Combining Radioimmunotherapy with Antihypoxia Therapy
2-Deoxy-d-Glucose Results in Reduction of Therapeutic Efficacy

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Abstract

Purpose: The efficacy of solid tumor radioimmunotherapy is reduced by heterogeneous tumor distribution of the radionuclide, with dose mainly deposited in the normoxic region and by the relative radioresistance of hypoxic tumor cells. In an attempt to overcome these challenges, radioimmunotherapy was combined with 2-deoxy-d-glucose (2DG), a hypoxia-selective cytotoxic inhibitor of glucose metabolism.

Experimental Design: In vitro toxicity of 2DG in LS174T cultures was tested using a colony-forming assay. The effect of combining 2DG with radioimmunotherapy in vivo was tested by administering radiolabeled anti-carinoembryonic antigen antibody ([131I]A5B7 IgG1 whole monoclonal) to nude mice bearing s.c. LS174T tumors, followed by 10 daily injections of 2DG (2.0 g/kg). Tumors were measured to assess therapeutic efficacy.

Results: Data from in vitro studies confirmed 2DG cytotoxicity in this cell line. Greater toxicity was observed under standard laboratory conditions and in hypoxic cultures than at intermediate, physiologically relevant levels of glucose and oxygen. Alone, 2DG had no effect on in vivo tumor growth ($P = 0.377$ compared with saline-treated controls). Combination of radioimmunotherapy with 2DG reduced the therapeutic effect of radioimmunotherapy (e.g., 150 $\mu$Ci $^{131}$I alone mean survival time, 48.33 ± 16.83 days; combined with 2DG, 30.67 ± 6.62 days, $P = 0.038$).

Conclusions: The combination investigated had a detrimental effect on survival. It is suggested that a cellular metabolic response to more aggressive therapy, previously reported in vitro, caused this. The results of this study have implications for the clinical application of combined cancer therapies with an antimetabolic modality component.

Radioimmunotherapy is a cancer treatment that uses a tumor-specific antibody to deliver a cytotoxic radionuclide. Delivered systemically, this therapy can access and treat tumor sites throughout the body, which might evade other therapies, such as surgery or locoregional external beam radiotherapy. Radioimmunotherapy has shown efficacy in model systems (1), lymphomas (2), and some common carcinomas (3), but has yet to achieve its clinical potential in solid tumors in which its effect has been suboptimal (4, 5).

Despite advances in antibody formats and radionuclide availability, use, and selection, radioimmunotherapy of common epithelial tumors is yet to achieve the success seen in the treatment of hematologic malignancies. Reasons for this include difficulty in delivering sufficient radiation dose to kill the tumor. Common epithelial tumors often have an abnormal vascular system leading to poor delivery of systemically delivered therapeutic molecules. Moreover, once the radionuclide has been delivered, its effect is hindered by heterogeneous resistance of tumor cells to therapy. Addressing these mechanisms of therapeutic resistance is critical to successful treatment in the clinic.

Oxygen is a potent radiosensitizer. Hypoxic cells require up to a 3-fold higher radiation dose than their aerobic counterparts to achieve the same level of toxic effect. This difference is called the oxygen enhancement ratio. The oxygen enhancement ratio for low-dose-rate therapies, such as radioimmunotherapy, is lower than for high-dose-rate external beam therapy (6–9). Although the effect of hypoxia on the efficacy of radioimmunotherapy is less than on external beam therapy, it still has an effect, as has been shown by the correlation between oxygen levels in tumors and tumor growth control in preclinical models given low-dose rate irradiation (10, 11), and increases in tumor control following combination of radioimmunotherapy with hypoxia-selective cytotoxins (12, 13). The heterogeneous distribution of the radionuclide throughout the tumor leads in turn to a heterogeneous distribution of dose.
In regions where relatively higher dose rates are delivered, the oxygen enhancement ratio will be higher, leading to greater hypoxic resistance to therapy.

A common feature of most solid tumors is raised glycolytic rate. This forms the basis of positron emission tomography imaging using $[^{18}F]$2-fluoro-2-deoxy-d-glucose, a radiolabeled structural analogue of glucose. Hypoxic cells rely on anaerobic glycolysis to maintain their energetic status, facilitated in part by increased expression of glucose transporters. Increased uptake of radiolabeled versions of 2-deoxy-d-glucose (2DG) by hypoxic cells has been reported both in vitro (14–16) and in vivo (17–19).

