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5-iodo-2-pyrimidinone-2'-deoxyribose (IPdR)-mediated cytotoxicity and radiosensitization in U87 human glioblastoma xenografts

Timothy J. Kinsella, M.D.¹, Michael T. Kinsella, B.S.¹, Yuji Seo, M.D.¹, and Gregory Berk, M.D.²

¹ Department of Radiation Oncology, University Hospitals Case Medical Center, Cleveland, OH

² Hana Biosciences, South San Francisco, CA

Abstract

Purpose—5-iodo-2-pyrimidinone-2'-deoxyribose (IPdR) is a novel orally administered (po) prodrug of 5-iododeoxyuridine (IUdR). As po IPdR is being considered for clinical testing as a radiosensitizer in patients with high grade gliomas, we performed this *in vivo* study of IPdR-mediated cytotoxicity and radiosensitization in a human glioblastoma xenograft model, U87.

Methods and Materials—Groups of 8–9 athymic male nude mice (6–8 weeks old) were implanted with sc U87 xenograft tumors (4×10^6 cells) and then randomized to 10 treatment groups receiving increasing doses of po IPdR (0, 100, 250, 500, and 1000 mg/kg/d) administered once daily (qd) $\times$ 14 d with or without radiation therapy (RT) (0 or 2 Gy/d $\times$ 4 d) on days 11–14 of IPdR treatment. Systemic toxicity was determined by body weight measurements during and following IPdR treatment. Tumor response was assessed by changes in tumor volumes.

Results—IPdR alone at doses of ≥ 500 mg/kg/d results in moderate inhibition of tumor growth. The combination of IPdR + RT results in a significant IPdR dose-dependent tumor growth delay with the maximum radiosensitization using ≥ 500 mg/kg/d. IPdR doses of 500 and 1000 mg/kg/d did result in transient 5–15% body weight loss during treatment.

Conclusions—In U87 human glioblastoma sc xenografts, po IPdR given qd $\times$ 14 d and RT given 2 Gy/d $\times$ 4 d (days 11–14 of IPdR treatment) results in a significant tumor growth delay in an IPdR dose-dependent pattern. The use of po IPdR + RT holds promise for phase I/II testing in patients with high grade gliomas.

Keywords

IPdR; U87 xenografts; radiosensitization

Correspondence to: Timothy J. Kinsella, M.D., Professor and Chairman, Department of Radiation Oncology, University Hospitals Case Medical Center, 11100 Euclid Avenue, B181, Cleveland, OH 44106-6068, Telephone: 216-844-2530, Facsimile: 216-844-4799, E-mail: timothy.kinsella@UHhospitals.org.

Conflict of Interest Statement

There are no conflicts of interest by the authors.

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INTRODUCTION

Glioblastoma multiforme (GBM) is a universally fatal malignancy typically seen in older (>40 years old) adult patients and accounts for approximately 40% of all brain tumors (18,820 total cases in the U.S. for 2006) (1). In spite of attempts at maximal tumor resection based on the location of the mass and the use of postoperative radiation therapy, the median survival is typically 9–10 months with <10% survival at 2 years. Nearly all patients die from locally recurrent/persistent disease. The current standard of care for postoperative treatment of GBM patients is conventional fractionated radiation therapy (60 Gy at 2 Gy fractions over 6 weeks) and daily oral temozolomide where the median and 2-year survival are modestly improved to 14 months and 20%, respectively (2). Thus, GBM remains a clinically “radio-resistant” disease.

