^{125}I-Iododeoxyuridine for the Treatment of a Brain Tumor Model: Selection of Conditions for Optimal Effectiveness

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Abstract: The intent of this study was to optimise conditions for the use of ^{125}IUdR in the treatment of cancer. The radiopharmaceutical plus a biomodulator, methotrexate (MTX) was delivered by intra-tumoral injection of a thermosensitive hydrogel forming a slow release depot of ^{125}IUdR and MTX in the tumor.

Methods: The C6 rat glioblastoma was implanted intra-cranially. A chitosan polymer was used to formulate a biodegradable and biocompatible implant for controlled intra-tumoral delivery of ^{125}IUdR plus MTX.

Results: Intratumoral implant of hydrogel loaded with 7.0 -7.4 MBq of ^{125}IUdR resulted in survival of 20% of treated animals to 180 days after tumor implant. Simultaneous delivery of MTX increased the number of rats that were effectively cured, to 40%.

Conclusion: Using an injectable thermolabile hydrogel as vehicle for ^{125}IUdR delivery a higher level of tumor control was achieved in a rat glioma model than had been previously reported.

Keywords: Glioblastoma, ^{125}IUdR, intratumoral drug delivery, thermosensitive hydrogel.

INTRODUCTION

Approximately 20,000 patients are diagnosed with gliomas each year in the United States. The majority of these are malignant, comprising glioblastomas (GBM) and anaplastic astrocytomas as well as other less common variants). GBM is the most common and most aggressive subtype. Low-grade gliomas also have the potential to become highly malignant neoplasms [1]. Advances in neurosurgical techniques [2] and novel approaches to increasing the effectiveness of radiotherapy which have been tried include interstitial and stereotactic brachytherapy [3], radiosurgery [4], immunotherapy [5], boron-capture therapy [6], molecularly targeted agents [7], and gene therapy [8]. In spite of the continuing research into innovative therapies the combination of surgery, radiation, and temozolomide chemotherapy remains the mainstay of treatment for high-grade gliomas [1]. The fundamental problem lies in the difficulty of total removal or effective eradication of the tumor and this situation motivates the search for alternative treatment modalities for targeting and selective killing of tumor cells.

One of the major challenges of chemotherapy for GBM is the achievement of adequate drug concentration within the tumor itself. The blood-brain barrier, although often impaired in areas of bulky tumor, still acts as a barrier against many drugs, particularly in the periphery of the tumor, which is often highly infiltrative. For this reason a variety of alternative delivery methods have been evaluated. One approach is selective increase of drug concentration in the tumor achieved by intralesional drug injection which can enhance both cytotoxicity and sensitization of the tumor cells while reducing normal tissue toxicity. The concept of biodegradable polymers for intratumoral controlled drug release was developed in the early 1970s. Of several polyanhydride-based controlled release systems that have been developed, one of the most successful is the copolymer of bis(*p*-carboxyphenoxy) propane (CPP) and sebacic acid [9, 10]. Wafers of this co-polymer impregnated with carmustine, a nitrosourea compound (Gliadel wafers; MGI Pharma, Bloomington, Minnesota) placed into the surgical cavity after tumor resection, have been modestly effective [11].

Results obtained with biodegradable polymers have been promising, but these devices have the disadvantage that insertion requires surgical intervention and, although in some cases, this can be done when the tumor is resected during the course of conventional treatment, dependence on an invasive procedure remains a drawback. Another mode of intra-tumoral drug delivery, biodegradable microspheres avoids the need for surgery for insertion since they can be introduced by intra-tumoral injection [12, 13]. However, microspheres do not form a continuous film or solid implant with the structural integrity needed for certain prostheses and, they may be poorly retained under certain circumstances because of their small size, discontinuous nature and lack of adhesiveness. During the last decade, injectable *in situ* gel-forming systems have received increased interest in drug delivery and tissue engineering. These devices can overcome many of the problems associated with polymers or microspheres in that they are both injectable and produce solid biodegradable implants with a range of mechanical characteristics in terms of rigidity and load bearing making

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1876-388X/11

them compatible with both soft and hard tissues [14-16]. In the study described here we have used a chitosan polymer to formulate a biodegradable and biocompatible implant for controlled delivery of a radiopharmaceutical in a slow-release manner directly into a intra-cranial implant of a rat glioblastoma.

