Comparison between Intracerebral Biodistribution of [^{125} I]IUdR in a Rat Glioma Model and [^{125} I]IUdR Uptake in Spheroid Multicellular Culture: Effect of Proliferative Heterogeneity

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Abstract

Introduction: Radioiodinated iodo deoxyuridine (IUdR) is a novel,cycle-specific agent that has potential for the treatment of residual malignant glioma after surgery. Because this thymidine analogue kills only roliferating cells. However, maligment cells which are not synthesizing DNA during exposure to the radio pharmaceutical will be spared. To determine whether tumour incroporation [^{125} I]IUdR could be enhanced by protracted administration we employed three different delivery methods such as single injection, polymer implant and using osmotic pump in an in vivo study in compare with an in vitro study using multicellular glioma spheroids of a range of sizes and incubation times.

Methods and Materials: We used a C6 cell line, growing in the brains of Wistar rats, as a glioma model and compared biodistribution of radiopharmaceutical in glioma cells by using autoraiogarphy method.Autoradiogarphy technique also used to discribe means of[^{125} I] IUdR incorporation at different times and depths within UVW multicellular glioma spheroids of a range of sizes.

Results: Twenty-four hours after administration of drug, autoradiography of brain section demonstrated nuclear uptake of the radiopharmaceutical in cells throughout tumour while normal brain cells remained free of radioactivity. The [^{125} I]IUdR labelling indices (%±s.e.m.) achieved were 6.2 (0.4) by single injection, 22.5 (4.1) using a sustained release polymer implant (poly lactide-co-glycolide) and 34.3 (2.0) mini-osmotic pump. The results of the spheroids study confirm that incorporation of [^{125} I]IUdR decreased markedly with increasing size of spheroid. The distribution of IUdR was uniform throught small spheroids (<200 μm), while the concentration of IUdR occurred predominantly in the peripheral cells of larger spheroids. The IUdR uptake enhancement occurred by increasing the incubation time from 52 hours to 104 hours i.e. one or two multiples of the initial volume doubling time.

Cunclusion: The results obtained from in vitro and in vivo studies emphasize the need for a sustained delivery system as a prerequisite for effective treatment. Thses findings are also encouraging for the development of a sustained release for radiolabelled IUdR for use in the treatment of intracranial tumours, particularly in the immediate postoperative setting.

Key Words: Radioiododeoxyuridine, Glioma, Intracerebral theraphy, Rat, Spheroid

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Introduction

Glioma constitues more than 40% of malignancies of the central nervous system and is associated with a very poor prognosis (1). Although this tumour dose not metastasize to distanat sites, it undergoes diffuse local spread, and total surgical resection with generous margin of adjacent normal tissue is rarely feasible. It is resistant to most cycotic drugs and, while some benefit has been reported with radiation treatment (2), external beam radiotherapy is limited by normal tissue intolerance. New treatment agents for glioma therapy are therefore urgently needed.

The Auger electron emission from ^{125} I have an effective range of only a few nanometers (3). Therefore this radionuclide must be associated with DNA to kill cells (4). A suitable targeting vehicle is the thymidine analogue 5- [^{125} I] iodo- 2- deoxyuridine. (i. e.[^{125} I] IUdR) which is incorporated into the DNA of cycling cells. Because of their rapid rate of growth, there should be preferential uptake into tumour cells. The efficacy of locoregional administration of this agent has been demonstrated in rodent models of gliosarcoma (5), meningeal carcinoma (6) and ovarian ascites(7). Because [^{125} I]IUdR deiodinates rapidly in vivo (8) it is anticipated that non intravenous routes of administration will be employed in the initial clinical use of this agent. Since nuclear incorporation of [^{125} I]IUdR occurs only in cells which are synthesizing DNA, a major limitation to this therapy is the presence within the targeted tumour of cells which are temporarily out of S phase during exposure to the drug (9). One way to overcome this restriction is to employ, in addition, radiohalogen conjugates of deoxyuridine, such as the long range ß-emitter ^{131} I (10) or that α-emitter ^{211} At (11), whose decay particles are lethal over ranges equivalent to several cell diameters. By this approach, untargeted cells adjacent to those which have accumulated radiopharmaceutical receive a radiation dose by cross-fire. The potential benefit of this radiological bystander effect has been demonstrated using multicellular tumour spheriods (12).

Since IUdR is incorporated into the DNA only of those cells in S-phase, a major limitation to the efficacy of the radiopharmacutical will be the presence of non-dividing cells in the targeted tumour. It is important that the effect of proliferative heterogeenity on IUdR targeted therapy should be evaluated because theoretical calculation suggests that this could be the dominant factor in the efficacy of treatment (13).

An autoradiographic technique using [^{125} I]IUdR is described as means of studying IUdR incorporation at different volume doubling times and depths within multicellular glioma spheroids of a range of sizes to compare the difference between uptake of [^{125} I]IUdR in different in vivo and in vitro models.

