demonstrated that brain tumors in rats could be visualized following direct intratumoral administration of [^{123}I]IUdR with high sensitivity (all tumors were visualized) and high specificity (uptake was observed in tumor tissue only) even when the tumors were invisible macroscopically (< 0.5 mm) (1). [^{123}I]IUdR with its high specific activity (8,510 TBq/mmol) and its favorable imaging characteristics (159 keV γ rays) could therefore become a useful agent for the diagnosis of residual or progressive tumor and could allow differential diagnosis between active tumor and scar, necrosis and edema. Our research effort is now being extended to a clinical trial in patients with primary brain tumors.

Our studies also emphasize the importance of the route of administration. Bagshawe and co-workers reported mixed results following the intravenous injection of radioiodinated IUdR in patients with various tumors (33-35). These observations can be explained by the well-known, rapid systemic degradation and short half-life in circulation of radiolabeled IUdR (13,14). In addition, nonselective uptake of IUdR into normal dividing cells precluded its effective use as a systemic agent and required the concomitant administration of drugs such as hydroxyurea and 5-fluorouracil in an attempt to improve selective uptake by tumor cells (36). The rationale for optimal delivery of this radiopharmaceutical rests, therefore, on intratumoral, intraarterial or intracavitary administration. These routes can potentially (i) lead to higher concentration of the radiopharmaceutical in the tumor while minimizing systemic distribution of the compound, (ii) bypass the main site of metabolism, *i.e.* the liver, (iii) limit the dilution factor resulting from delivery of the agent into the circulation and permit the delivery of more radioactive IUdR directly to the target, and (iv) allow delivery of a dose at the target that could otherwise be attained only if potentially toxic amounts of the radiopharmaceutical were administered systemically. In view of this, we compared direct intralesional administration of the radiopharmaceutical with intraarterial injection in rats bearing intracerebral gliosarcoma tumors. The data showed clearly that, within the limitations of our tumor model, the intraarterial injection did not meet our goals and led to uptake by the dividing epithelia of normal tissues without specific tumor targeting. Only the intralesional route of administration achieved specific cerebral tumor targeting with minimal normal tissue uptake, *i.e.* optimal target to nontarget

ratios. This is essential with cell-cycle-phase selective drugs not only for imaging purposes but also for therapeutic applications, since normal tissues containing proliferating cells, such as the bone marrow or intestinal mucosa, are frequently dose-limiting in cancer therapy. The *in vivo* instability of radiolabeled IUdR in the systemic circulation with its rapid dehalogenation becomes in this case a distinct advantage, since the direct intralesional administration of the radiopharmaceutical should allow high levels of intact radiolabeled IUdR to reach tumor cells while its short biologic half-life in circulation should limit its incorporation into the DNA of proliferating normal tissues. In addition, IUdR being a low molecular weight compound (MW=354.1), it will diffuse freely throughout the tissue. Since there are no reports of antibody response against this small molecule, it will lend itself to repeated injections.

Our results also indicate the feasibility of scintigraphic visualization of bladder tumors in humans following intravesical administration of [^{123}I]IUdR since (i) our autoradiographic studies have demonstrated uptake at an early stage of tumor development, *i.e.* the hyperplasia/dysplasia stage, (ii) IUdR-incorporated activity should remain associated with the tissue, (iii) unincorporated activity, *i.e.* free iodine or other radiolabeled metabolites in the urine, can be removed by voiding or catheterization and the bladder washed prior to imaging, and (iv) IUdR can also be radiolabeled with ^{131}I, an isotope with a longer half-life than ^{123}I (8 days versus 13.2 h), allowing imaging at a later time point, *i.e.* after the metabolism of IUdR has occurred and the radiolabeled metabolites have been excreted. Experimental [^{131}I]IUdR studies in animals are underway to test this hypothesis. Furthermore, intravesical administration of radiolabeled IUdR in patients with bladder cancer would permit not only direct delivery to the target but also a well controlled delivery system for diagnosis and/or therapy in which (i) tumor cells can be exposed for appropriate time intervals to precise concentrations of radiolabeled IUdR, a situation which is particularly desirable since IUdR uptake is proportional to the exposure period as well as to its extracellular concentration (12,22), and (ii) the possibility of removal of unincorporated IUdR will limit the radiation dose to the patient.

In addition to its scintigraphic potential for the diagnosis of active tumor, IUdR radiolabeled with the Auger electron emitters ^{125}I and ^{123}I has been shown to be highly toxic to various normal and tumor mammalian cell lines *in vitro* (12,15-17,21,22) and has demonstrated therapeutic effects following intraperitoneal injection into mice bearing an ascites tumor of