Iodine-125 is a prolific emitter of low-energy (<1 keV) electrons (~20 electrons per decay) that dissipate their energy typically within nanometer distances from the decay site [17]. As a result, when ^{125}I decays in close proximity to DNA the biological toxicity resembles that of high-LET radiations [18-21]. The iodine-substituted analogue 5-iodo-2'-deoxyuridine (IUdR) behaves like thymidine (dThd) [22, 23]. IUdR and dThd are phosphorylated intracellularly by thymidine kinase to IUdR monophosphate (IdUMP) and dThd monophosphate (dTMP), respectively. The latter is further phosphorylated in a stepwise reaction and incorporated into the DNA. 5-iodo-2'-deoxyuridine mono-phosphate can be similarly phosphorylated and incorporated into DNA or it may be dehalogenated by thymidylate synthetase (TS) to dUMP [24] which is further converted to dTMP *via* the ''*de novo*'' TS-catalysed reaction. When IUdR labeled with ^{125}I is incorporated into proliferating cells during DNA synthesis the radio-active decay of ^{125}IUdR which is retained in the cells and their progeny has been shown to be extremely toxic [18-20]. ^{125}IUdR administered intravenously is unlikely to be useful as an antitumor agent because of its nonspecific uptake by all proliferating cells [25, 26] and its rapid dehalogenation in the liver [25, 27, 28]. This problem can be addressed by localizing ^{125}IUdR to the tumor site by some form of intra-tumoral injection or implantation as described above. Locoregional administration of lUdR labeled with the radionuclides ^{125}I or ^{123}I has been shown to be therapeutically effective in mice with intraperitoneal ovarian tumors [29, 30] and in rats with solid brain tumors [31].

The effectiveness of ^{125}IUdR depends on efficient utilization of the thymidine analog during DNA synthesis. A number of biomodulators have been investigated to find the most effective for facilitation of analog incorporation into DNA. The antimetabolite methotrexate (MTX) is an effective inhibitor of dihydrofolate reductase and of thymidylate synthase, inhibits *de novo* synthesis of IUdR, and enhances incorporation of exogenous ^{125}IUdR into DNA [32].

For this study we investigated an intra-lesional drug delivery system designed to overcome the problems encountered in the use of ^{125}IUdR in the treatment of brain tumors. The radionuclide was delivered to the tumor *via* an injectable thermosensitive hydrogel implant, avoiding dehalogenation and ensuring that ^{125}IUdR was available for uptake into DNA during several cell cycles while the incorporation of ^{125}IUdR into DNA was facilitated by the simultaneous release from the hydrogel implant of the biomodulator, methotrexate.

MATERIALS AND METHODS

Preparation of 5- [^{125}I]Iodo-2'Deoxyuridine (^{125}IUdR)

^{125}IUdR was prepared by the method described by Macapinlac *et al*. [33]. 2-Deoxyuridine (*Sigma*, 0.5 mg) was dissolved in phosphate buffer (0.5 mL, 0.1 N, pH 7.0) and the solution was introduced into a 3cc serum vial containing 0.25 µg of iodogen. After addition of ^{125}I sodium iodide (740 MBq, 10 µL, *MDS Nordion*), the vial was sealed and heated in a 65ºC oil bath for 15 minutes, manually swirling it every 30 seconds. After cooling, the contents were loaded onto a 1 mL SepPak (*Waters*, C18), which had been pre-conditioned with ethanol (5 mL) and water (10 mL). The SepPak was subsequently rinsed with water (20 mL) to remove unreacted iodide and 5-iodouracil and the purified ^{125}IUdR (592-666 Mbq, 80 - 90 %) was obtained by rinsing the SepPak with 3-5 mL of ethanol. Samples were concentrated to ~1 mL under a flow of nitrogen at room temperature.

Analysis

Samples were analyzed by reverse phase HPLC (Luna ODS2, 150 x 4.6 mm) using 10: 90 methanol: water (1 mL/min) as the mobile phase. The identity of the labeled sample was verified by comparing the retention time of the labeled product (detected with a sodium iodide detector) to the UV trace of an authentic standard (at 288 nm). The radiochemical purity was routinely greater than 99.9 %.

Preparation of Biosyntech Hydrogel

The autogelling chitosan solution was prepared as previously described [14]. Chitosan (1.7% w/w) was dissolved in 0.1 M hydrochloric acid at room temperature and sterilized by autoclaving (121°C, 20 min) [15]. To the cooled solution chilled aqueous 45% (w/w) ß-glycerophosphate (ß-GP) solution (sterilized by filtration) was carefully added drop-wise to obtain clear and homogeneous liquid solution which was mixed for an additional 10 min at 4° C. The pH of the final cold solutions ranged from 6.9 to 7.2. This clear, autogelling system is proprietary and patented by BioSyntech [16].