2DG inhibits glycolysis, possibly through competitive inhibition of, among other glycolytic enzymes, phosphoglucosomerase, inhibiting formation of ketose from glucose-6-PO$_4$ (20). The depletion of tumor ATP by 2DG in vivo has been shown (21). 2DG has been shown to be cytotoxic in vitro; given alone, it can affect the growth of some tumors in vivo (22, 23). However, this effect has not been reported in all cases (22, 24). When included in the diet, it can also reduce tumor incidence and growth (25). In addition to causing cell death, 2DG contributes a radiosensitizing effect through inhibition of repair (26–28).

Local oxygen and glucose concentrations are important factors influencing the cellular toxicity of 2DG. Their penetration into tissue is limited by both diffusion and metabolic utilization. Factors influencing their rate of metabolic utilization are complex and interwoven. Where both are available in abundance, cells tend to utilize glucose (the Crabtree effect; i.e., inhibition of oxygen consumption upon addition of glucose to tissues or microorganisms having a high rate of aerobic glycolysis), resulting in a lower respiration rate (29). Therefore, we investigated the toxicity of 2DG in standard laboratory conditions and, in addition, attempted to recreate the nutrient gradient that arises in solid tumors.

In this study, we sought to combine the normoxia targeting of radioimmunotherapy with the hypoxia toxicity of 2DG, our hypothesis being the combination of radioimmunotherapy with 2DG would achieve a greater therapeutic effect by attacking physiologically distinct regions of the tumor.

Following in vitro work confirming the cellular toxicity of 2DG, the combined therapy was tested in an in vivo xenograft model of colorectal cancer. The predicted effect of the combined therapy would be greater than additive because 2DG contributes a radiosensitizing effect.

Materials and Methods

In vitro studies

LS174T human colorectal adenocarcinoma cells (30) were obtained from the European Collection of Animal Cell Cultures (Salisbury, Wiltshire, United Kingdom). Experiments were carried out in triplicate on three separate occasions. Culture media and additives were obtained from Cambrex (Nottingham City, Nottinghamshire, United Kingdom), unless otherwise indicated. RPMI 1640 ([glucose] = 2.0 g/L) was supplemented with 10% FCS (Cambrex), 2 mmol/L glutamine, 1% nonessential amino acids, 100 units/ml penicillin, and 100 μg/mL streptomycin (hereafter called "standard growth medium") for cell propagation and control flasks.

In vitro toxicity of 2DG

Medium used for toxicity studies varied in glucose concentration and included glucose-depleted FCS (obtained by dialysis), but all other supplements were added as in the standard growth medium. The availability of both oxygen and glucose have a great effect on the toxicity of 2DG. Standard laboratory conditions (i.e., glucose 2 g/L, oxygen 20.9%) supply both at higher amounts than are normally physiologically available. To simulate conditions in a peripheral, relatively well-nourished region of the tumor, glucose was included in amounts comparable with those found in blood during random sampling (1.2 g/L; 6.7 mmol/L; ref. 31), and this was paired with oxygen at the higher levels reported in LS174T tumors (38 mm Hg or 5% where atmospheric, 20.9%; ref. 32). A decrease in provision, as might be found in the internal perinecrotic region of the solid tumor, was simulated by assuming an 80% decrease in both nutrients (glucose 0.24 g/L, 1.34 mmol/L; oxygen 7.6 mm Hg or 1%). All certified gases were obtained from British Oxygen Company (Windlesham, Surrey, United Kingdom) and contained 5% CO$_2$ balance N$_2$.

As energy can be derived from l-glutamine (included in RPMI glucose-free medium), the glutaminolysis inhibitor (33) amino oxoacetic acid (Sigma Aldrich, Poole, Dorset, United Kingdom) was added to test medium to a final concentration of 10 mmol/L. A colony-forming assay was used to investigate the toxicity of 2DG. The plating efficiency of these cells in our hands is 30%, and therefore 600 cells were placed into each flask with 5 mL standard growth medium to obtain 200 colonies in the absence of a toxic effect. Flasks were then placed in a standard incubator (37°C, 5% CO$_2$).