IUdR is a halogenated thymidine analog which has been recognized as an *in vitro* and *in vivo* radiosensitizer for nearly 50 years (3). In experimental human tumor models including human glioblastoma, the extent of radiosensitization is correlated directly with the %IUdR-DNA incorporation where the mechanism of radiosensitization is related to enhanced ionizing radiation (IR)-induced DNA double strand breaks (DSBs) (3). IUdR must be administered intravenously (or intra-arterially) as a prolonged continuous infusion for effective radiosensitization as the drug undergoes rapid metabolism with a serum half-life of ≤ 5 minutes (4). A major drawback to continuous intravenous infusions of IUdR as a clinical radiosensitizer is DNA incorporation in rapidly proliferating normal tissues, which results principally in myelosuppression and gastrointestinal toxicities (4–9). These systemic toxicities have limited the daily dose and total IUdR dose (or duration of IUdR treatment). In prior clinical trials of intravenous infusions of IUdR [or the related thymidine analog, 5-bromodeoxyuridine (BUdR)], modest survival improvements were found in patients with anaplastic astrocytomas and with GBM, compared to data on radiation therapy alone (4–9).

IPdR is a pyrimidinone nucleoside which was originally synthesized as an antiviral agent based on the hypothesis that nucleosides without an amino- or keto- group at position 4 of the pyrimidine ring would be used as a substrate of viral thymidine kinase (TK) but not mammalian TK (10). However, these same investigators subsequently found that IPdR could be efficiently converted to IUdR by a hepatic aldehyde oxidase (11). Thus, IPdR is a prodrug of IUdR, which can be administered orally.

We have been involved in the pre-clinical development of po IPdR as a radiosensitizing drug for over a decade (12–18). We have determined the pharmacokinetics of po IPdR and the active metabolite, IUdR, in rodents (mice, rats) and non-rodents (ferrets, Rhesus monkeys) (12,13,15); confirmed efficient conversion of IPdR to IUdR using cytosolic extracts of normal human liver (13); and measured an improved therapeutic gain for human tumor radiosensitization by po IPdR given qd $\times$ 6–14 days compared to continuous intravenous infusions of IUdR using subcutaneous (sc) human colorectal (HT29; HCT116) (12,13,16) and human glioblastoma (U251) tumor xenografts (14,17) in athymic mice. Most recently, we completed a pre-clinical pharmacokinetic and toxicology study of a once-daily $\times$ 28 day po IPdR dose escalation schedule in anticipation of the use of this IPdR dosing schedule in the first phase I clinical trial (18).

In this *in vivo* study, we determine the systemic toxicity of po IPdR given once daily for 14 days using assessment of body weight during and following IPdR $\pm$ RT (2 Gy/d $\times$ 4 days) treatments as well as determining the extent of IPdR-mediated tumor cytotoxicity and radiosensitization using tumor growth delay measurements in a second clinically relevant human glioblastoma sc xenograft model, U87. We have previously demonstrated differential *in vitro* radiosensitization using clinically relevant IUdR exposures in both U251 and U87 human glioblastoma cell lines (19). In this present study, we report effective IPdR-dose related

radiosensitization with modest tumor growth delay using higher doses of po IPdR alone and modest but reversible systemic IPdR normal tissue toxicity (body weight loss). Thus, po IPdR continues to show promise as a potential clinical radiosensitizer for poorly radioresponsive (“radioresistant”) human tumors and will be tested in future phase I/II clinical trials in adult patients with high grade gliomas.

METHODS AND MATERIALS

Animals

Athymic male nude mice, aged 6–8 weeks, were supplied by Taconic Laboratories (Taconic, NJ). Animals were initially acclimated and quarantined for 3 days prior to subcutaneous (sc) inoculation with the U87 human tumor cells. Groups of 8–9 mice were housed in a micro-isolation cage system with wood chip bedding. Sterilized rodent food and water were provided ad libitum. Rodent housing and feeding guidelines conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99–198).