Preparation of BST-Gel^{TM} Loaded with ^{125}IUdR

200 µL aliquots of ^{125}IUdR, prepared as described, were added to a tube containing 200 µl chitosan solution and mixed well on ice. 3µL MTX solution (10mg/mL (Sigma) was added followed by slow addition of 33 µL of ß-glycerophosphate with stirring. The ^{125}IUdR/MTX loaded gel was transferred to a cooled 100 µL Hamilton syringe before injection. The actual activity of ^{125}IUdR injected was 7.0-7.4 MBq based on the count of a standard which was prepared from the same stock as the 30µL used for injection.

Tumor Implantation

All mice were handled according to the guidelines of the Canadian Council on Animal Care according to a protocol approved by the McGill University Animal Care Committee.

The C6 cell line is a clonal line developed from a rat glioblastoma induced in randomly bred Wistar rats by N-nitrosomethylurea [34]. Transplantation of cultured C6 cells into the brains of rats by stereotaxic procedure produces intra-cerebral tumors representing as closely as possible the characteristics of spontaneous gliomas with good reproducibility [35]. C6/Lac Z cells which constitutively express the LacZ reporter gene were obtained from ATCC. Culture of the C6/LacZ cell line and stereotactic injection of cells to produce an intracranial tumor have been described elsewhere [36].

Treatments

12 days after tumor implant 30 µl of hydrogel containing ^{125}IUdR and MTX was injected intra-cranially with a Hamilton syringe using the same co-ordinates which had been used to introduce the tumor cells. The amount of ^{125}IUdR was 0.037µg per tumor and the total radioactivity was 7.0-7.4 MBq. Following tumor implant the rat was examined daily for behavioral and neurological signs of tumor growth. Signs include decreased alertness, passivity, poor grooming, irritability, fearfulness and neurological deficits such as focal motor deficits and gait disturbance. Animals were sacrificed when 4 of these signs appeared.

Biodistribution and Measurement of Incorporation of ^{125}IUdR into DNA

Rats treated with ^{125}IUdR were sacrificed by CO_2 inhalation 1, 3, 8 and 11 days after treatment. The brain hemisphere containing the tumor, the contralateral brain hemisphere, liver, kidney spleen and heart were removed, weighed and 50-100mg tissue samples were taken from each organ and stored at -80 ^{0}C until processing. The tumor was dissected from the brain and weighed and at the same time the solid hydrogel pellet containing ^{125}IUdR/MTX was dissected out and removed.

For processing tissues were minced with scissors and digested overnight at 55^{o}C in 0.5 ml 50 mM Tris, 10 mM EDTA, 0.5%SDS, pH 8.0 containing 0.5 mg proteinase K. At this point any remaining fragments of the solid hydrogel pellet could be identified and removed since they are resistant to digestion. An aliquot of the homogenous digest obtained was counted in a gamma-counter (Cobra II Auto-Gamma Counting System, Packard, Canada) against standards of the injected activity.

The remainder of the digest was used for the extraction of DNA by the phenol-chloroform-isoamyl alcohol method. Precipitated DNA was dissolved in 0.5ml of water and the DNA-incorporated activity was determined by gamma counting. Standards prepared at the time of ^{125}IUdR implant were counted at the same time. The yield and purity of DNA were determined spectrophotometrically at 260nm and 280nm. The total amount of DNA-incorporated activity in liver, kidney, spleen heart and brain was calculated on the basis of the sample size and organ weight.

Statistics

Kaplan-Meier survival curves were derived from rat survival times and plotted for each group. The groups were compared overall using the log-rank test, and were considered as significantly different if the test provided a result lower than 0.05. PASW Statistics 18 for Windows & Vista (formerly SPSS Statistics, published by SPSS Inc.) was used for statistical analysis.

RESULTS

Biodistribution and Incorporation of ^{125}IUdR into DNA

After implantation of the hydrogel containing ^{125}IUdR + methotrexate rats were sacrificed at intervals up to 11 days after implant and organs were collected for determination of content of radioactivity. Table **1** shows the results for whole tissue extracts expressed as per cent of injected dose per gm of tissue. The highest amounts of ^{125}I were found in the tumor at one day after implant, levels subsequently declined but there was still 10% of injected dose/g present at 8 and 11 days after implant. For the other organs sampled the greatest amount of radioactivity on Day 1 after implant was in the spleen (5% injected dose/g compared with 1-2% for the other organs sampled).

The proportion of the radio-activity which is bound to DNA is shown in Table **2**. At Day 1 after implant the amount of ^{125}IUdR incorporated into DNA for the tumor and other represented 60-80% of the total radioactivity in whole tissue extracts. With increasing time after implant the total amount of ^{125}I in tumor and other tissues decreased rapidly (Table **1**) but the proportion of that activity which was isolated with DNA increased.

