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A GSK-3 β inhibitor protects against radiation necrosis in mouse brain

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Abstract

Purpose/Objectives—To quantify the effectiveness of SB415286, a specific inhibitor of GSK-3 β , as a neuroprotectant of radiation-induced, CNS (brain) necrosis in a mouse model.

Methods and Materials—Cohorts of mice were treated with SB415286 or DMSO prior to irradiation with a single 45-Gy fraction targeted to the left hemisphere (brain) using the Leksell Perfexion Gamma Knife. The onset and progression of radiation necrosis were monitored longitudinally by non-invasive *in vivo*, small-animal MRI, beginning 13 weeks post-irradiation. MRI-derived necrotic volumes for SB415286- and DMSO-treated mice were compared. MRI results were supported by correlative histology.

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Conflict of Interest: none

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Results—Mice treated with SB415286 showed significant protection from radiation-induced necrosis, as determined by *in vivo* MRI with histologic validation. MRI-derived necrotic volumes were significantly smaller at all post-irradiation time points in SB415286-treated animals. While the irradiated hemispheres of the DMSO-treated mice demonstrated many of the classic histologic features of RN, including fibrinoid vascular necrosis, vascular telangiectasia, hemorrhage, and tissue loss, the irradiated hemisphere of the SB415286-treated mice consistently showed only minimal tissue damage. These studies confirmed that treatment with a GSK-3 β inhibitor dramatically reduced delayed time-to-onset necrosis in irradiated brain.

Conclusions—The unilateral cerebral hemispheric stereotactic radiosurgery mouse model, in concert with longitudinal MRI monitoring, provides a powerful platform for studying the onset and progression of radiation necrosis and for developing and testing new neuroprotectants. SB415286's effectiveness as a neuroprotectant for necrosis motivates potential clinical trials of it or other GSK-3 β inhibitors.

Keywords

Magnetic resonance imaging; Radiation necrosis; Neuroprotection; Small animal models; Radiotherapy

Introduction

Radiation necrosis (RN) is a major complication of radiotherapy [1] that often limits radiation dose. The incidence of reported RN ranges from 3–24% of patients following focal irradiation [2–4] and may be up to threefold higher with concurrent chemotherapy [4]. A number of treatment options for RN are available [2,5–8], however, each therapy has limitations and introduces potential complications for patients. Current interventions have limited success in mitigating RN.

A recent review describing the pathobiology, diagnosis, and management of RN, outlined two mechanisms for the origin and development of cerebral RN, vascular injury and glial injury, concluding that neither alone fully explained RN pathogenesis [9]. RN has been associated with acute apoptosis of oligodendroglial precursor cells that results in: (i) loss of mature oligodendrocytes, (ii) demyelination and white-matter necrosis [10], and (iii) damaged astrocytes that result in breakdown of the blood brain barrier (BBB) [11,12]. Damage to endothelial cells could elicit sphingomyelinase-dependent apoptosis, leading to increased cytokine production. BBB breakdown is associated with elevated levels of vascular endothelial growth factor (VEGF) and, together with subsequent narrowing of vessels due to fibrosis, can result in edema and necrosis [13]. Inhibiting the apoptotic process of glial cells may prevent or ameliorate the development of radiation necrosis.

Others and we have studied neuroprotection by GSK-3 β inhibitors, which increase neuronal cell viability [14–16]. GSK-3 β is a multifunctional serine/threonine kinase originally identified as a regulator of glycogen metabolism [17]. GSK-3 β is widely expressed in most tissues, however, it is expressed abundantly in brain and especially in neurons, due to its vital role in neuronal signalling [18].

GSK-3 β induces apoptosis in response to a wide variety of conditions [19–21] including irradiation [16]. In cell culture studies, apoptosis was either attenuated or prevented by inhibiting GSK-3 β in primary [15] and hippocampal (HT-22) neurons [22]. GSK-3 β inhibition enhances survival of irradiated endothelial cells due to attenuated apoptosis and to protect cells *in vivo* from radiation-induced apoptosis [23].