After 24 h, flasks were removed from the incubator and washed with 2 × 5 mL PBS before 5 mL test medium was added. Control flasks were then replaced in the incubator, whereas test flasks were placed in a hypoxia chamber of our own devising so that selected hypoxic conditions could be controlled. A polycarbonate box was clamped to a chamber of the same dimensions with five sides and access ports covered by neoprene gauntlets (Glove Box Technology Ltd., Huntingdon, Cambridgeshire, United Kingdom) for manipulation of medium constituents under constant atmospheric conditions. The sealed hypoxia chamber was kept in a standard 37°C incubator between manipulations. The oxygen concentration in the exhaust was continuously monitored using a Mettler-Toledo DO sensor and a model 4300 DO microprocessor analyzer (Mettler-Toledo, Beaumont Leys, Leicester, United Kingdom). This was calibrated by setting 100% saturation as the concentration of oxygen in the atmosphere and 0% saturation as the concentration after allowing the system to stabilize in a saturated solution of sodium sulfite, both at room temperature (1.01 bar, 20°C). Tygon R3603 (low oxygen permeability) tubing was used throughout the system. Gas was humidified using distilled water in a Dreschel assembly. Cultures were equilibrated with gas mixtures for 30 min before 2DG (Sigma Aldrich) was added at the concentrations given, always in 50 μL PBS per flask. Following incubation for 2 or 24 h, the flasks were removed from the chamber, washed with 2 × 5 mL PBS, and then incubated in 5 mL standard growth medium. After 14 days, the colonies were washed with 2 × 5 mL PBS, fixed (3 mL methanol: acetic acid 3:1), and stained with 0.25% crystal violet solution; visible colonies were then counted.

In vivo studies

Animal studies. LS174T xenograft tumors were grown in the flanks of female nude mice (nu/nu, MF1), which were 2 to 3 months old and weighed 20 to 25 g. The United Kingdom Coordinating Committee on Cancer Research Guidelines for the Welfare of Animals in Experimental Neoplasia and the Animal (Scientific Procedures) Act 1986 were followed for all of the animal experiments. Food and water were available ad libitum, the water containing 0.1% potassium iodide to block thyroid uptake of radioactive iodine.

Antibody biodistribution studies

Antibody radiolabeling. The whole IgG monoclonal antibody A5B7 (34) was labeled with $[^{131}I]$ (T$_{1/2}$ 8.04 days, β-emission energy 0.606 MeV, range of β-energy deposition, X$_{100}$, 0.83 mm; ref. 35) using the chloramine-T method (36). Radiochemical purity was confirmed using
2DG Reduces RIT Efficacy

TLC (mobile phase methanol/H2O, 80:20, stationary phase Si60 gel) and antigen binding was confirmed by application of a dilution to a carcinoembryonic antigen affinity column.

Biodistribution. Biodistribution mice were given radioimmunoconjugate and either 2DG (2 g/kg in 0.2 mL, i.p.) or saline (0.2 mL, i.p.). At 24 and 48 h, mice (n = 4 for each treatment) were killed by cervical dislocation and tissues were taken for gamma counting to investigate the effect of 2DG on whole-body distribution of radioactivity. Samples of tumor were taken for histologic study. Following formalin fixation, paraffin-embedded tissue was sectioned (3 μm) and exposed to phosphor storage plates. After 14-day exposure, the latent images created were read using a Storm 860 phosphor image reader. Sections were stained using H&E to enable analysis of distribution of radionuclide throughout the tumor. For more details on this technique, see ref. 17.

Therapy studies

Preliminary toxicity study. Previous in vivo studies have reported that the LD50 of 2DG is in the range 3.85 to 4.5 g/kg (22, 37). Preliminary studies were undertaken to investigate the effect of 2DG in our model system. Mice (n = 2) were injected i.p. with 0.5, 1.0, and 2.0 g/kg 2DG in 0.9% NaCl solution, always in a volume of 0.2 mL, on alternate days for a total of three times. It was found that the 2DG had no long-term toxic effect on the mice. As previous investigators have found tumor growth retardation effects with 2.0 g/kg 2DG, this was selected for the combination study.