ovarian origin (2-4). Following the incubation of radiolabeled IUdR with cultured mammalian cells and the intranuclear localization of the radiopharmaceutical, there is an exponential reduction in cell survival with no shoulder on the survival curves, a high relative biological effectiveness (RBE) value of 7 (37), an oxygen enhancement ratio (OER) of 1.4 (21) (significantly smaller than the OER of 3 for X ray exposure), and the production of double strand breaks (38-40). In contrast, the decay of ^{125}I extranuclearly, for example within the cell cytoplasm (15,17,37), affixed to cell plasma membranes (16), or extracellularly (12,17,37) produces no extraordinary lethal effects and these survival curves resemble those observed with X rays, *i.e.* they have a distinct shoulder, shallower slopes, and a low RBE (< 2). Our experience, and that of others, with this and several other Auger electron emitters have reiterated the dependence of Auger-electron-emitting toxicity on the intranuclear DNA localization of the radionuclide (37,41). The radiotherapeutic effectiveness of [^{123}I]IUdR and [^{125}I]IUdR has been demonstrated *in vivo* in an experimental murine ascites tumor model of ovarian origin following intraperitoneal injection. In this instance the intracavitary administration of both radiopharmaceuticals led to rapid uptake by ascites tumor cells but not by normal cells and resulted in significant tumorcidal effect (2-4,42). Furthermore, when radioiodinated IUdR was given in fractionated doses synchronized to the cell cycle of these tumor cells, increases in median survival time were observed as well as some cures (2-4,42). These findings suggest that IUdR radiolabeled with Auger electron emitters may be an efficient therapeutic agent for the treatment of cancers, such as brain and bladder tumors, that are accessible to the direct intralesional or intracavitary delivery of the radiopharmaceutical.

Because of (i) the very short range of the electrons produced (nanometer dimensions) during the decay of these isotopes, (ii) their critical dependence on intranuclear localization for toxic effects, and (iii) the heterogeneous distribution of the radiopharmaceutical within the tissue of interest, classical dosimetry based on the MIRD schema and the ICRU procedures greatly underestimates the actual energy deposited in these cells (37,43-45). Studies are therefore currently being conducted in tumor-bearing animals following the intratumoral injection of the radiopharmaceutical to derive dosimetric estimates at the cellular level in order to define the tumoricidal and optimal therapeutic dose. In order to be therapeutically effective, this Auger-electron-emitting carrier must not only specifically target tumor cells in sufficient concentration to achieve tumoricidal dose, but must also target the entire tumor cell population. Because of its cell-cycle

dependency, this goal may only be attained by appropriate dose fractionation or continuous infusion in order to label all cells as they pass through the S phase of DNA synthesis. This requires knowledge of the intrinsic anatomical and biological characteristics (including cell population kinetics) of tumors in humans. Finally, since such an agent would allow a better understanding of tumor cell biology in individual patients, it might also play a major role in predicting and following response to therapy and thereby become an integral part of the decision-making process in the diagnosis and therapy of a variety of human cancers.

CONCLUSION

Animal and human studies have shown that the direct intratumoral or intracavitary administration of radiolabeled IUdR leads to specific incorporation of the Auger electron emitters ^{123}I and ^{125}I into the DNA of dividing tumor cells and high target to nontarget ratios. [^{123}I/^{125}I]IUdR may therefore allow differential diagnosis between active tumor and scar, necrosis and edema. In addition, being selective for proliferating cells, this radiopharmaceutical may promote a better understanding of the intrinsic characteristics of the tumor and define its degree of aggressiveness as well as its response to various therapeutic modalities. Finally, since [^{123}I]IUdR and [^{125}I]IUdR have strong antineoplastic potential, they could become an integral part of the therapeutic regimen of many human cancers that are accessible to direct intralesional or intracavitary administration.

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DISCUSSION

Humm, J. L. The administration of ^{123}IUdR or ^{125}IUdR intratumorally results, as you have shown, in enormous differential uptake in the tumor to nontumor tissues. The advantages of injecting ^{123}IUdR into the CSF for the

treatment of brain malignancies is clear, where the normal brain tissues are a nondividing cell population. But there are severe limitations of the intratumor route, in that the real goal of targeted therapy is the treatment of metastatic disease. It seems to me that the pharmacologic manipulation of cell cycle turnover as investigated by Bagshawe and colleagues is a more promising direction for the general approach of Auger emitters as a therapeutic modality. What are your long term plans for the continuation of this interesting work?

Van den Abbeele, A. D. At the present moment, our interest is confined to important problems in the treatment of brain, bladder and ovarian cancers, for which significant improvements at the primary site are highly desirable. The potential for targeted radionuclide therapy using Auger emitters as well as other radionuclides for microscopic metastatic disease is a challenge for the future.

DeSombre, E. R. If you are injecting intratumorally would not ^{123}I be the Auger emitter of choice (rather than ^{125}IUdR) if a single injection is used?

Van den Abbeele, A. D. A single injection would not be used for therapy since IUdR is a cell-cycle-dependent agent. For diagnosis, only ^{123}IUdR can be used for scintigraphic purposes. For therapy, either continuous infusion or repeated injections would be needed for those radiopharmaceuticals and there are arguments to be made on both sides for use of ^{123}IUdR or ^{125}IUdR although there may be more practical problems with the use of ^{123}IUdR.

Harapanhalli, R. S. What reasons do you ascribe to the fact that actively dividing epithelial cells do not take up IUdR as against the tumor cells in intratumoral injections?

Van den Abbeele, A. D. 1) In the rat brain tumor model, the tumor is surrounded by essentially nonproliferating tissue. 2) In the case of all the tumor models, once IUdR has percolated through the tumor tissue and reach the circulation, its half-life in circulation is very short (on the order of minutes). This can be used as an advantage since it has prevented uptake by distant dividing tissues in our experiments following a single intratumoral injection. 3) Finally, neoplastic tissue is usually more actively dividing than normal tissue thereby increasing the probability of specific targeting.

Schneiderman, M. What was the dose given to your patients?

Van den Abbeele, A. D. The patients with colon adenocarcinoma were injected intratumorally with 190-216 μCi (or 7-8 MBq) of ^{125}IUdR as a single injection during colonoscopy. The patients with primary brain tumors are being injected intratumorally with 5 mCi of ^{123}IUdR during stereotactic biopsy.