GSK-3 β inhibitors are utilized for the treatment of various diseases [24,25], with ongoing development of new GSK-3 β inhibitors that are in various phases of clinical trials [25]. Accordingly, GSK-3 β inhibition represents a potential therapeutic approach in the treatment of radiation necrosis.

A novel animal model of stereotactic radiosurgery-induced, delayed radiation necrosis developed in our lab [26] accurately recapitulates the classic histologic features of radiation necrosis observed clinically and provides a robust platform to test the efficacy of neuroprotective drugs. Building on the observation that inhibitors of GSK-3 β reduce radiation-induced cell death *in vitro*, this model is employed herein to validate the neuroprotective effect of SB415286, a specific GSK-3 β inhibitor, in mouse brain following high-dose radiation treatment. Further, the effect of SB415286 on the effectiveness of radiation therapy in tumor-bearing mice is investigated.

Materials and Methods

Animals

Six-week-old, female Balb/c mice were used for the study and observed daily and weighed weekly to ensure that interventions were well tolerated.

Irradiation and treatment

Mice were irradiated at dose rates of 2.7 to 1.8 Gy/min with the Leksell Gamma Knife® Perfexion™ (Elekta; Stockholm, Sweden; <http://www.elekta.com>), a state-of-the-art device used for stereotactic irradiation of patients with malignant brain tumors. Mice were supported on a specially designed platform mounted to the stereotactic frame that attaches to the treatment couch of the Gamma Knife (GK). Mice were anesthetized with a mixture of ketamine (25 mg/kg), acepromazine (5 mg/kg), and xylazine (5 mg/kg), injected intraperitoneally 5 min before the start of irradiation. For studies of radiation necrosis, mice were irradiated with single fractions of either 60-Gy or 45-Gy of radiation targeted to the left hemisphere (brain). 45 Gy represents the lowest radiation dose that consistently produced delayed radiation necrosis and approaches doses that may be used clinically in Gamma Knife radiosurgery for treatment of malignant tumors and arteriovenous malformations [27,28]. The resulting brain parenchymal changes were monitored and characterized by MRI and histology. For tumor therapy studies, mice received 22.5 Gy of radiation in three equal fractions in the hemisphere in which the tumor was implanted.

GSK-3 β inhibitor

SB415286 (GlaxoSmithKline; London, UK) is a potent and selective cell-permeable, ATP-competitive GSK-3 β inhibitor with a half-maximal inhibitory concentration (IC₅₀) and an inhibitory constant (K_i) of 78 and 31 nM, respectively [29].

Experimental Outline

Two different sets of experiments, designated as “A” and “B” were performed. Experiment “A” was designed to validate the efficacy of GSK-3 β inhibition in the treatment of radiation necrosis. Two cohorts of mice (n=12 each) received a single 45-Gy dose (50% isodose) of GK radiation. At this dose, the onset of radiation necrosis typically occurs 10–12 weeks post-irradiation. Mouse cohort #1 was an irradiated, DMSO-treated, control group; cohort #2 was treated with SB415286 (1 mg/kg in DMSO) i.p. at 24 h, 12 h and 30 min prior to irradiation.

Experiment “B” was designed to test whether GSK-3 β inhibition would also have protective effects in tumor tissue. Orthotopic tumors were generated using DBT glioma cells, transduced for stable expression of both firefly luciferase and green fluorescent protein (DBT-FG cells), as described previously [30]. Post-contrast T1-weighted imaging was performed on post-implantation day (PID) 9 to confirm tumor implantation. Mice were randomly assigned into three cohorts: cohort #1 (n=10) was an untreated control group (no irradiation, no SB415286); cohort #2 received a total 22.5 Gy dose (50% isodose) of GK radiation targeted to the tumor site over 3 fractions given at PID 10, 12 and 14 (but no SB415286); cohort #3 received the exact same radiation treatment as cohort #2 but was also treated with SB415286 (1 mg/kg in DMSO), at 24 h, 12 h and 30 min prior to the first irradiation fraction and then 30 min prior to the subsequent irradiation fractions. Mice were sacrificed if/when they lost more than 20% bodyweight, suffered obvious behavioral detriments, such as ataxia.