Cell Culture and Tumor Xenografts

The U87 human glioblastoma cell line (American Type Culture Collection, Rockville, MD) was maintained in Minimum Essential Media (MEM, Sigma, St. Louis, MO), supplemented with 10% fetal bovine serum, L-glutamine and penicillin/streptomycin at 37° C in a humidified 10% CO₂ atmosphere. Exponentially growing U87 cells were harvested from plastic cell culture plates using a 0.25% trypsin-EDTA solution (Sigma). Four × 10⁶ cells in 100 µL of sterile phosphate buffered saline (PBS) were injected into the sc tissue of the right hind limb. When palpable (usually within 4–6 days), tumor measurements using two dimensions including the maximum diameter and its rectangular diameter were determined twice weekly. Once the tumor dimensions reached 50–75 mm³ (typically day 12 following inoculation), IPdR drug treatment was begun (day 1 of protocol). An initial group of 125 athymic mice underwent tumor cell inoculation and a subgroup of 85 mice with optimal tumor volumes (50–75 mm³ at day 12 following inoculation) were used for study. Study and non-study mice were sacrificed by intraperitoneal pentobarbital anesthesia (40–45 mg/kg body weight) (Sigma) and cervical dislocation for compassionate reasons.

Drug Preparation and Administration

IPdR was synthesized by Ash-Stevens (Detroit, MI) and provided as a sterile powder form with greater than 99% purity. Drug was stored in colored glass vials at –20° C and prepared daily in a vehicle solution of 0.5% carboxyl-methyl carbonate (Sigma) in sterile PBS. Tumor bearing mice were randomly assigned to vehicle control or IPdR treatment groups, as described below, and administered a fixed volume (10 ml/kg) in all groups by daily gastric gavage for 14 consecutive days.

Experimental Treatment Groups

The 85 mice with optimal tumor volumes (≈75 mm³ by day 12) were randomly assigned to 10 experimental groups. There were 2 control groups receiving no IPdR (vehicle control); 2 IPdR dose groups receiving 100 mg/kg/d; 2 IPdR dose groups receiving 250 mg/kg/d; 2 IPdR dose groups receiving 500 mg/kg/d; and 2 IPdR dose groups receiving 1000 mg/kg/d. One half of the groups received radiation therapy (RT) on days 11–14 of po IPdR treatment (IPdR or vehicle control + RT groups with 9 animals/group), while the other half of the groups received no RT (IPdR or vehicle control alone groups with 8 animals/group).

Radiation Therapy (RT)

To ensure uniform treatment conditions, all animal groups were brought to the radiation therapy room each day on days 11 to 14 of IPdR (or vehicle control) treatment and were anesthetized using an intraperitoneal injection (ip) with sodium pentobarbital (40–45 mg/kg body weight). All animals then positioned for sham (control) or actual local irradiation to the right hind limb tumor site using customized lead collimation with a ^{137}Cs gamma irradiator (Model Mark 1-68A, Shepherd and Associates, San Fernando, CA) at a dose rate of 2.9 Gy/min.

Analysis of System Toxicity

Serial body weight measurements were performed every 3–4 days during treatment (days 1–14) and following treatment to day 31. Changes in body weight were compared using the 2 control groups (no drug $\pm$ RT) and the 8 treatment groups (IPdR $\pm$ RT). Baseline weights in control and treatment groups ranged from 26–28 grams (G).

Tumor Regrowth Assay

Two dimensional tumor measurements, using the maximum diameter and its rectangular diameter, were calculated according to the equation:

$$TV = R_1 \times R_2 \times R_2 / 2$$

where: TV is the tumor volume, R_1 is the maximum diameter and R_2 is the rectangular diameter. The measurements of tumor regrowth were performed twice weekly until tumor volumes approached 1,500 mm³, which was the maximum volume allowed by the Institutional Animal Care and Use Committee. By day 31 of study, 6 out of 8 mice in the untreated control group had tumor volumes ≥ 1500 mm³, which resulted in study termination. For some analyses, tumor volumes at the completion of study (day 31) were compared between the treatment groups (IPdR alone; IPdR + RT) to the control groups (no treatment; RT alone). ANOVA and Dunnett's Multiple Comparison Tests were conducted using GraphPad's InStat Biostatistics Program (version 3.0). A p-value of less than 0.05 is considered to be statistically significant.