Combined therapy study. Eight groups of mice (n = 6) were involved in the combined experiments, which commenced when the tumors reached a mean of 0.047 cm3 (±0.029 cm3), allowing sufficient time to study relative growth rates under different treatment regimes. Antibody was injected i.v. and 2DG was injected i.p., both diluted in saline (0.9% NaCl solution), so that all i.v. injections were 0.1 mL and all i.p. injections were 0.2 mL. Where a group did not receive either of these treatments, saline was given. Group 1 received saline only and group 2 received 2 g/kg 2DG (e.g., 50 mg for a 25 g mouse) i.p. and saline (0.9% NaCl solution) i.v. Groups 3, 4, and 5 received [131I]A5B7 therapy alone (50, 100, and 150 μCi respectively). Groups 6, 7, and 8 received [131I]A5B7 therapy followed after 1 h with 2DG. Mice in these groups were given 2DG at this level, at the same time, for a further 9 days (i.e., 10 in total). [Unlabeled antibody and radiolabeled nonspecific antibody have been shown to have little effect on tumor growth control in this system and poor localization, respectively (38); therefore, these controls were not included in this study.] Mice were weighed and tumor dimensions were measured [volume was calculated by (length × breadth × depth × π) / 6] on the day that therapy commenced and then on alternate days for 2 weeks, then twice a week for the remainder of the experiment. Mice were sacrificed when the tumor volume reached or exceeded 1.5 cm3. Survival data were analyzed and log-rank analysis was carried out using Sigma Plot V8 for Windows.

Results

In vitro studies

The deoxygenation of the hypoxic chamber is shown in Fig. 1. Most (95%) of the change was reached after 10 to 12 min using a flow rate of 4 L/min. Following dialysis, FCS protein levels were unchanged (from 31 to 30, mean of 30.75 ± 0.96 g/L), but glucose decreased after three buffer changes from an original 6.8 mmol/L to 0.1 mmol/L; that is, below detection range limit for the assay.

In vitro toxicity of 2DG

Data from a colony-forming assay, used to investigate the toxicity of 2DG in physiologically relevant levels of oxygen and glucose, are presented in Fig. 2. No toxicity was detected after 2-h incubation (data not shown). After 24 h incubation with 2DG, toxicity was detected in the cultures with standard cell medium glucose and atmospheric oxygen. It has been reported that 5 mmol/L glucose can inhibit oxidative phosphorylation by 54% (29). The Crabtree effect made the cells in standard laboratory conditions (11.17 mmol/L glucose) more susceptible to glycolytic inhibition, overstating the cellular toxicity of 2DG. Little or no toxicity was found in intermediate levels of glucose (6.7 and 1.34 mmol/L glucose) and oxygen (Fig. 2B and C). Under these conditions, similar to conditions found in solid tumor, the Crabtree effect had less influence on energy metabolism, and 2DG consequently had less toxicity effect. Greater toxicity was shown under anoxic and glucose-deprived conditions (Fig. 2D) even at low levels of 2DG. Although multiple tests were used, the significance was impressive at higher doses of 2DG, so type I errors (i.e., due to chance) are unlikely.

Antibody labeling and biodistribution studies

Radiopharmaceutical purity of the radioimmunoconjugate was found by TLC to be ≥99%, and antigen binding was ≥87%.

Fig. 1. Deoxygenation of the hypoxic chamber by gas containing 5% (short dashed line), 1% (long dashed line), and 0% (solid line) O2 (5% CO2, balance nitrogen).
Biodistribution. Biodistribution data confirmed that radio-labeled antibody localized selectively in the tumor (Table 1). Mice injected with saline or 2DG (2 g/kg) gave similar whole-body biodistributions and tumor uptakes \((P = 0.86, 48 \text{ h} P = 0.62, \text{all tissues})\). Radionuclide localization to the tumor was higher in the 2DG-treated group at 48 h \((\text{tumor } \% \text{ID/g, saline and 2DG treated 10.63} \pm 4.16, 24.33 \pm 12.62 \% \text{ID/g, respectively})\) although the difference was not statistically significant \((P = 0.08)\). Figure 3 shows tumor sections that have been stained with H&E to show morphology, and their corresponding radioluminographs showing heterogeneous antibody localization throughout the tumor. The loss of therapeutic effect could have been attributed to greater residence of radionuclide in the necrotic region of the tumor, but these images do not support this. From these results, we conclude that 2DG did not deleteriously affect therapy by reducing radionuclide uptake in, or retention by, the tumor.