MR imaging

Images were collected in an Agilent/Varian (Santa Clara, CA) 4.7-T small-animal MR scanner equipped with a DirectDrive console. The scanner is built around an Oxford Instruments (Oxford, United Kingdom) 33-cm, clear-bore magnet equipped with a 21-cm inner diameter, actively shielded Agilent/Magnex gradient coil assembly (maximum gradient, 28 G/cm; rise time approximately 200 ms) and Oy International Electric Company (IEC; Helsinki, Finland) model A-240 amplifiers (300 V and 300 A).

Data were collected with an actively decoupled coil pair: 1.5-cm outer diameter surface coil (receive) and a 9-cm inner diameter Helmholtz coil (transmit). Mice were anesthetized with isoflurane/O₂ (3% [vol/vol]) before the imaging experiments and maintained on isoflurane/O₂ (1% [vol/vol]) throughout the experiments. Mice were restrained in a laboratory-constructed Teflon head holder with ear bars and a tooth bar and were placed on a water pad with circulating warm water to maintain body temperature at approximately 37°C. They were injected intraperitoneally with 0.5 mL Omniscan (gadodiamide; GE Healthcare, Princeton, NJ) contrast agent, diluted 1:10 in sterile saline, 15 min before being placed in the magnet.

Mice in experiment “A” that received 45 Gy of radiation were imaged 10, 13, 16, 19 and 22 weeks post-irradiation. For cohort #1 in experiment “B”, the untreated control group, mice were imaged at PID 10 and then every 3 days until they were sacrificed. For cohorts #2 and #3 of experiment “B”, the treated groups, mice were imaged at PID 10 (before receiving GK radiation), 17, and then every 3 days until they were sacrificed. Multislice, T2-weighted, spin-echo transaxial images were obtained during the 3–15 minutes immediately following gadodiamide administration with the following parameters: time to repetition (TR) = 1.5 s, time to echo (TE) = 0.05 s, field of view (FOV) = $1.5 \times 1.5 \text{ cm}^2$, slice thickness = 0.5 mm, 21 slices to cover the whole brain. Multislice, T1-weighted, spin-echo transaxial images were then obtained over 5 minutes with the following parameters: TR = 0.65 s, TE = 0.02 s, FOV = $1.5 \times 1.5 \text{ cm}^2$, slice thickness = 0.5 mm, 21 slices.

Histology

Immediately after the last MR imaging session, mice were perfused intracardially with 0.1 M Phosphate-buffered saline and formalin. The heads were dissected and fixed in formalin for 24 hrs. The brains were removed from skulls and embedded in paraffin. The brain was coronally sectioned and 8-micron-thick tissue sections were stained with hematoxylin and eosin (H&E) according to standard protocols. For each brain, a single slice reflecting the same anatomic features was chosen for comparison.

Clonogenic survival assay

Clonogenic survival assays were performed as described previously [31]. Briefly, the DBT-FG cells were treated with either SB415286 (25 μM) or DMSO for 16h and calculated numbers of cells were plated to enable normalization for plating efficiencies. Cells were allowed to attach for 5 hours and then irradiated with 0, 2, 4, 6 or 8 Gy. After 7–10 days of incubation, plates were fixed with 70% ethanol and stained with 1% methylene blue. Colonies consisting of >50 cells were counted by viewing the plates under a microscope. The survival fractions were calculated as (number of colonies/number of cells plated)/(number of colonies for corresponding control/number of cells plated). The dose-modifying factor at 10% survival (DMF_{10}) was calculated from the survival curves by computing the ratio of the IR doses that reduced survival to 10% with and without inhibitor.