We also used the time to regrow to 300% of the initial volume as a measure of IPdR dose + RT tumor response. In this analysis, tumor volumes were normalized to the volumes of one day before initiation of RT (day 10 of IPdR). The regression curve of tumor regrowth was generated by a least squares method, as we previously published (17). The time to grow to 300% of the initial volume was then calculated. Tumor regrowth delay in days was quantified by the tumor regrowth of the irradiated group (IPdR + RT and RT alone) minus that of the corresponding nonirradiated groups (IPdR alone and vehicle alone).

RESULTS

Treatment Toxicity

Throughout the entire study, all animals except one remained healthy and active. One high-dose IPdR (1000 mg/kg/d) mouse without RT was found dead on day 13 for unknown reasons. Moderate (500 mg/kg/d) and high-dose IPdR (1000 mg/kg/d) mice with and without RT experienced increasing weight loss between days 11 to 17 which ranged from 5–15% with a return to control weights by days 20–27 (Fig. 1). These toxicity data are comparable to our prior pre-clinical toxicology studies in mice, rats and ferrets given high-dose IPdR (up to 2000 mg/kg/d) for 14–28 days (13,15,18). In these prior studies, no IPdR related deaths occurred and a recent full necropsy study in rats receiving up to 2000 mg/kg/d $\times$ 28 days demonstrated no gross pathological findings and only mild reversible histopathological changes in lymph nodes, liver, and bone marrow (18).

Treatment Effect on Tumor Growth

The study was terminated on day 31 as the tumor volumes in 6 of 8 control mice reached 1500 mm³, which was the maximum volume allowed by the Institutional Animal Care and Use Committee. Consequently, the day 31 tumor volumes are used as one end-point to assess effects of treatment with RT alone, IPdR alone, and combined IPdR + RT compared to control (Fig. 2).

In panel A of Fig. 2 and in Table 1, we compare the tumor volumes (measured in mm³) at day 31 for the two control groups (no drug; RT alone) to the eight IPdR ± RT treatment groups. The mean tumor volume (± standard errors; S.E.) at day 31 for the untreated (no IPdR, no RT) control group was 1609 ± 285 mm³; while for the RT alone (2 Gy/d × 4 d) group, the mean tumor volume was 821 ± 113 mm³. With the use of low dose IPdR (100 mg/kg/d × 14 d), no differences in tumor volumes at day 31 were found with IPdR alone (1658 ± 191 mm³) nor with IPdR + RT (845 ± 149 mm³) compared to their respective controls. However, with the use of higher IPdR doses (≥ 250 mg/kg/d × 14 d), a clear IPdR dose response effect on mean tumor volumes at day 31 was found using both IPdR alone and IPdR + RT as follows: using 250 mg/kg/d × 14 d (IPdR alone, 1040 ± 105 mm³; IPdR + RT, 551 ± 86 mm³); using 500 mg/kg/d × 14 d (IPdR alone, 960 ± 131 mm³; IPdR + RT, 268 ± 69 mm³); and finally using 1000 mg/kg/d × 14 d (IPdR alone, 718 ± 99 mm³; IPdR + RT, 309 ± 60 mm³).

Using ANOVA, the p-values are 0.009 for the IPdR alone effect on change in mean tumor volumes at day 31 and 0.002 for the IPdR + RT effect on change in mean tumor volumes at day 31. Using the Dunnett Multiple Comparison Test, the effect of IPdR alone compared to no treatment at day 31 was significant at doses of 500 mg/kg/d ($p < 0.05$) and 1000 mg/kg/d ($p < 0.01$). Similarly, the effect of IPdR + RT compared to RT alone at day 31 was significant in both higher IPdR + RT treatment groups ($p < 0.01$). In panel B of Figure 2, we plot the mean percentage (%) of inhibition of tumor growth at day 31 in the various groups following treatment with IPdR alone compared to no treatment and with combined treatment (IPdR + RT) compared to RT alone. Again in the U87 sc xenografts, IPdR alone and IPdR + RT groups receiving doses of >250 mg/kg/d × 14 d showed significant effects on tumor growth inhibition.

