

## Efficacy of local polymer-based and systemic delivery of the anti-glutamatergic agents riluzole and memantine in rat glioma models

### Laboratory investigation

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**Object.** The poor outcome of malignant gliomas is largely due to local invasiveness. Previous studies suggest that gliomas secrete excess glutamate and destroy surrounding normal peritumoral brain by means of excitotoxic mechanisms. In this study the authors assessed the effect on survival of 2 glutamate modulators (riluzole and memantine) in rodent glioma models.

**Methods.** In an in vitro growth inhibition assay, F98 and 9L cells were exposed to riluzole and memantine. Mouse cerebellar organotypic cultures were implanted with F98 glioma cells and treated with radiation, radiation + riluzole, or vehicle and assessed for tumor growth. Safety and tolerability of intracranially implanted riluzole and memantine CPP:SA polymers were tested in F344 rats. The efficacy of these drugs was tested against the 9L model and riluzole was further tested with and without radiation therapy (RT).

**Results.** In vitro assays showed effective growth inhibition of both drugs on F98 and 9L cell lines. F98 organotypic cultures showed reduced growth of tumors treated with radiation and riluzole in comparison with untreated cultures or cultures treated with radiation or riluzole alone. Three separate efficacy experiments all showed that localized delivery of riluzole or memantine is efficacious against the 9L gliosarcoma tumor in vivo. Systemic riluzole monotherapy was ineffective; however, riluzole given with RT resulted in improved survival.

**Conclusions.** Riluzole and memantine can be safely and effectively delivered intracranially via polymer in rat glioma models. Both drugs demonstrate efficacy against the 9L gliosarcoma and F98 glioma in vitro and in vivo. Although systemic riluzole proved ineffective in increasing survival, riluzole acted synergistically with radiation and increased survival compared with RT or riluzole alone.  
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**KEY WORDS** • riluzole • memantine • glioma • radiation • oncology

APPROXIMATELY 24,000 new malignant CNS neoplasms are diagnosed annually in the United States, and over 13,000 people will die of these tumors.<sup>7</sup> The vast majority of these tumors are high-grade infiltrating gliomas such as anaplastic astrocytoma or glioblastoma. Recent advances in therapy have increased median survival in patients with glioblastoma from ap-

*Abbreviations used in this paper:* ALS = amyotrophic lateral sclerosis; DMSO = dimethyl sulfoxide; HPLC = high-performance liquid chromatography; NMDA = *N*-methyl-D-aspartate; PBS = phosphate-buffered saline; pCPP:SA = bis-(*p*-carboxyphenoxy)propane:sebacic acid; RT = radiation therapy; TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.

proximately 9 months to approximately 20 months.<sup>20</sup> Local delivery of antitumor agents, either in the form of radiation or chemotherapy, is considered the mainstay of postoperative adjuvant therapy for both initial and recurrent high-grade glioma.<sup>6,33,36</sup> Nonetheless, these improvements in survival are measured in months, and patients ultimately succumb to their disease. This is largely due to the highly infiltrative nature of these tumors and the current inability to distinguish and treat functionally intact brain regions harboring infiltrating tumor. Much work has addressed the infiltrative nature of these tumors, but no current therapies exist to limit parenchymal invasion.<sup>12</sup>

Growth and invasion of gliomas in the brain may be aided by alterations in glutamate regulation, lead-

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ing to damage of surrounding normal functional tissue via excitotoxic mechanisms and enhancement of tumor cell motility. Glioma cell lines release excitotoxic levels of glutamate<sup>3,38</sup> and also abrogate the normal astrocytic function of glutamate uptake from the synaptic cleft and extracellular milieu.<sup>37</sup> The resulting excess of glutamate promotes the growth of gliomas.<sup>32</sup> In addition, the migration of glioma cells has been shown to be inhibited by calcium-permeable AMPA receptor blockade.<sup>14</sup> Blockade of glutamate metabolism through inhibition of synthesis, enhancement of extracellular transport, and/or blockade of glutamate receptors could act to inhibit glioma growth and invasion.<sup>26,27,31,34</sup>