Statistical analysis

For experiment “A”, the weekly changes of necrotic volumes for each group were summarized using means and standard deviations. Because of the relatively large variability in these volumes, a logarithm transformation was performed to better satisfy the assumption of a normal distribution. Two-way ANOVA was used to compare the mean volumes of radiation necrosis among treatment groups, followed by *ad hoc* multiple comparisons for between-group differences at specific time points. For experiment “B”, “Last Observation Carried Forward (LOCF)” analysis, an established statistical method that assumes that an outcome remains frozen in time after a subject drops out of a study – i.e., the last observed value is carried forward to the end of the study [32], was used for visualization of tumor growth. Two-way ANOVA was used to compare the mean tumor volumes among treatment groups, followed by *ad hoc* multiple comparisons for between-group differences at specific

time points. All tests were two-sided and a p-value of 0.05 or less was taken to indicate statistical significance. The statistical analysis was performed using SAS 9.3 (SAS Institutes, Cary, NC). The correlation between T1-weighted- and T2-weighted-derived slopes was assessed using linear mixed-effect models. Specifically, a mixed model for repeated measurement data was fitted to T1-weighted- and T2-weighted-derived volumes separately and the slope of the radiation necrosis “growth curve” was estimated for each mouse.

Results

MRI detects radiation necrosis as image hyperintensity in T2-weighted images

Representative T2-weighted spin-echo images of SB415286-treated and DMSO-treated mice, covering the same anatomic region of the brain and collected at 13, 16, 19, and 22 weeks following a single 45-Gy dose of radiation are shown in Figure 1. Hyperintense areas in these images correspond with regions of RN in the brain. Evident hyperintense regions are clearly seen in DMSO-treated, control mice at 13 weeks post-irradiation and these regions expand significantly in extent by 22 weeks. Images of SB415286-treated mice show minimal hyperintensity through 22 weeks post-irradiation.

Necrosis volumes can be measured quantitatively from MR images

The development of RN is associated with hyperintensity in both T2-weighted and contrast-enhanced, T1-weighted images. At each time point, regions of interest were drawn around the entire brain in several contiguous image slices, chosen to include the entire hyperintense region. Each brain was divided along the midline into left (irradiated) and right (non-irradiated) hemispheres. The image intensity for each individual pixel in the left hemisphere was normalized by the average of the 25 pixels (5×5 square) including and immediately surrounding its mirror-image pixel in the right hemisphere, and histograms of normalized intensity for the irradiated hemisphere were constructed. We performed this same analysis in a cohort (n=10) of non-irradiated mice and showed that the resulting histogram is symmetric; with 99% of the pixels being distributed in the intensity range 0.6 – 1.4 about a normalized mean of 1.0. Therefore, an intensity threshold of 1.4 (normalized ratio) was chosen as the cutoff for normal brain tissue. The number of pixels exceeding this threshold measures the necrotic volume at each time point for irradiated mice.

GSK-3 β inhibitor slows the progression of radiation necrosis in brain tissue

Cohorts of mice, treated with either SB415286 (1mg/kg) or DMSO (control) received a single, 45-Gy dose of radiation. Seven out of 12 mice treated with SB415286 had smaller necrotic regions, measured from T2-weighted MR images, at 22 weeks compared with control mice. The neuroprotective effect is clearly evident in the progression of the mean volumes (Figure 2A), in which the treated cohort (red) had a greatly diminished volume of necrosis at weeks 16, 19 and 22 post-irradiation compared to DMSO-treated controls (blue) (p=0.0024, 0.0003, and 0.0056 for weeks 16, 19 and 22, respectively). Figure 2B shows the correlation between the slopes of the necrotic-volume “growth curves” of necrotic volume derived from contrast-enhanced T1- and T2-weighted images. The excellent correlation (r=0.98; p<0.001) demonstrates the congruence of these measurements. In contrast to the 45-Gy irradiations, mice irradiated with a single 60-Gy dose of radiation showed no

neuroprotective effect from SB415286 treatment (slope of linear growth curves = $6.21 \text{ mm}^3/\text{week}$ for controls and $6.17 \text{ mm}^3/\text{week}$ for SB415286-treated mice, $p=0.97$).