Microencapsulation allows the preparation of biodegradable microspheres that can be precisely implanted in a small area of the brain by stereotactic techniques (14, 15, 16). IUdR can be incorporated into thin films of biodegradable polymer (17). These findings suggest that significant sparing of non-cycling maligment glioma cells would results from treatment delivered as a single injection of [^{125} I]IUdR and that uptake of drug would be enhanced by prolonged exposure. We have undertaken an investigation to test this hypothesis in vivo and in vitro. [^{125} I]IUdR was administered directly into tumours produced by intracerebral implantation in Wistar rats of C6 rat glioma cells. Three different means of delivery were imployed: single injection, slow release from biodegradable inserts and diffusion from implanted osmotic-pumps. We demonstrate that the tumour cell uptake was greatly improved by prolonged delivery of the radiopharmaceutical in both in vivo and in vitro studies and the most efficient method was increasing the incubation time or administration by osmotic-pump.

Materials and Methods

Tissue culture media and supplements were purchased from Gibco-BRL (Paisley, UK). All other reagents were obtained from Sigma-Aldrich Co Ltd (Dorest, UK), unless otherwise stated. No-carrier-added [^{125} I]IUdR of specific activity 74 TBq mmol-1 was obtained from Amersham International plc, UK.

* *Cell culture*

The C6 rat glioma cell line was obtained from the American Type Cultures Collection (Rockville, MD,

USA) and UVW glioma cell line obtained from Department of medical oncology (UK). Cells were grown in F10-DMEM (Dulbeccos medium) supplemented with 10% (v/v) fetal bovine serum. The modified Eagle cells were passaged after reaching 90% confluency (every fourth day) and plated at an initial density of 104 cells cm-2 at 370C in 5% carbon dioxide at 90% relative humidity. Caltures were routinely tested and found to be free from mycoplasma contamination.

* *Spheroid culture*

One milion cells from UVW glioblastoma cell line were harvested from exponetial monlayer cultures, seeded into biological petri dishes (Ston, Staffs, UK) containing 15 ml of medium and kept in a 5% CO_2 incubator for 48 hours. Cells were then transferred to spinner flasks (250 ml) (Techne, UK) containing 100 ml medium in 5% CO_2 at 37°C and cultured until spheroids of the required size were obtained.

* *Uptake of [^{125} I]IUdR by UVW spheroids*

Cells aggregate of approximatly100 diameter, wer transferred to spinner flasks containing 150 ml of medium. The contents of the vessels were equilibrated with a mixture of 5% CO_2 and 95% air for 3 min. The flasks were 'sealed and placed on a magnetic stirrer platform in a hot room at 37°C. Half of the medium was changed 3 times per week. After 2-6 weeks of grow,spheroids of 150 μm to 1000 μm diameter were transferred from the spinner flask into 50ml tubes, each containing 10 ml of medium with 0.06 MBq/ml of [^{125} I]IUdR. Each tube contained 10-20 spheroids, depending on size.After equilibration with 5% CO_2 the tubes were placed on a roller shaker and incubated at 37°C for either 52 or 104 hours, i.e. one or two multiples of the initial volume doubling time. The spheroids were then washed several times in culture medium until no further soluble radioactivity could be eluted. They were embedded in mounting medium on cork discs, and frozen by cooling in liquid nitrogen. Sections (5 μm) were cut in a cryostat (Bright) at -20°C and thawed onto silanised slides. After drying at room temperature, the sections were stored desiccated at -20°C.

* *Experimental animals*

Wistar male and female rats weighing 250-300 gwere obtained from Harlan Olac (Bicester, UK).

* *Intracranial implantation of C6 cells*

The implanation procedure was similar to those described previously (18, 19). Briefly, after the induction of anaesthesia by halothane inhalation, animals were placed in Kopf stereotaxic frame; the head was shaved and disinfected; a midline incision was made using a scalpel blade and underlying tissue removed using blunt dissection. Using a dentist drill fitted with a size 018 burr, a hole was drilled 4 mm posterior to the coronal suture and 3 mm to the right of the sagittal suture. The needle was advanced to a depth of 6 mm from the top of the skull and then withdrawn to a depth of 4 mm.

Exponentially growing C6 cells were detached from tissue culture plates using 0.1%trypsin in phosphate-buffered saline (PBS)-EDTA, suspended in PBS and counted in a Coulter counter(Coulter Electronic, Luton, UK). Using a Hamilton syringe (Hamiltone, Bonaduz, Switzerland) fitted with a 23G needle, 5x10 cells contained in a 10 ml volume were injected slowly into the cavity. After1 min the needle was removed.

A piece of surgicel was placed over the injection site to aid healing and prevent infection. The head wound was then closed with Dexon sutures (Davis & Geck, Gosport, Hants, UK).

The procedure was carried out under sterile conditions. The animals were observed for three weeks. Those showing signs of ill health or distress were immediately sacrificed.

* *Biodistribution of [^{125} I]IUdR*

These experiments were performed on groups of six rate. To minimize thyroidal of free radioiodide, the drinking water was supplemented with 0.1% (w/v) KI 2 days before administration of [^{125} I]IUdR. One week after initiation of tumour growth and using the same coordinated. 0.37 as those for implanation of C6 cells, rats were injected intracranially with MBq of [^{125} I]IUdR in 10 ml. The injection time was 1 min. After injection,

the Hamilton syrrng needle was left in place for a futher minute befor being slowly with drawn. This procedure resulted in no leakage of injectate.