Specific activity of DNA (MBq/µg) extracted from the tumor and other tissues is also shown in Table **2**. Specific activity was highest for tumor DNA and for all tissues specific activity fell rapidly between day 1 and 3 after implant.

Survival

The survival of tumor bearing rats that were untreated or treated with hydrogel alone, ^{127}IUdR/hydrogel, ^{125}IUdR/hydrogel or ^{125}IUdR/hydrogel/MTX is shown in Table **3** and Fig. (**1**). Intratumoral implant of hydrogel loaded with ^{125}IUdR resulted in an increase in mean survival time and 20% of treated animals survived for180 days after tumor implant. Addition of MTX to the implant increased the anti-tumor activity of the hyrogel/^{125}IUdR formulation and the number of treated rats that survived to a least 180 days post-implant, was increased to 40%.

Table 1. ^{125}I Expressed as a Percentage of the Injected Dose/g in Rat Organs at Various Times After Intra-Tumoral Implant of Biosyntech Hydrogel Containing ^{125}IUdR

Days After Implant	^{125}I Percent Injected Dose/g					
	Tumor	Brain	Liver	Spleen	Kidney	Heart
1	262 ± 69	0.96 ± 0.42	1.9 ± 0.78	5.28 ± 2.4	2.05 ± 0.65	2.11 ± 0.83
3	70.4 ± 19.6	0.1 ± 0.01	0.24 ± 0.04	0.28 ± 0.04	0.26 ± 0.07	0.24 + 0.05
8	9.9 ± 2.9	0.30 ± 0.09	0.21 ± 0.06	0.25 ± 0.07	0.18 ± 0.06	0.20 ± 0.06
11	10.3 ±3.0	0.12 ± 0.04	0.14 ± 0.04	0.03 ± 0.001	0.26 ± 0.08	0.28 ± 0.08

There were 3 rats in each experimental group. The errors are expressed as Standard Error of the Mean (SEM).

Table 2. Percentage of Total Radio-Activity in Tumor and Other Tissues which is Incorporated into DNA and Specific Activity of Extracted DNA (Mbq/μg) at Various Time After Implantation of Hydrogel/^{125}IUdR/MTX

Days After Implant		Per cent of Total Activity Incorporated into DNA (% ± SEM) Specific Activity of Extracted DNA (Mbq/μg ± SEM)					
		Tumor	Brain	Liver	Spleen	Kidney	Heart
1	%	60.8 ± 11.3	77.7 ± 7.4	62.2 ± 4.3	80.8 ± 4.5	80.8 ± 4.5	60.3± 5.4
	Mbq/μg	10.3 ± 3.0	0.26 ± 0.05	0.41 ± 0.13	0.81 ± 0.13	0.26 ± 0.15	0.44 ± 0.023
3	%	60.1 ± 12.2	65.1± 3.6	76.1 ± 3.6	75.2 ± 6.6	78.7 ± 7.1	66.9 ± 1.2
	Mbq/μg	2.0 ± 0.56	0.04 ± 0.008	0.074 ± 0.023	0.07 ± 0.04	0.04 ± 0.02	0.04 ± 0.02
8	%	86.2 ± 9.5	64.4 ± 8.0	84.6 ± 5.3	89.9 ± 5.5	98.1 ± 5.7	95.4 ± 5.6
	Mbq/μg	0.30 ± 0.10	0.15 ± 0.03	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
11	%	93.0 ± 9.7	93.0 ± 9.5	90.1 ± 5.5	91.9 ± 5.5	89.6 ± 5.5	80.5 ± 5.2
	Mbq/μg	0.33 ± 0.09	0.04 ± 0.012	0.00	0.04 ± 0.01	0.04 ± 0.015	0.04 ± 0.01

DISCUSSION

From the early 1960s Auger-emitting radio-isotopes have been explored for a variety of applications in biomedical research. The important feature of these radionuclides is their ability to deposit energy in a very small volume and presentation of an Auger emitter (^{125}I or ^{123}I) as a thymidine analog, which can be incorporated into DNA, is a means for the radionuclide to decay where it can cause massive highly localized damage with a Relative Biological Effectiveness (RBE) relative to x-rays estimated to be 7.3 [37, 38]. The potential of this process to cause highly selective killing of proliferating cells is the basis for the frequent proposition that iodine-125 and other Auger-electron emitting radionuclides could be clinically effective in the treatment of malignant disease.