In Fig. 3, we compare the time to growth to 300% of the initial volume from 1 day prior to the start of RT for the 10 groups (2 control and 8 treatment groups). In this analysis, the tumor volume was normalized to the volume one day before the start of RT (i.e. day 10 of IPdR or vehicle control administration), with a regression curve for each treatment group generated by a least square method. The growth inhibitory effect of RT was quantitated by a growth delay in days with or without IPdR. As shown in Fig. 3, IPdR enhanced the RT-induced tumor growth delay in a dose-dependent fashion at all IPdR doses, although the radiosensitizing effect of IPdR appears to plateau at 500 mg/kg/d × 14 days. A sensitizer (IPdR) enhancement ratio, defined as the ratio of time to regrow to 300% original tumor volume for IPdR + RT treatment groups over time to regrow to 300% original tumor volume for RT treatment alone approximates 2–3 for the two lower IPdR dose groups and 5–6 for the two higher IPdR dose groups.

Discussion

In this study, we demonstrate IPdR dose-dependent tumor cytotoxicity and radiosensitization in U87 human glioblastoma sc xenografts using analyses of tumor growth delay following a once-daily × 14 d po IPdR dosing schedule. Two different end-points were selected to assess the effects of RT alone, IPdR alone and IPdR + RT on U87 xenograft tumor growth i.e. tumor volumes at day 31 of study when the control (untreated) tumors reached a volume of 1500 mm³ (Fig. 2; Table 1) and time to regrow to 300% of the original tumor volume for IPdR + RT versus RT alone using tumor volumes at the start of RT (day 10 of IPdR treatment) (Fig.

3). Both analyses show significant IPdR-mediated radiosensitization using po IPdR dosing schedules of ≥ 500 mg/kg/d $\times$ 14 d. Additionally, po IPdR alone results in modest cytotoxicity in U87 glioblastoma sc xenografts (Fig. 2; Table 1).

Significant IPdR-mediated radiosensitization using tumor growth delay was previously reported by our group using a similar po IPdR dosing schedule in U251 human glioblastoma sc xenografts (15). Modest IPdR cytotoxicity was also found. In that study, we also found >2 -fold higher %IUdR-DNA incorporation in tumor cells following the 14 day po IPdR treatment compared to a 14 day continuous infusion of IUdR using the maximum tolerable dose (100 mg/kg/d). In this prior study, the use of continuous infusional IUdR treatment did not result in significant radiosensitization in the U251 sc xenografts, based on analysis of tumor regrowth delay to 300% of initial tumor volume as also used in this current study (Fig. 3). Thus, based on these data of IPdR-mediated radiosensitization in U87 human glioblastoma xenografts in this study (Figs. 2 and 3) and in U251 human glioblastoma xenografts from our prior study (14), we suggest that high-grade brain tumors are ideal clinical targets for this approach to radiosensitization as adjacent normal brain (which is non-proliferating) should show little to no IUdR-DNA cellular incorporation. Indeed, even with the use of continuous infusional IUdR in some recent phase I and II clinical trials, an improved survival outcome was reported in patients with anaplastic astrocytomas and possibly in patients with glioblastoma (5–7).

A second potential advantage to the use of po IPdR as a clinical radiosensitizer compared to the use of continuous intravenous infusions of IUdR is reduced systemic toxicity to normal proliferating normal tissues. In this current study, we found modest systemic toxicity to higher doses of IPdR (≥ 500 mg/kg/d) given once-daily $\times$ 14 d as manifest by a 5–15% body weight loss during drug treatment which was readily reversible within 1–2 weeks following treatment (Fig. 1). In prior pre-clinical studies, we have compared the %IUdR-DNA cellular incorporation in normal bone marrow and normal intestine in athymic mice with human brain tumor (U251) and human colorectal cancer (HT29; HCT116) xenografts treated with once daily po IPdR versus continuous infusional IUdR for periods of 6–14 days (12–14). In these studies, we found ≥ 2 -fold lower %IUdR-DNA incorporation in these dose-limiting normal tissues following po IPdR treatment compared to continuous infusional IUdR (12–14). In our most recent pre-clinical study of the systemic normal tissue toxicities to IPdR given once daily $\times$ 28 d, Fischer-344 rats tolerated IPdR doses as high as 2000 mg/kg/d without significant weight loss and no significant clinical (including analyses of blood counts and liver function tests) toxicities were found as determined weekly during and for 28 days following drug treatment (18). Additionally, no significant gross or histopathological changes were found at full necropsy in half of the study rats sacrificed immediately following the 28 day drug treatment nor in the other half of study rats sacrificed after 28 days of observation following IPdR treatment (18). Based on these 28 day toxicology data (18), the Food and Drug Administration (FDA) approved an investigator-initiated Investigational New Drug (IND) application (#70,333) for phase I clinical testing of po IPdR as a radiosensitizer.