Histology reveals that GSK-3 β inhibitor protects against radiation necrosis in irradiated brain tissue

Figure 3 shows 2x (top) and 10x (middle) H&E histologic images and corresponding T1-weighted MR images (bottom) for DMSO-treated, and SB415286-treated mice at 22 weeks following a single, 45-Gy dose of radiation. The irradiated hemispheres of the DMSO-treated mice demonstrated many of the classic histologic features of RN, including fibrinoid vascular necrosis (black arrow), vascular telangiectasia (yellow arrows), hemorrhage (red arrow), and loss of neurons and edema (blue arrows) [33]. In contrast, the irradiated hemisphere of the SB415286-treated mouse consistently showed only minimal tissue damage. These histologic findings support the MR data shown in Figure 2, demonstrating a significant neuroprotective effect of GSK-3 β inhibition.

GSK-3 β inhibitor does not affect the therapeutic efficacy of radiation on tumor tissue

Figure 4 shows representative T1-weighted, spin-echo images of non-irradiated, radiation only, and radiation plus SB415286-treated tumor-bearing mice; images cover the same anatomic region of the brain and were obtained on PID 10 and 20. Hyperintense areas in these images correspond with regions of tumor in the brain. Evident hyperintensity is clearly seen in all three mice at PID 10, but only in the non-irradiated mice did the hyperintense region grow significantly in extent between PID 10 and 20. This is seen clearly in the tumor growth curves for the three cohorts (Figure 5). Tumors in non-irradiated mice grew quickly compared to the irradiated mice, while no significant difference was observed between radiation-treated mice with and without pre-treatment with SB415286, demonstrating that SB415286 does not affect the therapeutic efficacy of radiation.

Survival data are shown in Figure 6A for all cohorts. If SB415286 fully protected tumor cells from radiation, the survival curves of cohorts 1 and 3 would be the same. Instead, treatment with radiation plus SB415286 leads to a significant increase in median survival compared with non-irradiated controls ($P=0.0024$), a survival increase that was statistically indistinguishable from radiation treatment alone ($P=0.9974$). This observation demonstrates that administering the GSK-3 β inhibitor SB415286 has no effect on this glioma model's sensitivity to radiation. We further evaluated the effect of the GSK-3 β inhibitor SB415286 on irradiated DBT-FG cells in a clonogenic assay. As shown in Figure 6B, the DBT-FG glioma cells treated with $25 \mu\text{M}$ SB415286 showed significantly decreased survival following irradiation. SB415286 did not protect, but in fact radiosensitized, the DBT-FG cells in culture with a DMF_{10} of 1.18.

Discussion

SB415286, a small molecule inhibitor of GSK-3 β , provides significant protection from radiation-induced apoptosis in neuronal cells in culture [16,23]. MRI monitoring of RN after GK irradiation offers an attractive strategy for validating the *in vivo* neuroprotective effect of this GSK-3 β inhibitor with laboratory animals. We have established a mouse model that

consistently reproduces the histology of RN observed in patients. Herein, we demonstrate that exposure to SB415286 reduces the progression of RN in the mouse. The onset and progression of RN in mouse brain were characterized by measuring the volume of hyperintense regions on contrast-enhanced T1-weighted images. Characterization with T2-weighted images yielded essentially equivalent findings (data not shown). The greatly reduced progression of RN in SB415286-treated mice measured longitudinally by *in vivo* MR imaging, and corresponding histologic findings, clearly demonstrate the efficacy of this GSK-3 β inhibitor. In addition, tumor-bearing mice treated with SB415286 prior to irradiation show no difference in median survival compared with untreated, irradiated mice, suggesting that SB415286 did not reduce the therapeutic efficacy of radiation on tumor tissue. These findings are entirely consistent with a colony formation assay of DBT-FG glioma cells in culture.

The neuroprotective effect of SB415286 is dependent on the radiation-dosing scheme. Mice receiving a single, 60-Gy dose of radiation showed no significant difference in the progression of RN between control and treated cohorts was observed (data not shown). The radiation dose-response relationship has been modeled as a sigmoid function [34]. The results of these studies imply that 45 Gy of radiation occurs on the portion of the dose-response where the slope is significant, i.e., where small shifts in the treatment-modified (*via* SB415286) effective dose generate a significant protective effect.