At various times (0.5, 1, 2, 24 and 48 h) after injection of radiopharmaceutical, the rats were killed. Tissues of interest were removed, washed with PBS, bolt-dried, weighed and the radioactivity measured in an automated gamma counter (Canberra packard, Berkshire, UK). DNA was also isolated from these tissues by phenol-chloroform extraction and the incorporated radioactivity was determined by gamma counting. Statisticals analysis of biodistribution data was by student t-test.

* [^{125} I]IUdR-loaded biodegradable thin films

Poly (D,- L-lactic-co-glycolic acid) (PLGA), 85:15 (low intrinsic viscosity, Mw 62000), was purchased from medisorb (Cincinnati, OH, USA). PLGA was chosen for this work because of its clinical acceptability and its biodegradable and biocompatible into PLGA. Thin films of [^{125} I]IUdR were prepared by dissolving 10-30 mg PLGA in dichloromethane, mixing in small volumes (5-20 ml) of [^{125} I]IUdR by sonication and casting into a small silicoized petridish. The dichloromethane was allowed to evaporate overnight and then dried at 400C in a vacuum oven for 24 h. Radiolabelled IUdR within the thin films has been shown to be protected from degradation during incubation in PBS, serum and plasma (17).

* Implantation of [^{125} I]IUdR-loaded PLGA polymer

Thin films of PLGA for intracranial implantation had the dimensions 2.5x 1.5x 0.19 mm. These were loaded with 0.37 MBq of [^{125} I]IUdR. Using a dentist drill, the existing hole in the skull of a tumour-bearing rat was enlarged to a diameter of 2.5 mm. The drilling was performed in short bursts and the skull cooled with saline to prevent heat damage to the underlying brain. A fine scalpel (NO. 11) was used to make an incision in the dura at a depth of 4 mm. The thin film was then gently lowered into this space. Surgicel was then placed over the craniectomy site to protect the exposed brain, prevent infection and aid healing. The wound was then closed using Dexon sutures.

* Implantation of osmotic pump

Alzet miniosmotic pumps (Charles River Ltd, kent, UK) with a capacity of 200 ml and flow rate of 0.14 ml per min, were filled with 200 ml of solution containing 0.37 MBq of [^{125} I]IUdR in sterile saline, taking care to avoid the entrapment of air bubbles. A brain infusion cannula was then attached to the pump and the whole assembly incubated in sterile saline overnight at 37°C. The scalp was washed, disinfected with chlorhexidine and a midline incision was made. The underlying tissue was cleared around the existing burr hole. A subcutaneous tunnel, leading from the scalp incision to the midscapular space, was made using blunt dissecting scissors. The pump was located in this space, with the catheter tubing pointing towards the skull. A plastic spacer was attached. 4.5 to the cannula before it was inserted into the burr hole to achieve a depth of mm. The cannula was attached to the skull using Histoacryl (Cyanamid, hampshire, UK). This was allowed to harden for a few minutes before the scalp was closed with Dexon sutures. This procedure was carried out under sterile conditions.

* Intracerebral distribution of [^{125} I]IUdR

Twenty-foure hours after the initiation of the administration of 0.37 MBq [^{125} I]IUdR by intracranial single injection, polymer implantation or osmotic pump delivery, animal were sacrificed. Brains were prepared for sectioning and autoradiography by freezing in isopentane (BDH, Poole, UK) cooled to -42°C for 10 min and then covering in Lipshaw embeding medium. They were then stored at -200°C unit required. After sectioning (10 mm thick) and mounting.

* Autoradiography

The slides of either spheroids or rat brains were dipped in a 1:1 dilution of kodak NTB-2 emulsion in distilled water at 43°C. After drying, the sections were exposed in light-proof desiccating boxes for 92 h. Sections were developed in 1:1 dilution of kodak D 19 developer at 10°C for 4 min. After a brief wash in distilled water, the emulsion was fixed in kodafix

Table 1: Biodisteribution data for a single intracranial injection of 0.37 MBq[^{125}]IUDR into normal (upper values) and tumour-bearing rats

Tim(h) organ	0.5	1.0	2.0	24.0	48.0
Brain	12.7±0.82	10.6±0.51	1.9±0.30	0.14±0.02	0.13±0.02
	11.6±1.46	11.0±0.45	2.2±0.42	0.9±0.18	1.1±0.20
Blood	0.70±0.12	0.52±0.08	0.34±008	0.02±0.01	0.01
	0.66±0.08	0.46±0.06	0.38±0.05	0.02±0.01	0.01
Thyroid	0.56±0.09	0.69±0.15	0.47±0.05	0.44±0.06	0.46±0.05
	0.61±0.10	0.58±0.11	0.66±0.07	0.70±0.05	0.42±0.04
Liver	0.46±0.07	0.28±0.05	0.19±0.4	0.02±0.01	0.01
	0.53±0.09	0.33±0.07	0.22±0.04	0.02±0.01	0.01
small	0.97±0.18	0.59±0.14	0.39±0.07	0.20±0.05	0.07±0.02
intestine	1.12±0.23	0.670.10	0.30±±0.06	0.24±0.05	0.08±0.0.2
	0.41±0.03	0.44±0.07	0.33±0.06	0.020.01	0.02±±0.01
kidney	0.48±0.11	0.40±0.02	0.29±0.04	0.01	0.01
	1.83±0.22	2.07±0.38	1.05±0.08	0.37±0.09	0.12±0.02
stomach	1.84±0.25	1.94±0.19	1.10±0.28	0.29±0.4	0.10±0.02
	0.20±0.03	0.19±0.03	0.16±0.02	0.03±0.01	0.03±0.01
Bonemarrow	0.22±0.04	0.17±0.02	0.18±0.032	0.03±0.01	0.032