Another planned pre-clinical study of po IPdR by our group involves analyzing the %IUdR-DNA tumor cell incorporation compared to the IUdR-DNA cellular incorporation in normal brain tissues using both U251 and U87 human glioblastoma cell lines grown as intracerebral (ic) xenografts in athymic mice. In this planned pre-clinical study using ic xenografts, we will assess the potential local therapeutic ratio for IPdR-mediated radiosensitization in human glioblastoma using these two representative human tumor cell lines. Endpoints for analysis of radiosensitization by po IPdR will be a comparison of %IUdR-DNA incorporation in tumor tissue and normal brain tissue as well as an analysis of IPdR + RT compared to RT alone on mouse survival. Recent DNA microarray analyses of these two human glioblastoma cell lines in tissue culture (in vitro), as sc xenografts, and as ic xenografts suggested a convergence of gene expression, particularly in the ic xenograft model, compared to in vitro growth (20). Since

we have already demonstrated significant IUdR (IPdR)-mediated radiosensitization in these cell lines under *in vitro* growth conditions (19) and under sc xenograft growth conditions (this study; ref. 14), we anticipate effective IPdR-mediated radiosensitization in the two glioblastoma cell lines under ic xenograft growth with “sparing” of normal brain.

Clinical trials of po IPdR as a radiosensitizer in patients with high grade brain tumors are being proposed based, in part, on the promising pre-clinical data in this study of U87 sc xenografts. In our future clinical trials of po IPdR in patients with high grade gliomas, we plan to analyze IUdR-DNA incorporation in tumor and normal brain tissue using I^{124} -UdR PET scanning during drug treatment. This PET assay has been used to measure brain tumor proliferation by other investigators (21) and these PET analyses may provide insight into how best to dose po IPdR to maximize clinical radiosensitization in patients with high grade gliomas. We recently assessed the impact of different po IPdR dosing schedules using the sc U251 human glioblastoma xenograft model, where increased %IUdR-DNA tumor cell incorporation was found with a thrice daily dosing schedule but also with enhanced normal tissue %IUdR-DNA incorporation compared to a once-daily po IPdR $\times$ 14 day dosing schedule, using the same total po IPdR dose (17). Interestingly, an every other day (qod) dosing schedule of po IPdR showed the best therapeutic gain of %IUdR-DNA tumor cell incorporation compared to normal tissue cellular incorporation (17). Since the typical duration of radiation therapy is 6 weeks in patients with high grade gliomas, a qod po IPdR dosing schedule before (1–2 weeks) and during (6 weeks) radiation therapy is also being considered for clinical testing.