Glutamate excitotoxicity has been shown to play a role in CNS injury and some neurodegenerative diseases. Therapies directed at ameliorating injury through reduction of glutamate-mediated excitotoxicity have shown promise. Riluzole is a substituted benzothiazole that is approved by the FDA for the treatment of amyotrophic lateral sclerosis (ALS) and is thought to inhibit glutamate release.<sup>11</sup> It has been shown to have neuroprotective effects in animal models of CNS injury,<sup>21</sup> in neurodegenerative diseases such as Parkinson's disease<sup>14</sup> and ALS, and in brain trauma.<sup>5</sup> Riluzole, therefore, is a candidate for modulating the effects of glutamate in and around gliomas, and, if effective, could reduce excitotoxic brain injury, decrease tumor growth, and reduce tumor migration and invasion. Upregulation of glutamate transport could provide additional benefit in decreasing the negative effects of glutamate in and around gliomas. Transport by EAAT2 (GLT-1) is the primary mode of glutamate uptake in the mammalian forebrain,<sup>8</sup> and EAAT2 expression is downregulated in high-grade gliomas.<sup>10</sup> In fact, riluzole has been shown to prolong survival when delivered locally in a rat intracranial glioma model.<sup>14</sup>

Memantine, a noncompetitive inhibitor of *N*-methyl-D-aspartate (NMDA) receptors, has demonstrated neuroprotective effects in animal models of CNS injury<sup>29</sup> and in neurodegenerative diseases such as Alzheimer's disease.<sup>15</sup> Memantine is, therefore, a candidate for modulating the effects of glutamate at the edges of gliomas.

In vitro studies have shown that memantine only inhibits the growth of cultured glioma cells at concentrations above 100 μM. When rats with glutamate-releasing glioma implants were treated with 25 mg/kg of memantine twice daily, their implants were smaller than implants in untreated rats.<sup>32</sup> Because microdialysis studies have shown that these doses of memantine result in concentrations of only 1–2 μM in the brain,<sup>9</sup> it is believed that memantine is not directly inhibiting the growth of the tumor and may be acting through some other mechanism. We hypothesize that local delivery of these agents directly to the tumor and brain might enhance their efficacy, and we have therefore developed a local delivery system for riluzole and memantine utilizing biodegradable polymers.

The goal of the current study was to evaluate the use of glutamate metabolism modifiers (riluzole and memantine) via both systemic and local intracranial (polymer-based) delivery in animal models of intracranial glioma and gliosarcoma.

## Methods

### Riluzole and Memantine Cytotoxicity in Rodent Glioma and Gliosarcoma Cell Lines

The rodent glioma cell line F98 and rodent gliosarcoma cell line 9L were grown to 50% confluence in 96-well plates using Dulbecco's modified Eagle's medium (Gibco/Invitrogen Corp.) supplemented with 10% fetal bovine serum (Gemini BioProducts). Riluzole or memantine (A.G. Scientific, Inc.) was dissolved in the same medium at concentrations of 5, 10, 50, 100, 500, and 1000 μM. Five wells were treated with each concentration. Supplemented medium was used as a control. Cells were then incubated with the drug-containing medium at 37°C for 24 hours. Next, the medium was aspirated, the cells were washed once with sterile phosphate-buffered saline (PBS), and placed into fresh medium. The cells were washed again 72 hours after initial exposure, and cytotoxicity was determined by MTT assay. At the time of assay, cells were washed with PBS and incubated with 100 μl of 0.5 mg/ml 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT reagent) in medium for 2 hours at 37°C. The resulting formazan precipitate was dissolved in 100 μl of dimethyl sulfoxide (DMSO) per well. The optical density (OD<sub>570–650</sub>) was then read on a Bio-Rad microplate reader (Bio-Rad Laboratories).

### Organotypic Culture

Cerebellar slices were prepared from P8 EAAT4 or EAAT2 BACdSRed and eGFP reporter transgenic mice at postnatal Day 8 (P8).<sup>13,23</sup> After the mice were decapitated, the cerebellum was dissected out into cold Hank's balanced salt solution containing 6.4 mg/ml glucose. Cerebellar sagittal slices (350 μm thick) were cut using a vibratome and transferred onto membranes of 30-mm Millipore culture inserts with 0.4-μm pore size (Millicell, Millipore). Slices were maintained in culture in 6-well plates containing 1 ml of medium at 37°C in an atmosphere of humidified 5% CO<sub>2</sub>. Culture medium was changed twice weekly. After establishment of the culture, 25,000 F98 cells suspended on 0.5 μl Tris-buffered saline were injected within the organotypic culture using a microinjector. Riluzole was dissolved in DMSO. Twenty-four hours prior to irradiation, the medium was changed, and 10 μM of the tested drug was added. Cultures were irradiated with a single dose of 5 Gy using a cesium source. Tissue slices were photographed immediately prior to irradiation and at 24 hours, 48 hours, 72 hours, 96 hours, and 8 days after irradiation. Morphometric analysis was performed using MetaMorph Microscopy Imaging Software (Molecular Devices, Inc.), and the total area of tumor was determined at each time point and compared with the area prior to irradiation.