A group of publications describing results of the therapeutic use of ^{125}IUdR for brain and spinal cord tumors in animal models are particularly relevant for comparison to the study presented here. In one case the anti-tumor effect of the radionuclide was examined in a rat model of leptomeningeal metastases treated with ^{125}IUdR administered intrathecally. ^{125}IUdR (18.5MBq/rat) delayed the median time of paralysis from 9.2 days for controls to 11.2, 12.3, and 15.2 days for single dose, 5 daily injections and continuous infusion groups respectively [39]. In another study ^{125}IUdR was infused intra-cerebrally into rats bearing a one day old tumor (6.9 MBq) or over a six day period (8.7MBq), into animals with 9 day old tumors. Rats treated with cold IUdR survived for 17-24 days whereas tumor bearing animals treated with ^{125}IUdR survived for significantly longer and 10-20% were cured [31]. Finally, human rhabdomyosarcoma cells were implanted as a model for leptomeningeal metastases [40]. Treatment commencing at 10 days after implant of 5 x 10^6 tumor cells was by intra-thecal injection of ^{125}IUdR plus MTX over 12 days. Three protocols were used MTX (31μg) on alternate days, ^{125}IUdR (7.4 MBq) on alternate days or alternating MTX and ^{125}IUdR. The last was the most effective protocol and paralysis was delayed for up to 47 days after tumor implant compared with 20 days for saline injection. These investigators recognized the importance of delivery of the radionuclide by prolonged infusion and of the inclusion of an anti-metabolite (methotrexate) for optimization of the incorporation of ^{125}IUdR into DNA and the prolongation of the therapeutic response.

The protocol described in this paper took a similar approach to optimization of the therapeutic effect of ^{125}IUdR but proved to be more successful than the treatments of brain and spinal cord tumors described above. Thus, 40% of treated animals were "cured" of their tumors, surviving to 180 days post-implant, this in spite of the fact that the tumors were relatively larger when treated (12 days after implant) and the amount of ^{125}IUdR was relatively lower, not more than 7.4 MBq/tumor. The relative success of the procedure described here may be attributable to the use of an implanted pellet of hydrogel as delivery vehicle for the ^{125}IUdR. This continuous release device maintained a significant level of the radionuclide in the tumor over a period of several days and may be more effective in terms of radionuclide delivery than the local injection or infusion methods used in other studies.

Table 3. Survival of Rats Implanted Intracranially with Hydrogel Loaded with ^{125}IUdR with or without Methotrexate

Group	No Treatment (Group 1)	30μl Hydrogel	^{127}IUdR/ Hydrogel (Group 2)	Hydrogel ^{125}IUdR (Group 3)	Hydrogel ^{125}IUdR/ Methotrexate (Group 4)
Median Survival (days)	20	21	20	35	35
Surviving Ratios	0/8	0/25	0/8	2/10	5/13

Kaplan Meier Survival Analysis:
Between groups 1, 2, 3 and 4 P = 0.0032.
Between groups 1 and 2 P = 0.757.
Between groups 1, 3, 4 P= 0.0107.
Between groups 2, 3 and 4 P = 0.015.

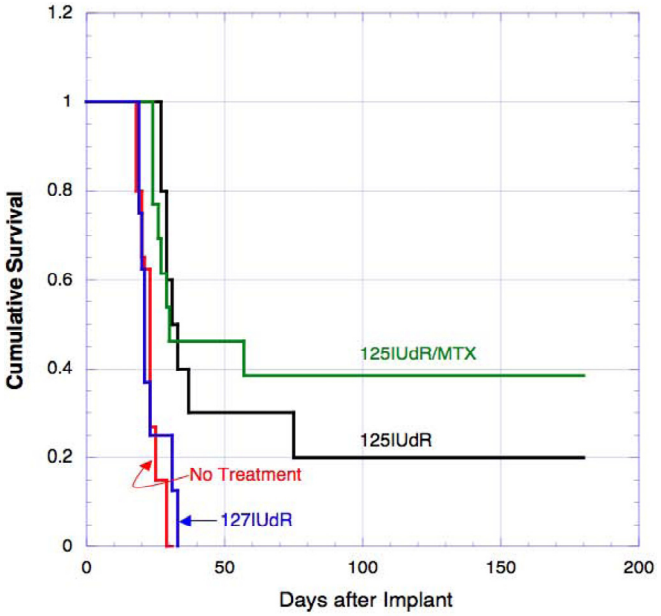

Fig. (1). Survival of rats with C6 glioblastoma following:

No treatment:

Biosyntech Hydrogel with ^{127}IUdR:

Biosyntech Hydrogel with ^{125}IUdR:

Biosyntech Hydrogel with ^{125}IUdR + methotrexate:

Treatments were given at 12 days after tumor implant.