Findings of the present study motivate consideration of SB415286 as a potential neuroprotectant. However, its half-life is shorter than 24 hours. Assuming the inhibitor acts by targeting the acute apoptosis of glial cells and endothelial cells, a process that lasts longer than 24 hours, continuous use of SB415286 following irradiation might, therefore, increase its therapeutic efficacy. Studies to optimize dosing schemes are a logical next step.

Conclusion

The data in this study demonstrate that (i) SB415286, a small molecule inhibitor of GSK-3 β , confers significant neuroprotection in a mouse model of RN and (ii) SB415286 does not reduce the therapeutic efficacy of radiation in the mouse DBT glioma model of glioblastoma. Extrapolating to the clinic, should SB415286 or other GSK-3 β inhibitors reduce/prevent the development of necrosis in normal CNS tissue following irradiation, the development of more aggressive/effective radiation paradigms would likely follow.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Giglio P, Gilbert MR. Cerebral radiation necrosis. *Neurologist*. 2003; 9:180–188. [PubMed: 12864928]
2. Siu A, Wind JJ, Iorgulescu JB, et al. Radiation necrosis following treatment of high grade glioma - a review of the literature and current understanding. *Acta Neurochirurgica*. 2012; 154:191–201. [PubMed: 22130634]
3. Brandsma D, Stalpers L, Taal W, et al. Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. *Lancet Oncol*. 2008; 9:453–461. [PubMed: 18452856]
4. Ruben JD, Dally M, Bailey M, et al. Cerebral radiation necrosis: Incidence, outcomes, and risk factors with emphasis on radiation parameters and chemotherapy. *Int J Radiat Oncol Biol Phys*. 2006; 65:499–508. [PubMed: 16517093]
5. Shaw PJ, Bates D. Conservative treatment of delayed cerebral radiation necrosis. *Journal Neurol Neurosurg and Psych*. 1984; 47:1338–1341.
6. Levin VA, Bidaut L, Hou P, et al. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. *Int J Radiat Oncol Biol Phys*. 2011; 79:1487–1495. [PubMed: 20399573]
7. Jeyaretna DS, Curry WT, Batchelor TT, et al. Exacerbation of cerebral radiation necrosis by bevacizumab. *J Clin Oncol*. 2011; 29:E159–E162.
8. But QC, Lieber M, Withers HR, et al. The efficacy of hyperbaric oxygen therapy in the treatment of radiation-induced late side effects. *Int J Radiat Oncol Biol Phys*. 2004; 60:871–878. [PubMed: 15465205]
9. Rahmathulla G, Marko NF, Weil RJ. Cerebral radiation necrosis: A review of the pathobiology, diagnosis and management considerations. *J Clin Neurosci*. 2013; 20:485–502. [PubMed: 23416129]
10. Shi L, Linville MC, Iversen E, et al. Maintenance of white matter integrity in a rat model of radiation-induced cognitive impairment. *J Neurolog Sci*. 2009; 285:178–184.
11. Zhou H, Liu Z, Liu J, et al. Fractionated radiation-induced acute encephalopathy in a young rat model: Cognitive dysfunction and histologic findings. *American Journal of Neuroradiology*. 2011; 32:1795–1800. [PubMed: 21920857]
12. Wilson CM, Gaber MW, Sabek OM, et al. Radiation-induced astrogliosis and blood-brain barrier damage can be abrogated using anti-tnf treatment. *Int J Radiat Oncol Biol Phys*. 2009; 74:934–941.
13. Wong CS, Van der Kogel AJ. Mechanisms of radiation injury to the central nervous system: Implications for neuroprotection. *Molecular Interventions*. 2004; 4:273–284. [PubMed: 15471910]
14. Bhat RV, Budd Haerberlein SL, Avila J. Glycogen synthase kinase 3: A drug target for cns therapies. *Journal of neurochemistry*. 2004; 89:1313–1317. [PubMed: 15189333]
15. Cross DA, Culbert AA, Chalmers KA, et al. Selective small-molecule inhibitors of glycogen synthase kinase-3 activity protect primary neurones from death. *Journal of neurochemistry*. 2001; 77:94–102. [PubMed: 11279265]
16. Thotala DK, Hallahan DE, Yazlovitskaya EM. Inhibition of glycogen synthase kinase 3 beta attenuates neurocognitive dysfunction resulting from cranial irradiation. *Cancer Res*. 2008; 68:5859–5868. [PubMed: 18632640]
17. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-amp-dependent protein kinase and phosphorylase kinase. *Eur J Biochem*. 1980; 107:519–527. [PubMed: 6249596]
18. Leroy K, Brion JP. Developmental expression and localization of glycogen synthase kinase-3beta in rat brain. *J Chem Neuroanat*. 1999; 16:279–293. [PubMed: 10450875]
19. Watcharasit P, Bijur GN, Zmijewski JW, et al. Direct, activating interaction between glycogen synthase kinase-3beta and p53 after DNA damage. *Proc Natl Acad Sci U S A*. 2002; 99:7951–7955. [PubMed: 12048243]
20. Loberg RD, Vesely E, Brosius FC 3rd. Enhanced glycogen synthase kinase-3beta activity mediates hypoxia-induced apoptosis of vascular smooth muscle cells and is prevented by glucose transport and metabolism. *J Biol Chem*. 2002; 277:41667–41673. [PubMed: 12200436]