Based on other pre-clinical data from our group on IUdR (or IPdR)-mediated radiosensitization of human tumors (16,22,23), we also hypothesize that human glioblastoma tumors which have DNA mismatch repair defects (MMR⁻) can be more “selectively” targeted for IPdR-mediated radiosensitization (24). Importantly, MMR⁻ glioblastomas show drug resistance to methylating drugs such as temozolomide (TMZ) and the nitrosoureas, using *in vivo* pre-clinical models and in clinical trials (25–27). Since the current standard of care for glioblastoma is to use RT (60 Gy/6 weeks) and concomitant po TMZ (2), we predict that RT + IPdR will be more effective than RT + TMZ in MMR⁻ glioblastomas. Finally, we have also demonstrated in pre-clinical studies that the concomitant use of a small molecule chemical inhibitor of DNA base excision repair (BER), called methoxyamine (MX), can enhance the cytotoxicity of TMZ (28) and the extent of IUdR (or IPdR)-mediated human tumor radiosensitization (29,30). Currently, a clinical phase I trial of TMZ + MX is ongoing at our cancer center. A potential future trial of a multi-agent radiosensitizing drug combination (i.e. TMZ + MX + IPdR) and RT for glioblastoma is under consideration, pending the results on systemic toxicities and tumor responses found in our ongoing TMZ + MX clinical trial.

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Figure 1. Body Weight Averages **Figure 1.**

Systemic toxicity [measured by change in body weight measured in grams (G)] of po IPdR administered by gastric gavage, once daily $\times$ 14 d with or without concomitant radiation therapy (RT) (2 Gy/day $\times$ 4 d; days 11–14 of po IPdR). Day 0 is the start of the 14 day IPdR treatment. Note reversible systemic toxicity in the higher ($\geq$ 500 mg/kg/d) IPdR dose groups. Concomitant RT did not enhance systemic toxicity. (Points and error bars; mean $\pm$ standard error of mean)

Figure 2A. Tumor Volumes (mm³) in the 10 experimental groups during and following treatment.

Figure 2B. IPdR Dose Response Curves: IPdR alone vs. IPdR with radiation therapy (RT)

Figure 2.

IPdR-mediated radiosensitization in U87 human glioblastoma xenografts as determined by the tumor volume at day 31 of study (panel A) (Points and error bars; mean $\pm$ standard error of mean) and by the mean % inhibition of tumor growth at day 31 with IPdR alone versus IPdR + radiation therapy (RT) (2 Gy/d $\times$ 4 d) (panel B).

Figure 3. Effect of IPdR Treatment (day 10) on RT-induced Tumor Growth Delay

Figure 3.

Effect of IPdR pre-treatment on radiation therapy (RT)-induced tumor growth delay using 2 Gy/d $\times$ 4 days. The 5 plots (A–E) represent tumor growth inhibition following RT (0 or 2 Gy/d $\times$ 4 d on days 11–14) with daily IPdR dosing (0, 100, 250, 500, 1000 mg/kg/day) on days 1 through 14. Tumor volume was normalized to the volume at one day before RT (day 10) of po IPdR. Time to growth to 300% of the initial volume (one day before RT) was then determined for each curve using a least squares regression method. The growth inhibitory effect by RT was quantitated in days by comparison of the growth delay with or without IPdR. IPdR augmented radiation-induced tumor growth delay in a dose-dependent manner. (Points and error bars; mean $\pm$ standard error of mean)

Table 1Day 31 tumor volumes (mean $\pm$ standard errors; SE) for control and treatment groups.

Control/Treatment Groups	Tumor Volumes
Control (no IPdR, no RT)	1609 $\pm$ 285 mm ³
RT alone (2 Gy/d $\times$ 4 d)	821 $\pm$ 113 mm ³
Low dose IPdR alone (100mg/kg/d $\times$ 14d)	1658 $\pm$ 191 mm ³
Low dose IPdR + RT	845 $\pm$ 149 mm ³
Intermediate dose IPdR alone (250mg/kg/d $\times$ 14d)	1040 $\pm$ 105 mm ³
Intermediate dose IPdR + RT	551 $\pm$ 86 mm ³
High dose IPdR alone (500mg/kg/d $\times$ 14d)	960 $\pm$ 131 mm ³
High dose IPdR + RT	268 $\pm$ 69 mm ³
Highest dose IPdR alone (1000mg/kg/d $\times$ 14d)	718 $\pm$ 99 mm ³
Highest dose IPdR + RT	309 $\pm$ 60 mm ³