Combined therapy

Systemic toxicity. Total body weight was used as a surrogate of toxicity, in addition to general observation of well being. In a preliminary study, 2DG showed little toxicity at the highest level tested \((2.0 \text{ g/kg})\), which was therefore chosen for therapy studies.

Mice given 2DG showed no acute toxicity, merely somnolence for 0.5 to 1.0 h, followed by full recovery. The only two groups to show weight loss were those given radioimmunotherapy alone at 50 and 150 \(\mu\text{Ci}\) (means at nadir of 95.4\% on day 7 and 95.1\% of pretreatment weight on day 3, respectively). The combined 2DG + 150 \(\mu\text{Ci}\) group showed no weight loss \((e.g., 103.5\% \text{ of starting weight on day } 7)\).

Tumor growth control. Individual tumor growth for all mice is presented in Fig. 4. The four graphs compare the growth delay of tumors given a range of radioactivity \((0-150 \mu\text{Ci})\) either alone \((\text{dashed lines})\) or in combination with 2DG \((\text{solid lines})\). Survival data for all groups is presented in the form of a Kaplan-Meier plot in Fig. 5 \((\text{data for untreated and radioimmunotherapy alone is shown in Fig. 5A; data for 2DG alone treated and in combination with radioimmunotherapy is shown in Fig. 5B})\). There was no significant difference between the tumor growth and survival of the untreated and 2DG alone–treated groups \((\text{maximum survival 14 and 12 days, respectively, } P = 0.37)\). As would be expected, radioimmunotherapy alone delayed tumor growth, and thus extended survival, in a dose-dependent manner.

Combination with 2DG treatment neither further delayed tumor growth nor enhanced survival. Indeed, in some groups, it significantly reduced the effect of radioimmunotherapy. For the 50 \(\mu\text{Ci}\) group, survival times with and without 2DG were not significantly different \((31 \text{ versus 20 days with 2DG treatment, } P = 0.91)\). For the 100 \(\mu\text{Ci}\) groups, there was a noticeable loss of tumor growth control when radioimmunotherapy was combined with 2DG, which was not yet significant \((\text{maximum survival for radioimmunotherapy})\).

**Table 1.** Biodistribution \((% \text{ID/g})\) of \(^{131}\text{I}-\text{labeled anti–carcinoembryonic antigen antibody}\) in MF1 nude mice bearing s.c. LS174T human adenocarcinoma xenografts \((\text{mean of } n = 4, \text{ SD in parentheses})\)

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<tr>
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<th>24 h</th>
<th>48 h</th>
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<tr>
<td></td>
<td>Saline</td>
<td>2DG</td>
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<tr>
<td>Blood</td>
<td>4.42 (1.86)</td>
<td>6.26 (3.47)</td>
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<tr>
<td>Liver</td>
<td>2.14 (0.36)</td>
<td>2.42 (0.95)</td>
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<tr>
<td>Kidney</td>
<td>1.07 (0.34)</td>
<td>1.30 (0.53)</td>
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<tr>
<td>Lung</td>
<td>1.80 (0.85)</td>
<td>1.96 (0.83)</td>
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<tr>
<td>Spleen</td>
<td>1.15 (0.24)</td>
<td>1.18 (0.53)</td>
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<tr>
<td>Colon</td>
<td>0.57 (0.21)</td>
<td>0.57 (0.20)</td>
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<tr>
<td>Muscle</td>
<td>0.61 (0.11)</td>
<td>0.69 (0.42)</td>
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<tr>
<td>Tumor</td>
<td>21.67 (8.81)</td>
<td>21.63 (7.11)</td>
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**NOTE:** Data presented confirm that labeled antibody localized in the tumor and that the 2DG therapy regimen used did not decrease radionuclide localization to the tumor.
alone-treated group 46 versus 34 days radioimmunotherapy combined with 2DG treatment, \( P = 0.092 \). However, for the 150 \( \mu \text{Ci} \) groups, combination of 2DG with radioimmunotherapy reduced therapeutic efficacy significantly (66 days maximum survival radioimmunotherapy alone versus 41 days with 2DG treatment, \( P = 0.038 \)). Four of the six mice in the 150 \( \mu \text{Ci} \) group outlived all six mice in the 150 \( \mu \text{Ci} \) + 2DG group. No sign of tumor was found in the final surviving mouse at necropsy. The difference between the 100 \( \mu \text{Ci} \) radioimmunotherapy alone group and the 150 \( \mu \text{Ci} \) + 2DG group was not significant (\( P = 0.487 \)). Addition of 2DG to radioimmunotherapy effectively reduced the dose by one third in terms of survival.