Signifficance of diference between uptake in normal and tumour-bearing rats: ap<0.05; bp<0.01; cp<0.001

for 5 min. The slides were washed and counterstained with haematoxylin, dehydrated through graded ethanols and mounted in DPX synthetic resinmountant (BDH, Poole, UK). To determine the depth within the tumour where maximal uptake of [^{125} I]IUdR had taken place, the tissue of every third section was solubilized with 1 M sodium hydroxide and radioactivity was measured in a gamma counter.For each exprimental animal, four sections, adjecent to the depth associated with peak activity, were selected for the estimation of IUdR labelling indices.Sections were examined using a Leitz microscope with 10x10 square grid eyepiece graticule at 400x magnification, providing a square field measuring 0.26x 0.26 mm (area=0.068 mm2). Four representative fields,at loci within the center of the tumour mass,were evaluated per section.Cells which had greater than ten nuclear-associated grains were scored positively.The labelling index for each filed was calculated by expressing the number of labelled cells as a percentage of all cells within the field, and the mean labelling index for each animal was determined. For statistical analysis,labelling indices were logarithmically transformed to stabilize the variance in different treatment groups, and s method for multiple pairwise compared by analysis of variance with Tukey comparisons.

Results

* *Biodistribution*

After intratumoural injection of [^{125} I]IUdR,no toxicity was observed in any animal. At 24 and 48 h,the retained fraction of injection dose per gram of brain was significantly higher in tumour bearing rats ($P<0.001$). After 24h, thyroid, stomach and, to a lesser extent, small intestine were the only normal tissues examined which retined substantial amounts of radioactivity (Table 1). The retention of activity by thyroid was significantly greater in test animals than in control animals at 2 and 24 h ($P< 0.001$ for both time points) after administration of radiopharmaceutical. However, by 48 h, there was no significant difference between thyroid activity in the two groups of rats. It is conceivable that thyroid function could be altered by the presence of an intracranial tumour which could vary the pressure experienced by pituitary or other regulatory ganglia in the brain. This, is turn, could

induce variations in thyroid hormone output thereby effecting radiopharmaceutical uptake by thyroid in tumour-bearing rats. We could detect no activity in DNA isolated from normal tissues of either 24th test or control group of animals whereas in rats with intracranial tumours, h after injection of radio-pharmaceutical, 86. 4±2.4% (mean±s.d) of the activity associated with brain was DNA-bound. This value increased to 90.1±3.6% (mean±s.d.) at 48 h. These observations are encouraging from the point of view of therapy. However, the initial rapid loss of IUdR from the brain emphasizes the need for a slow release delivery system.

* *IUdR labelling index of in vivo experimental tumours*

All three delivery systems were well tolerated by the animal; none of the animals exhibited sign of either discomfort or distress. The animals were sacrificed 24 h after the initiation of the administration of [^{125} I]IUdR. At this time, the remaining activity associated with PLGA polymer was, in all cases, less than 9% of the loaded dose.The corresponding residual activity in the osmotic pump reservoirs was less than 6% of the loaded dose.From duallabelling pilot studies (autoradiography and immunohisto-chemistry) it was clear that the immunohistochemical label could not be visualized underneath the silver grains of cell with heavy IUdR uptake. Because the immune cells associated with the intercranial C6 lesions were located mainly at the periphery of the malignant cellular mass, labelling indices were assessed in regions whithin the body of the tumour. Autoradiograms of brain sections demonstrated nuclear uptake of the radiopharma- ceutical in cells throughout tumour, while normal brain cells remained free from radioactivity. The [^{125} I]IUdR labelling indices (%±s.e.m.) achieved were 6.2 (0.4) by single injection 22.5 (4.1) by slow-release polymer 2 implant (polylactide-glycolide) and 34.3 (2.0) by mini-osmotic pump (Fig. 1 and 2). These differences were allstatistically significant (pump versus single injection, P< 0.001; polymer implant versus single injection, P< 0.001); pump versus polymer implant, (P<0.05).