21. Song L, De Sarno P, Joje RS. Central role of glycogen synthase kinase-3 β in endoplasmic reticulum stress-induced caspase-3 activation. *J Biol Chem*. 2002; 277:44701–44708. [PubMed: 12228224]
22. Yazlovitskaya EM, Edwards E, Thotala D, et al. Lithium treatment prevents neurocognitive deficit resulting from cranial irradiation. *Cancer Res*. 2006; 66:11179–11186. [PubMed: 17145862]
23. Thotala DK, Geng L, Dickey AK, et al. A new class of molecular targeted radioprotectors: Gsk-3 β inhibitors. *Int J Radiat Oncol Biol Phys*. 2010; 76:557–565. [PubMed: 20117291]
24. Medina M, Avila J. Glycogen synthase kinase-3 (gsk-3) inhibitors for the treatment of alzheimer's disease. *Curr Pharm Des*. 2010; 16:2790–2798. [PubMed: 20698823]
25. Phukan S, Babu VS, Kannoji A, et al. Gsk3 β : Role in therapeutic landscape and development of modulators. *Brit J Pharmacol*. 2010; 160:1–19. [PubMed: 20331603]
26. Jiang X, Engelbach JA, Yuan L, et al. Anti-VEGF Antibodies Mitigate the Development of Radiation Necrosis in Mouse Brain. *Clin Cancer Res*. 2014; 20:2695–2702. [PubMed: 24647570]
27. Flickinger JC, Kondziolka D, Maitz AH, et al. An analysis of the dose-response for arteriovenous malformation radiosurgery and other factors affecting obliteration. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2002; 63:347–354. [PubMed: 12142099]
28. Shaw E, Scott C, Souhami L, et al. Single dose radiosurgical treatment of recurrent previously irradiated primary brain tumors and brain metastases: Final report of rtog protocol 90-05. *International journal of radiation oncology, biology, physics*. 2000; 47:291–298.
29. Coghlan MP, Culbert AA, Cross DA, et al. Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem Biol*. 2000; 7:793–803. [PubMed: 11033082]
30. Jost SC, Wanebo JE, Song SK, et al. In vivo imaging in a murine model of glioblastoma. *Neurosurgery*. 2007; 60:360–370. discussion 370-361. [PubMed: 17290188]
31. Franken NA, Rodermond HM, Stap J, et al. Clonogenic assay of cells in vitro. *Nature protocols*. 2006; 1:2315–2319.
32. Mallinckrodt C, Clark W, Savid S. Type i error rates from mixed effects model repeated measures versus fixed effects anova with missing values imputed via last observation carried forward. *Drug Information Journal*. 2001; 35:1215–1225.
33. Perry A, Schmidt RE. Cancer therapy-associated cns neuropathology: An update and review of the literature. *Acta Neuropathologica*. 2006; 111:197–212. [PubMed: 16463065]
34. Liauw SL, Connell PP, Weichselbaum RR. New paradigms and future challenges in radiation oncology: An update of biological targets and technology. *Science translational medicine*. 2013; 5:173sr172.