**Discussion**

Having investigated the increased uptake of radiolabeled deoxyglucose in hypoxic regions of tumors (17), we anticipated that complementing the radiotoxicity delivered by the radioimmunotherapy to the normoxic region with antimetabolic therapy targeted toward the hypoxic region would lead to a more effective combination cancer therapy.

However, this study combining radioimmunotherapy therapy with 2DG found no advantage over each treatment alone. On the contrary, as radioimmunotherapy dose increased, producing a dose-dependent delay in terms of tumor growth control and, hence, extended survival times when used alone, the addition of 2DG led to a reduction in therapeutic efficacy.

The glucose structural analogue 2DG has been the focus of much interest. Once the higher uptake of glucose by tumors was documented, the potential for cancer therapy based on this effectively targeted glucoprivation was clear. The greater understanding of tumor heterogeneity, production of hypoxia, and in turn the reliance of hypoxic cell on glucose metabolism, which 2DG can inhibit, suggests that this is an attractive target for therapy. Combining this with therapies that are more effective in the normoxic region should be a significant advantage in the clinic.

Although the current study is the first to report the detrimental effect of combining 2DG with radioimmunotherapy in vivo, previous in vitro studies support our findings. For example, Seymour et al. (39) reported an increased recovery by cells following split dose irradiation while incubated with 2DG compared with controls. Although 2DG did have a radiosensitizing effect during primary irradiation, recovery after secondary irradiation was increased (increasing with time up to \( 20 \times \) at 4 h) by the addition of 2DG. Split dose recovery kinetics might be considered to be more relevant to the delivery of toxicity by radioimmunotherapy than those from single irradiations. Similar observations have been made by Hunter and Blekkenhorst (33). This suggests an increased response by the cell under a more aggressive therapeutic regimen, possibly leading to increased repair and survival. Furthermore, data extracted from a previous publication seem to agree with our findings (22). Here, tumors given external beam therapy (\( \leq \text{2 Gy} \)) alone were smaller than when the same irradiation regime was combined with 1 g/kg 2DG.

In vivo studies of 2DG alone have reported either no effect (22, 24) or slight tumor growth delay (22, 23). These differences may be explained in part in the light of our in vitro results, which suggest that only the very hypoxic regions of solid tumor are susceptible to 2DG toxicity. Both the level of hypoxia (\( \text{pO}_2 \text{ mmHg} \)) and its extent within the tumor are important. For example, literature data suggest that the EMT6 tumor (as used in ref. 23) is more hypoxic than the LS174T tumor. Data have been obtained using the Eppendorf oxygen electrode, which suggests that the population of cells in the LS174T tumor that are hypoxic is smaller than in the EMT6 tumor. In 10 LS174T tumors, no values lower than 2.5 mm Hg were recorded (40). However, for the EMT6 tumor, 34.7% of values were below 1 mm Hg, and 50.9% of values were lower than 2 mm Hg (\( n = 3 \); ref. 41). Consequently, a greater proportion of the tumor cell parenchyma was adversely affected by 2DG toxicity in the EMT6 study (23), resulting in the dose-dependent effects on tumor growth reported. Furthermore, our in vitro data suggest that only the very hypoxic populations of cells in the LS174T tumor would be susceptible to 2DG toxicity. If the tumor cell does not survive hypoxia, leading to the more oxygenated character of the solid tumor, then the LS174T cells affected by the toxicity of 2DG might be dying anyway, and not contributing to tumor growth.