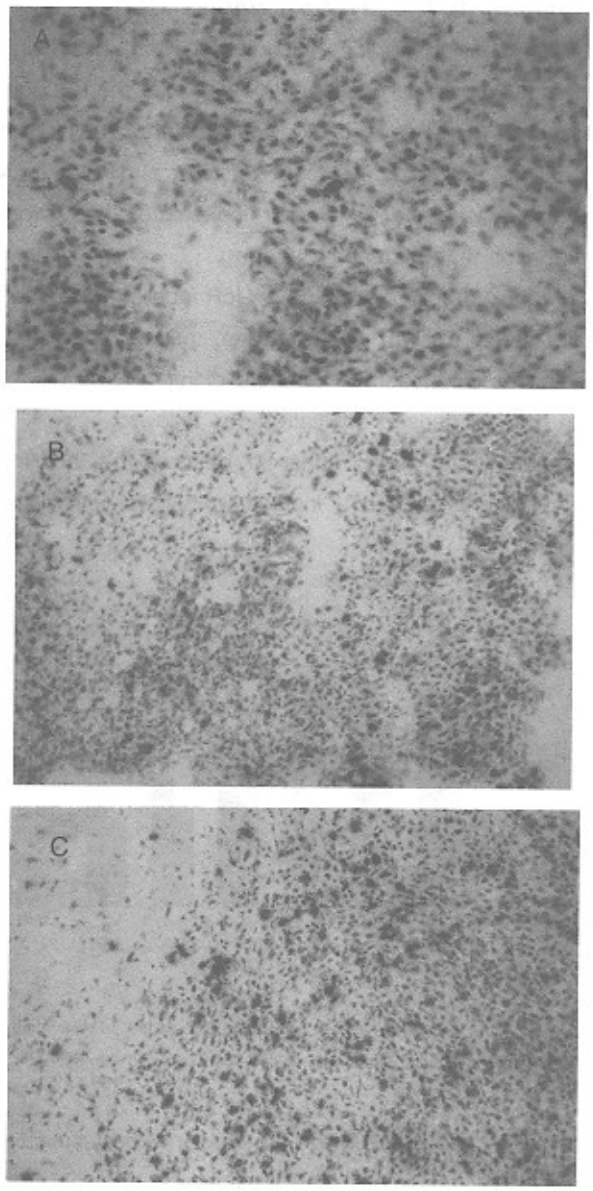

Fig. 1: Autoradiograms of sections of 7-days old C6 tumours exposed to [^{125} I]IUdR using defferent methods: A Single injection; B Slow release PLGA polymer implant C Mini osmotic pump

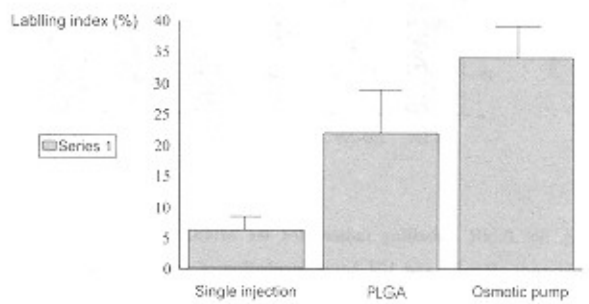

Fig. 2: Labelling index (mean and s.e.m) of three different delivery methods for [^{125} I]IUdR. The analysis was performed 24 hours after the initiation of the delivery of radiopharmaceutical

* Spheroids [^{125} I]IUdR uptake studies

All labelling indices decreased with increasing spheroid diameter. For spheroids of less than 300μm diameter, there was no significant difference between the IUdR labelling indices for 52 and104 hours incubation,whereas for larger spheroids the longer incubation time increased labelling.

Figure 3 (a, b, c) show the IUdR labelling indices in each three 25 μm layers (outer, middle and inner layers) as a function of spheroid diameter. The results can be summarised qualitatively as follows:

a) For any given spheroid diameter,the labelling index decreased with increasing depth within the spheroids.

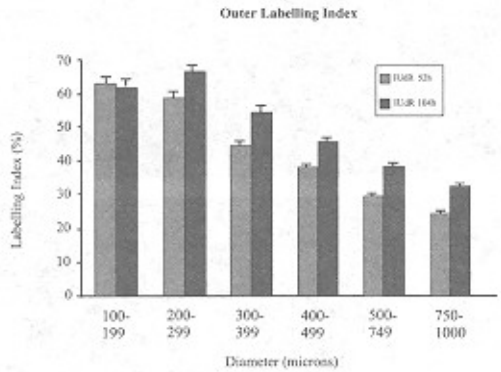

Fig. 3a: IUdR Labelling indices for the outer region of the tumour spheroids after 52 and 104 hours incubation of spheroid diameter

b) The reduction in the labelling indices increasing spheroid diameter occurred in all layers, but was more

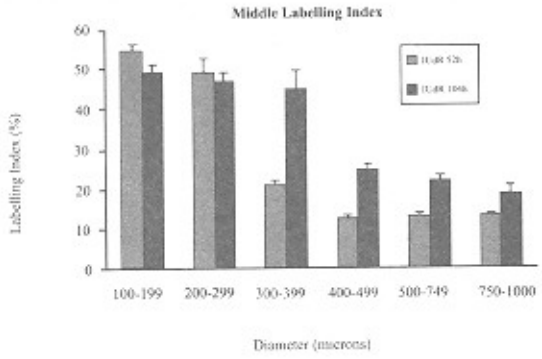

Fig. 3b: IUdR Labelling indices for the Middle region of the tumour spheroids after 52 and 104 hours incubation of spheroid diameter

c)The effect of increasing the IudR incubation time was greatest in the middle and inner layers of 300-400 μm diameter, where adoubling in the number of labelled cells was observed.