Summary

The effectiveness of SB415286, a specific inhibitor of GSK-3 β , as a neuroprotectant against radiation-induced necrosis is investigated after single-dose (45 Gy) stereotactic radiosurgery in a mouse model. Mice were irradiated with the Leksell Perfexion Gamma Knife and the development and progression of radiation necrosis were monitored longitudinally *in vivo* with MRI. Imaging results were supported with correlative histology. Treatment of mice with SB415286 prior to irradiation produced a statistically significant reduction in necrotic volume, compared with control animals.

Figure 1. Representative transaxial T2-weighted spin-echo images

DMSO-treated (top) and SB415286-treated (1 mg/kg in DMSO, bottom) mice at (from left to right) 13, 16, 19, and 22 weeks following a single 45-Gy dose, at 50% isodose, of radiation. Slices were chosen to display the same anatomic region of the brain at two time points.

Figure 2. Time course plots of MRI-derived necrotic volumes in mice irradiated hemispherically with a single 45-Gy dose of GK radiation

(A) Summary data mean $\pm$ standard error, $n=13$ and 12 for control and SB415286-treated mice, respectively, derived from T2-weighted images. (B) Scatter plot of the slopes of the linear fit to radiation necrosis “growth curves” for individual animals derived from T1- and T2-weighted data ($r=0.98$, $p<0.001$).

Figure 3. H&E-stained sections display characteristic histologic features of RN and demonstrate neuroprotection by SB415286

Representative 2x (top) and 10x (middle) H&E histology slices chosen near the radiation isocenter, and corresponding contrast-enhanced T2-weighted images (bottom) DMSO-treated and (two different) SB415286 treated mice at 22 weeks following 45-Gy fraction of radiation. The irradiated hemispheres of the control mice show many of the histologic features that are characteristic of RN, including fibrinoid vascular necrosis (black arrow), vascular telangiectasia (yellow arrows), hemorrhage (red arrow), loss of neurons and edema (blue arrows). In addition, the tissue injury observed on the histology slices is highly correlated with the hyperintense regions on T2-weighted images. These features are largely lacking or are reduced in severity in the SB415286-treated mice.

Figure 4. Representative transaxial T1-weighted spin-echo images

Nonirradiated, tumor-bearing (left), radiation-treated, tumor-bearing (middle) and radiation with SB415286 treated, tumor bearing (right) mice at 10 and 20 (from top to bottom) days post-implantation with DBT-FG glioma cells. Slices are chosen to display the same anatomic region of the brain at two time points.

Figure 5. Time course of MRI-derived tumor volumes for mice implanted with DBT-FG glioma cells

Mean $\pm$ standard error, $n = 10$ each for untreated, radiation-treated, and irradiated SB415286-treated mice derived from contrast-enhanced, T1-weighted images.

Figure 6. A. Survival of tumor bearing mice

Percent surviving fraction is presented for DMSO-treated mice (solid line, n=10), radiation-treated mice (dashed line, n=9), and combined radiation and SB415286-treated mice (dotted line, n=9). Both treatment regimens significantly increased median survival over the untreated group. Survival curves for the two treatment groups were statistically indistinguishable. **B. Survival of cultured DBT-FG glioma cells.** Cells were treated with DMSO (○) or 25 μ M of SB415286 (●) for 16 h, irradiated with 0, 2, 4, 6 and 8 Gy and plated for clonogenic survival assay. After 10 days, colonies were fixed, stained with 1%

methylene blue, and counted. Shown are the percent surviving fractions for DMSO- or SB415286-treated DBT-FG cells.