Aft et al. (23) also report on combined therapies using molecularly targeted radiotherapy and 2DG but, in contrast to the current study, found an improvement in therapeutic response over targeted radiotherapy alone. EMT6 tumors were treated with a combination of tumor-targeted radiotherapy (the
hypoxia-selective compound $^{64}\text{Cu}$-ATSM) and a similar 2DG regimen to ours. Following injection of 2 mCi $^{64}\text{Cu}$-ATSM and daily injections of 2DG (40 mg per mouse), tumors were three times smaller than controls and half the size of tumors that had received each treatment alone by day 17. Two major differences between that work and our own are that the radionuclide $^{64}\text{Cu}$ is an emitter of high-LET particles (Auger electrons), which cause different patterns of cellular damage compared with the

Fig. 4. Tumor growth data for control and therapy combination groups ($n = 6$ in all groups). A, data for the untreated group (dashed line) and the 2DG-treated group (solid line, in all cases). B to D, data for the 50, 100, and 150 μCi groups, respectively. Treatment with 2DG had no significant effect on tumor growth alone (saline-treated versus 2DG-treated, $P = 0.37$), but did significantly reduce the efficacy of therapy in the 150 μCi group (radioimmunotherapy alone versus radioimmunotherapy + 2DG, $P = 0.038$).
The temporal element of the Seymour et al. study is observed, the effects to be accounted for are the temporal aspect of radioresistance data from Seymour et al., the reduction in the effect of targeted radiotherapy seen in this study, and the increase in the effect of 2DG with increasing injected activity. It would be imprudent to attribute the observed effect to any particular agent or agents. However, we propose a mechanism based on the known behavior of nuclear factor-κB (NF-κB). The actual mechanism might involve NF-κB, or other molecules with similar activities.

NF-κB is a transcription factor in, among other things, radioresistance (42). This is a cytoplasm-based molecule, which, under normal conditions, is inhibited by binding to IκB-α. Upon stimulation, such as ionizing radiation, IκB kinase is activated, which phosphorylates IκB-α, which is then ubiquitinylated and degraded. NF-κB translocates to the nucleus where it binds to the promoter regions of genes to activate transcription. The amount of NF-κB translocating to the nucleus increases with time up to a maximum at 4 to 6 h, after which it declines (43). Binding of NF-κB to its promoter sequence also leads to production of IκB-α to replace that degraded (44, 45), controlling activity in a negative feedback loop.

The temporal element of the Seymour et al. study is explained by the increase in NF-κB nuclear translocation with time—highest radioresistance was observed at 4 h, which was their longest time point. If they had continued their study and used time intervals of >4 h between doses, then the increase in survival might have decreased after 4 to 6 h, depending on the cell line.

The decrease in effect of radioimmunotherapy when combined with 2DG in our experiment might be explained by the effect of decreased energetic cellular status on protein production under hypoxia. Synthesis of inhibitor might be decreased under challenging energetic conditions.

The amplified effect of 2DG with increasing radiation dose is explained by the response of NF-κB being increased under greater stimulation—therefore, it had a greater effect conferring radioresistance on the cells, and in the presence of 2DG its inhibition mechanism was decreased. The activity of NF-κB does not seem to be affected by 2DG (46).

In addition to other factors, the discordance in cellular response to the combinations of irradiative therapies and 2DG might be partly explained by variations in NF-κB activities between cell lines, which can vary markedly.

The findings of this study have implications for targeted radiotherapy of solid tumors. Effectively, the 2DG-treated tumor has been glucoprivated; that is, the inability to use glucose has been imposed by the intracellular activity of 2DG. This has therefore increased the population of tumor cells under conditions already experienced by a proportion of the tumor at any one time (i.e., having lower glucose than is required). As our tumors have responded to this by resisting targeted radiotherapy, it suggests that a population of solid tumors might always respond to targeted therapy in this manner. Were this response to be confirmed in a range of relevant cell lines, and a marker identified so that cells responding in this way in vivo could be detected, then this could help to explain, in part, the resistance of solid tumors to targeted radiotherapy. In addition, if methods of blocking or circumventing the response could be developed, then improved therapeutic efficacy could be attained. The use of 2DG in combination with irradiative cancer therapy has been advocated clinically (for example, see ref. 47). This study suggests that more should be known about the mechanisms of interaction of these agents, as well as the pathophysiology of the target tumor, before such a therapy regime is widely applied.

In this study, we have sought to combine the strength of radioimmunotherapy, which effectively treats the normoxic region of the tumor, with a therapy targeted toward the hypoxic region, which can survive radiotherapy. The combination of 2DG with radioimmunotherapy resulted in a poorer response of the cell to radioactivity under different environmental conditions and should provide new therapeutic targets.

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References


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