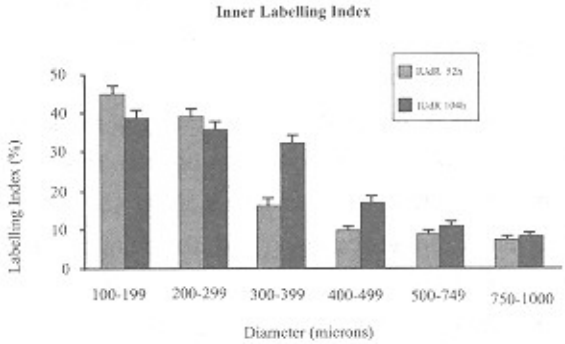

Fig. 3c: IUdR Labelling indices for the inner region of the tumour spheroids after 52 and 104 hours incubation as a function of spheroid diameter

Discussion

The purpose of these studies were to assess the limitations to IudR-tareted Auger electron therapy for glioma imposed by heterogeneity of cellular proliferation.Auger electron emitters such as ^{125} I, are radiotoxic only to cells which incorporate them into DNA (4). This is because the effective range of Auger electrons is only a few nanometers (3). The thymidine analogue [^{125} I] IUdR facilitates the delivery of lethal radiation to dividing cells while sparing quiescent cells. Therefore there is considerable interest in the intralesional delivery of this radiopharmaceutical for the treatment of residual glioma following surgery (6).

However, it is recognized that a limitation of short-term administration of [^{125} I]IUdR would be sparing of malignant cells not involved in DNA synthesis during the time of exposure to the drug.

The results of the present study with spheroids indicate that, in this model, there is an inverse relationship between the number of cycling cells and spheroid diameter.The growth fraction in the smallest spheroids studied was approximately 70% and this fraction decreased progressively with increasing spheroid size. For the largest sheroids, only 40% of cells in the proliferating rim were cycling. IudR labelling indices in spheroids after incubation for 52 hours (the doubling time of the initial monoexponential growth phase) were significantly low. This suggest that, of the

cycling fraction of cells, a considerable proportion had a cycle time longer than 52 hours. In addition, the present in vitro study demonstrates that IUdR incorporation is further reduced at depth within the proliferation region of spheroids relative to the superficial cell layers. Similar findings for radiolabelled thymidine incorporation were reported for spheroids derived from human breast carcinoma cell line MCF-7 (13). Increasing the period of incubation with IUdR from 52 to 104 hours increased the proportion of cells that incorporated IudR in most size classes, although the IUdR labelling index remained low,it is therefore likely that a higher uptake of IUdR could be achieved by further prolongation of the incubation time. This particularly true for the treatment of cells which are proliferating less rapidly as exemplified by cells existing in the hypoxic and nutrient deprived regions of large spheroids. This suggested that protracted delivery of the radiopharmaceutical may be used to overcome the difficulty caused by proliferative heterogeneity of gliomas. Brem and co- workers (20, 21, 22) have pioneered the use of sustained release biodegradable polymeric implants containing cytotoxic agents (e.g. carmustine) for the treatment of brain tumours and it has been demonstrated that synthetic implantable, biodegradable polymers hold promise for the controlled release and local delivery of IUdR for radiosensitization of gliomas (23). In a recent study involving a rat model of leptomeningeal metastases. continuous infusion of [^{125} I] IUdR by means of micro-osmotic pump was found to be more effective than single or intermittent applications of the radiopharmaceutical (24).

The present investigation was designed to compare the extent of cellular uptake of [^{125} I] IUdR in experimental tumours achieved by single intralesional injection, slow- release polymer implant and diffusion from osmotic pump. We have demonstrated that significantly greater accumulation of [^{125} I] IUdR was achieved in malignant cells by means of sustained delivery than by single intralesional injection and that administration by osmotic pump was superior to release from a biodegradable implant. This confirms findings of enhancement of [^{125} I] IUdR uptake in vitro, by prolonged exposure of glioma cell spheroids and is in agreement with the previously observed superiority of osmotic pump delivery (24). Recent studies have shown that in vitro there is a rapid release of 40% IUdR in 4 h followed by a slow sustained loss of drug. However, the rate of release of IUdR from PLGA thin films can be finely regulated by varying the thickness of the polymre (17). Further refinements to the design of this delivery system are in progress to achieve sustained release of IUdR over a range of time intervals. Extension of the infusion time beyond 24 h to several days should enable greater enhancement of radiopharmaceutical uptake in tumour provided radiolabelled IUdR remains stable.

Twenty- four hours after intratumoural injection of [^{125} I] IUdR, 99% of the activity observed after 30 min had cleared from the brains of control animals whereas nuclear uptake occurred in the brains of tumour-bearing animals. After intratumoural injection of [^{125} I] IUdR, no toxicity was apparent and the greatest accumulation of the radiopharmaceutical after 48 h occurred in tumour. Activity was evident in the thyroid (indicating incomplete blockade by potassium iodide), in the stomach and small intestine after 2 days. The latter observation is in agreement with pharmacokinetic studies using a rat brain tumour model derived from the rat glioma cell line 9L (18). Encouragingly, concentration of [^{125} I]IUdR did not occur in bone marrow.