Although these results are gratifying the question that remains is, since everything possible had been done to optimize the uptake of ^{125}IUdR, why was the radionuclide treatment not even more effective? The answer probably is related to the fact that the radionuclide was not incorporated into a sufficiently high proportion of tumor cells and the reasons for this are implicit in the cell kinetics of the C6 tumor. Based on measurements in this laboratory of the morphology of C6 tumors between 10 and 20 days after implant we know that the volume doubling time is 2.5-3.0 days. The cell doubling time however is considerably less. This is indicated by the doubling time of C6 cells in tissue culture which is 18 hours. In addition, the doubling time of C6 cells *in vivo* can be estimated from the measurements of specific activity of DNA labeled with ^{125}IUdR (Table **2**). Between 1 and 3 days after hydrogel/^{125}IUdR implanted into the tumor the mean amount of ^{125}IUdR incorporated into DNA declines with a half-life of 22 hours. Since the only means by which the ^{125}IUdR label can be lost is by cell division, 22 hours also represents the doubling time of the cycling cells in the tumor. Thus there is a considerable discrepancy between the volume doubling time of the tumor and the doubling time of the cells in cycle. Part of this could be accounted for by the cell loss factor which in C6 which is an unknown quantity, however even taking that into account the results imply that *in vivo* a sizeable fraction of the tumor cells are not in the Growth Fraction and do not incorporate ^{125}IUdR. This is a problem which cannot be dealt with by improving radionuclide incorporation with biomodulators nor by improving delivery with biodegradable implants.

This conclusion is supported by consideration of the radiation dose per labeled cell. Based on the method described by Kassis *et al.* 1998 [31] the dose per cell can be estimated from the observed specific activity of DNA (Table **2**) assuming the amount of nuclear DNA/cell to be 7pg [41]. One day after hydrogel /^{125}IUdR implant the specific activity is 10.3 MBq/µg DNA. From the formula

$$A_{total} = A_{C6} \times T_e/\ln 2$$

where A_{total} is the cumulative activity A_{C6} is the activity at 1 day after hydrogel /^{125}IUdR implant and T_e is the effective half life of the nuclide elimination from the tumor cells, assumed to be 22 hours, the *in vivo* C6 cell doubling time A_{total} is calculated to be 8240Bq-sec/cell (8240decays/ ^{125}I labeled cell per 7.4 MBq injected). (This is compares with 89 Bq sec/cell (89 decays /^{125}I-labelled cell per 0.46MBq injected obtained by Kassis and co-workers [31]). Our results indicate a very high dose to the labeled C6 cell but in effect the dose will be even higher because of the relative biological effectiveness (RBE) of ^{125}IUdR relative to x-rays [37, 38]. This result reinforces the conclusion that the uptake ^{125}IUdR must be heterogenous with a proportion of C6 cells not incorporating any ^{125}IUdR.

Over the last few years there has been relatively little published on the potential therapeutic application of ^{125}IUdR. The reasons for this may be related to the difficulties which have described in targeting tumor cells. Another impediment may have arisen as a result of concerns, which have been exacerbated in recent years, about radio-protection and security risks when radio-active isotopes are used in larger than trace amounts. From this point of view the system described has two advantages. Firstly, the hydrogel was chosen as a delivery vehicle because of the ease with which it could be formulated with ^{125}IUdR. The process requires mixing only with no other manipulation of radio-active material. Secondly, the approach used was more effective than any other reported in the literature while using a relatively small amount of radio-active material, no more than 7.4 MBq per animal. It is clear from the results reported here and elsewhere that cells which are labeled incorporate radio-active material much in excess of the amount required for cell kill. The proportion of cells labeled is related to the Growth fraction of the tumor (number of cells in cycle) which is influenced by the type and size of tumor and should be independent of the amount of ^{125}IUdR supplied. If this assumption is correct it implies that reduction in the amount of ^{125}IUdR injected would result in the same number of cells being killed and thus the same level of tumor control. This would of course have to be verified by experiment. If it is correct it would imply firstly that response to therapy using ^{125}IUdR would not be improved simply by loading in more radionuclide and secondly that the amount of radio-active material used could be further reduced without further affecting the level of tumor control.

CONCLUSION

Using an injectable thermolabile hydrogel as vehicle for ^{125}IUdR we have achieved a higher level of tumor control in a rat glioma model than has previously been reported. There are indications that tumor cell kinetics place an insurmountable limit on what can be achieved with this therapeutic approach. Nevertheless the extent of tumor control represents a substantial gain on what is achievable by other methods used in the

treatment of gliomas and suggests these studies should be followed up.