Heterogeneous proliferative activity of glioma cells is one of the main barriers to the therapeutic use of radiolabelled IUdR. We compared the in vitro toxicities of three radioiodoanalogues of IUdR. Ultra- short range Auger electron emitters (^{125} I and ^{123} I) coupled to IUdR were more toxic than [^{131} I] IUdR to clonogens derived from single cell cultures and treated in exponential growth phase. In contrast, the long- range ß-emitter conjugate [^{131} I] IUdR, which provides some 'cross fire'irradiation between cells, was more efficient than the short- rangeradionuclides in reducing the survival of clonogens derived from multicellular spheroids (10). It was concluded that only cells which were in S phase during the period of incubation with radiopharmaceutical were killed by IUdR conjugated to

Auger electron emitters ([123] I and [125] I), whereas [[131] I]IUdR had superior toxicity to clonogenic cells in spheroids due to cross-fird ß-irradiat ion of G0 cells. These findings suggest that a combination of [[131] I]IUdR and [[125] I] IUdR or [[123] I] IUdR might bemore effective than [[125] I] IUdR or [[123] I] IUdR alone for the treatment ofresidual glioma. It is also important to recognize that in the setting of immediate post-surgical resection, regrowth is rapid (26) and a large fraction of cells are actively replicating. In this situation an S phase-specific agent would be valuable, especially if administered by a slow delivery system. The success of curative targeting strategis is governed by the ability to sterilise all clonogens. Therefore, therapeutic regimes must be designed to overcome the limitations imposed by proliferative heterogeneity, including the presence of viable tumour cells which are temporarily out of cycle or very slowly. Such conditions were apparent using spheroid model, in which substatial variation in proliferative was observed.This study clearly demonstrates that increased time of incubation with IUdR partially overcomes this obstacle. The optimal benefit from radioiodinated IUdR therapy is likely to be obtained by prolonged exposure to the drug so that the maximum number of potentially cycling cells will incorporate the Auger emitter as we demonstrated in our in vivo study. Another means of overcoming the incomplete dose distribution associated with [125] I therapy has been demonstrated using the a-emitter conjugate 5-[[211] At] astato-2'-deoxyuridine ([[211] At] AUdR) (27). Not only does tumour cell uptake of [211] At enable alpha particle bombardment of neighbouring, untargeted, out-of-cycle cells by cross-fire but each decay of [211] At produces at least ten times the number of DNA double-strand breaks as that obtained per [125] I decay (28). The optimal therapeutic use of radiolabelled deoxyuridine may consist of mixtures of short and long radionuclide derivatives including [[125] I]IUdR. [[131] I]IUdR. [[211] At] AUdR and [[123] I]IUdR. The latter agent as well as delivering toxic Auger electrons to tumour cells, could, by virtue of its favourable Y-emissions, facilitate imaging to direct external beam irradiation.

In conclusion, a variety of methods of intra delivery of IUdR in vivo and in vitro were studied to determine which procedure would result in the maximal trmour uptake. Direct injection, implantable osmotic pumps and biodegradable ploymers were used to administer [[125] I] IUdR. Autoradiography of brain sections demonstrated nuclear uptake of the radiophrmaceutical in cells throughout trmour while normal brain cells remained free from radioactivity. Labelling was optimal for the pump delivering a given load over 24 h and was substantially better than for a single injection. These results reinforce our in vitro findings and emphasize the need for a protracted delivery system (perhas [125] I extending over several days provided this is within the limits of the stability of [[125] I] IUdR) as a prerequisite for effective treatment. Both direct DNA damage and sensitization to external beam radiotherapy should be increased by prolonged administration of [[125] I] IUdR.

References

1. MacDonald DR: Low-grade gliomas,and oligodendrogliomas. Semin ncol 1994; 21: 236-248

2. Leibel SA,Scott CB and Loeffler JS: Contemporary approaches to he treatment of malignant gliomas with radiation therapy. Semin Oncol 1994; 1: 198-219

3. Martin RF and Haseltine WA: Range of radiochemical damage to NA with decay of iodine- 125. Science 1981; 213: 896-898

4. Kassis AI, Fayad F, Kinsey BM, Sastry KSR, Taube RA, Adelstein SJ: Radotoxicity of 123 I in mammalian cells, Radiat Res 1987; 111: 305-318

5. Makrigiorgos GM, Meta KR, Matalka KZ, Cook CU, Sahu SK, Black PM, Adelstein SJ: 5- [[125] I] ioso- 2-deoxyuridine in the radioterapy of rain tumors in rats. J Nucl Med 1998; 39: 1148-1154

6. Kassis AI, Tumeh SS, Wen PYC, Baranowska- Kortylewicz J, Van Den bbeele AD, Zimmerman RE, Carvalho PA, Garada BM, Desisto WC, Bailey o, Castronovo FP, Mariani G, Bladk PM, Adelstein SJ: Intratomoral dministration of 5 [[125] I]iodo-2'- deoxyuridine in a patient with a brain tumor. J ucl Med 1996; 37: 19S-22S

7. Barnowska-Kortylewicz J, Makrigiorgos GM, Van Den Abbeele D, Berman RM, Adelstein SJ, Kassis AI: 5[[123] I]iodo-2-deoxyuridine in the radiotherapy of an early ascites tumour model.Int J Radiat Oncol Biol Phys 1991; 21:

1541-1551
8. Klecker RW Jr, Jenkins JF, Kinsella TJ, Fine RL, Strong JM, Collins M: Clinical pharmacology of 5-iodo-2'- deoxyuridine and 5-iodo-uracil and endogenous pyrimidine modulation, Clin Pharmacol Ther 1989; 38: 45-51
9. O'Donoghue JA and Whedon TE: Targeted radiotherapy using uger electron emitters. Phys Med Biol 1996; 41: 1973-1979
10. Neshasteh- Riz A, Mairs RJ, Angerson WJ, Stavton PD, Reeves JR, ampling R, Owens J, Whedon TE: Differential cytotoxicity of [^{123} I] UdR. [^{125} I] IUdR and [^{131} I] IUdR to human glioma cells in monolayer of pheroid culture: effect of proliferative bererogenneity and radiation cross- fire, Br J Cancer 1998; 77: 385-390
11. Larsen RH, Vaidyanathan G, Zalutsky MR: Cytotoxicity of a- article- emitter 5- [^{211} At] astato- 2'- deoxyuridine in human cancer cells. Int J adiat Biol 1997; 72: 79-90
12. Cunningham SH, Mairs RJ, Wheldon TE, Welsh PC. Vaidayanathan G, Zalutsky MR: Radiotoxicity to neuroblastoma cells and spheroids of eta-, alpha- and Auger electron- Emitting conjugayes ofbenzylguanidine. Br J Cancer 1998; 77: 2061-2068
\13. Olea, N, Villalobos, M, Ruiz de Almodovar, J M, Pedraza V: MCF-7 breast cancer cells grown asmulticellular spheroids in vitro: effect of 17]-estradiol. Int J Cancer 1992; 50: 112-117
14. Whateley TL, Rampling R, Robertson L, Crossan IM, Fallon PA, Plumb JA, Kerr DJ: Biodegradable systems for sustaineddelivery fodrugs to brain- tomors, J Cell Biockem 1995; S19A: p 178
15. Menei P, Boi sdron-Celle M, Croue A, Guy G, Benoit JP: Effect f stereotaxic implantation of biodegradable 5-fluorouracil- loaded icrospheres in healthy and C6 glioma-bearing rats. Neurosurgery 1996; I39: 117-123
16. Reza MS, Whateley TL: Iodo- 2'- deoxyuridine (IUdR) and UdR loaded biodegradable microspheres for controlled delivery to the brain. J icroencapsulation 1998; 15: 789-125
17. Reza S and Whateley TL: Biodegradable thin films for sustained elivery to the brain. Drug Delivery 1999; 82(1): 74-80
18. Adelstein SJ, Kassis AI: 5 [^{123} I] iodo-2'- deoxyuridine in the adiotherapy of an early ascites tumor model. Int J Radiat Oncol Biol Phys 1991; 21: 541-1551
19. Zhu J, Zhang L. Hanisch UK. Felgner PL and Reszka RA (1996) ontinuous intracerebral gene delivery system for in vivo liposome-mediated ene therapy. Cene Ther 3: 472- 476
20. Brem H, Piantadose S, Burger PC, Walker M, Selker R, Vick NA, Black , Sisti M, Brem S, Motr G, Muller P, Morawetz R, Schole SC: Lacebo-controlled trial of safety and efficacy of intraoperative controlled elivery by biodegradable polymers of chemotherapy for recurreni gliomas. Lancel 1995; 345: 1008-1012
21. Olivi A, Ewend MG, Utsuki T, Tyler B, Domb AJ, Brat DJ, Brem H: Intersitial delivery of carboplatin via biodegradable polymers is effective gainst experimental glioma in the rat. Caner Chem Phrmacol 1996; 39: 90-96
22. Sipos EP, Tyler B, Piantadosi S, Burger PC, Brem H: Ptimizing interstitial delivery of BCNU from controlled release polymers for he treatment of brain tumors, Cancer Chem Pharmacol 1997; 39: 383-389
23. Williams JA, Dillehay LE, Tabassi K, Sipos E, Fahlman C, Brem H: Implantable biodegradable polymers for IUdR radiosensitisation of xperimental human malignantglioma. J Neurooncol 1997; 32: 181-192
24. Sahu SK, Wen PYC, Fouloa CF, Nagel JS, Black PMcL,Adelstein SJ, Kassis AI: Intrathecal 5-[^{125} I] iodo-2'-deoxyufidine in a rat model of ptomeningeal metastases. J Nucl Med 1997; 38: 386-390l
25. Beutler AS, Banck MS, Wedekind D, Hedrich HJ: Tumor gene therapy Made easy: allogeneic major histocompatibibity complex in the C at glioma model. Human Cene Ther 1999; 10: 95-101
26. Cruickshank GS: The use of SPECT in the analysis of brain umours. In SPECT Imaging of the Brain, Kuncan R (ed), Kluwer cademic: Dodrecht, 1997, pp 161- 178
27. Vaidyanathan G, Larsen RH, Zalutsky MR: (5- [^{211} At] astato)'- deoxyuridine, an a-Particle- emitting endoradiotherapeutic agent undergoing NA incorporation, Cancer Res 1996; 56: 1204-1209
28. Walicka MA, Vaidyanathan G, Zalutsky MR, Adelstein SJ, Kassis I: Survival and DNA damage in chinese hamster V79 cells exposed to Ipha particles emitted by DNA- incoroprated astatine- 211, Radial Res 1998; 150: 63-268