ACKNOWLEDGEMENTS

This work was supported by the Canadian Institutes of Health Research and by the Cancer Research Society of Montreal Inc.

REFERENCES

[1] Khasraw M, Lassman A. Advances in the treatment of malignant gliomas. Curr Oncol Rep 2010; 12: 26-33.

[2] Jenkinson M, Plessis DD, Walker C, Smith T. Advanced MRI in the management of adult gliomas. Br J Neurosurg 2007; 21(6): 550-61.

[3] Kreth F, Thon N, Siefert A, Tonn J. The place of interstitial brachytherapy and radiosurgery for low-grade gliomas. Adv Tech Stand Neurosurg 2010; 35: 183-212.

[4] Chang C, Chen W, Wei K, *et al*. High-dose-rate stereotactic brachytherapy for patients with newly diagnosed glioblastoma multiformes. J Neurooncol 2003; 61(1): 45-55.

[5] Chow K, Gottschalk S. Cellular immunotherapy for high-grade glioma. Immunotherapy 2011; 3(3): 423-34.

[6] Sköld K, Gorlia T, Pellettieri L, Giusti V, H-Stenstam B, Hopewell J. Boron neutron capture therapy for newly diagnosed glioblastoma multiforme: an assessment of clinical potential. Br J Radiol 2010; 83: 596-603.

[7] Kanu O, Mehta A, Di C, *et al.* Glioblastoma multiforme: a review of therapeutic targets. Expert Opin Ther Targets 2009; 13: 701-18.

[8] Candolfi M, Xiong W, Yagiz K, *et al.* Gene therapy-mediated delivery of targeted cytotoxins for glioma therapeutics. Proc Natl Acad Sci USA 2010; 107: 20021-6.

[9] Leong K, Brott B, Langer R. Bioerodible polyanhydrides as drug carrier matrices. I: characterization, degradation and release characteristics. J Biomed Mater Res 1985; 19: 941-55.

[10] Leong K, D'Amore P, Marletta M, Langer R. Bioerodible polyanhydrides as drug-carrier matrices. II Biocompatibility and chemical reactivity. Biomed Mater Res 1986; 20: 51-64.

[11] Sipos E, Tyler B, Piantadosi S, Burger P, Brem H. Optimizing interstitial delivery of BCNU from controlled release polymers for the treatment of brain tumors. Cancer Chemother Pharmacol 1997; 39: 383-9.

[12] LaVan D, Lynn D, Langer R. Moving smaller in drug discovery and delivery. Nat Rev Drug Discov 2002; 1:77-84.

[13] Jain R. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 2000; 21: 2475-90.

[14] Chenite A, Buschmann M, Wang D, Chaput C, Kandani N. Rheological characterization of thermogelling chitosan/glycerol-phosphate solutions. Carbohydr Polym 2001; 46: 39-47.

[15] Jarry C, Chaput C, Chenite A, Renaud M, Buschmann M, Leroux J. Effects of steam sterilization on thermogelling chitosan-based gels. J Biomed Mater Res 2001; 58: 127-35.

[16] Chenite A, Chaput C, Wang D, *et al.* Novel injectable neutral solutions. Biomaterials 2000; 78: 187-97.

[17] Charlton D, Booz J. A Monte Carlo treatment of the decay of 125I. Radiat Res l981; 87: 10-23.

[18] Kassis A, Sastry K, Adelstein S. Kinetics of uptake retention and radiotoxicity of 125IUdR in mammalian cells, implications of localized energy deposition by Auger processes. Radiat Res 1987; 109: 78-99.

[19] Hofer K, Harris C, Smith J. Radiotoxicity of intracellular "Ga, 125I and 'H. Nuclear *versus* cytoplasmic radiation effects in murine L1210 leukaemia. Int J Radiat Biol 1975; 28: 225-41.

[20] Kassis A, Howell R, Sastry K, Adelstein S. Positional effects of Auger decays in mammalian cells in culture. In: Baverstock K, Charlton D, Eds. DNA damage by Auger emitters. London: Taylor and Francis; 1988; pp. 1-13.

[21] Kassis A, Fayad F, Kinsey B, Sastry K, Adelstein S. Radiotoxicity of an '"l-labeled DNA intercalator in mammalian cells. Radiat Res 1989; 18: 283-94.

[22] Eidinoff M, Cheong L, Gurpide EG, Benua R, Ellison R. Incorporation of 5-iodo-uracil labelled with iodine-131 into the deoxyribonucleic acid of human leukaemic leucocytes following in vivo administration of 5-iododeoxyuridine labelled with iodine-131. Nature 1959; 183: 1686-7.

[23] Kassis, A, Fayad F, Kinsey B, Sastry K, Taube R, Adelstein S. Radiotoxicity of 125I in mammalian cells. Radiat Res 1987; 111: 305-18.

[24] Garrett C, Wataya Y, Santi D. Thymidylate synthetase. Catalysis of dehalogenation of 5-bromo- and 5-iodo-2'-deoxyuridylate. Biochemistry 1979; 18: 2798-804.

[25] Calabresi P, Cardoso S, Finch S, *et al*. Initial clinical studies with 5-iodo-2'- deoxyuridine. Cancer Res 1961; 21: 550-9.

[26] Prusoff W. A review of some aspects of 5-iododeoxyuridine and azauridine. . Cancer Res 1963; 23: 1246-59.

[27] Lee D, Prensky W, Krause G, Hughes W. Blood thymidine level and iododeoxyuridine incorporation and reutilization in DNA in mice given long-acting thymidine pellets. Cancer Res 1976; 36: 4577-83.

[28] Klecker R, Jenkins J, Kinsella T, Fine R, Strong J, Collins J. Clinical pharmacology of 5-iodo-2'-deoxyuridine and 5-iodouracil and endogenous pyrimidine modulation. Pharmacol Ther 1985; 38: 45-51.

[29] Bloomer W, Adelstein S. 5-125I-iododeoxyuridine as prototype for radionuclide therapy with Auger emitters. Nature 1977; 265: 620-1.

[30] Baranowska-Kortylewicz J, Makrigiorgos G, van den Abbeele A, Berman R, Adelstein S, Kassis A. 5-[123I]iodo-2'-deoxyuridine in the radiotherapy of an early ascites tumor model. Int J Radial Oncol Biol Phys 1991; 21: 1541-51.

[31] Kassis A, Wen P, van den Abeele A, *et al*. 5-[125I]iodo-2'-deoxyuridine in the radiotherapy of brain tumors in rats. J Nucl Med 1998; 39: 1148-54.

[32] Mester J, DeGoeij K, Sluyser M. Modulation of [5-125I]iododeoxyuridine incorporation into tumour and normal tissue DNA by methotrexate and thymidylate synthase inhibitors. Eur J Cancer 1996; 32A: 1603-8.

[33] Macapinlac H, Kemeny N, Daghighian F, *et al*. Pilot clinical trial of 5-[125I]iodo-2'-deoxyuridine in the treatment of colorectal cancer metastatic to the liver. J Nucl Med 1996; 37: 25S-9S.

[34] Benda P, Someda K, Messer J. Morphological and immunochemical studies of rat glial tumor and clonal strains propagated in tissue culture. J Neurosurg 1971; 34: 310-23.

[35] San-Galli F, Vrignaud P, Robert J, Coindre J, Cohadon F. Assessment of the experimental model of transplanted C6 glioblastoma in Wistar rats. J Neurooncol 1989; 7: 299-304.

[36] Li Y, Owusu A, Lehnert S. Treatment of intracranial rat glioma model with implant of radiosensitizer and biomodulator drug combined with external beam radiotherapy. Int J Radiat Oncol Biol Phys 2004; 58: 519-27.

[37] Kassis A, Makrigiorgos G, Adelstein S. Dosimetric considerations and therapeutic potential of Auger electron emitters. In: Adelstein S, Kassis A, Burt R, Eds. Frontiers in nuclear medicine: dosimetry of administered radionuclides, proceedings of a symposium, 1989. Washington, DC: American College of Nuclear Physicians 1990: pp. 257-74.

[38] Kassis A. Dosimetric aspects of administered radionuclides. In: Carpi A, Sagripanti A, Mittermayer C, Eds. Progress in clinical oncology. Munich: Sympomed Medical Publishers 1992: pp. 112-24.

[39] Sahu S, Wen P, Foulon C, *et al.* Intrathecal 5-[125I]Iodo-2'-Deoxyuridine in a Rat Model of Leptomeningeal Metastases. J Nucl Med 1997; 38: 386-90.

[40] Kassis A, Dahman B, Adelstein S. *In vivo* therapy of neoplastic meningitis with methotrexate and 5-[125I]iodo-20-deoxyuridine. Acta Oncol 2000; 39: 731-7.

[41] Araki T, Yamamoto A, Yamada M. Accurate determination of DNA content in single cell nuclei stained with Hoechst 33258 fluorochrome at high salt concentration. Histochemistry 1987; 87: 331-8.

Received: April 14, 2011 Revised: July 6, 2011 Accepted: July 12, 2011