### Riluzole Release Kinetics From Polymer Wafer

Polymer wafers were manufactured by dissolving the appropriate amount of the polymer bis-(*p*-carboxyphenoxy)propane:sebacic acid (pCPP:SA) in 300–500 μl methylene chloride and 100 ml DMSO. Next, the drug was added to create 10-mg polymer wafers with 10%, 25%, and

40% weight drug/weight pCPP:SA. Control wafers had no riluzole added. The organic solvent was evaporated in a vacuum chamber, and wafers were pressed using a custom die and mechanic's vise. Wafers were incubated in triplicate in 1 ml PBS at 37°C. Following the incubation, the supernatant was carefully aspirated, stored, and replaced with fresh PBS. Sampling times were at 1, 4, 8, 12, and 24 hours and then daily for 7 days. The riluzole concentration in supernatant was measured with high-performance liquid chromatography (HPLC) (Beckman System Gold, Beckman Coulter Corp.) utilizing a C18 Waters reverse phase column and a mobile phase of 80:20 water/methanol at a flow rate of 1 ml per minute. Cumulative release curves were then constructed for each riluzole/polymer concentration tested, following creation of a standard curve of known drug concentrations using these methods.

#### *Intracranial Safety of the Riluzole and Memantine Polymers*

All animal studies were approved by the Johns Hopkins University Animal Care and Use Committee and carried out in accordance with its standards. Female Fisher 344 rats (Charles River Laboratories), weighing 125–175 g were anesthetized with an intraperitoneal injection of 3 ml/kg of a stock solution composed of ketamine hydrochloride (Abbott Laboratories) 25 mg/ml, xylazine (Phoenix Pharmaceutical) 2.5 mg/ml, and 14.25% ethyl alcohol in 0.9% NaCl (Pharmaceuticals, Inc.). The superior scalp was clipped and prepared with iodine surgical preparation solution. A 1-cm incision was made in the midline posterior to bregma. A high-speed electric drill with a 2-mm bur was used to create a 3-mm-diameter craniectomy centered in the left parietal bone. Suction aspiration was then used to create a small corticectomy under the bone defect into which 1 polymer wafer was placed. Hemostasis was attained and the scalp incision closed with surgical clips. Postoperative analgesia was provided with 100 µl of buprenorphine administered intraperitoneally. For the riluzole polymer, animals were divided into 3 groups and each received a 10%, 20%, or 40% polymer implanted intracranially. The same method was used for the memantine polymer wafers, with 9 rats divided into 3 groups. Each group then received a memantine/pCPP:SA wafer loaded with 10%, 20%, or 40% polymer. Following surgery, animals were evaluated daily for signs of neurotoxicity.

#### *Safety of Intraperitoneally Delivered Riluzole in Rats*

Twelve female Fischer 344 rats, 125–175 g (Charles River Laboratories), were assigned to one of the 4 following groups ( $n = 3$  per group): 100 mg/kg riluzole administered intraperitoneally twice daily; 80 mg/kg riluzole administered intraperitoneally twice daily; 40 mg/kg riluzole administered intraperitoneally twice daily; or 20 mg/kg riluzole administered intraperitoneally twice daily. Each group was given twice daily intraperitoneal injections of their respective dosage of riluzole dissolved in 200 µl DMSO. Animals were monitored daily. Any animals that were deemed to be anorexic, in extremis, or to have severe neurological deficit were killed by carbon dioxide asphyxiation. Due to

toxicity, this safety experiment was repeated with animals receiving 10 mg/kg of riluzole twice daily.

#### *Systemic Dosing of Memantine in Rats*

Based on published reports, the dose used for systemically delivered memantine was 25 mg/kg, twice daily.<sup>19,35</sup>

#### *Local and Systemic Memantine Efficacy Against Intracranial 9L Gliosarcoma*

Fisher 344 rats were anesthetized and underwent a craniectomy, both as previously described. Following the suction corticectomy, a 3-mm<sup>3</sup> 9L gliosarcoma explant was placed into the defect. Animals were then assigned to one of 3 treatment groups: no treatment (control) ( $n = 10$ ); intraperitoneal injections of memantine, 25 mg/kg twice daily ( $n = 4$ ); 40% memantine polymer implant (Day 0) ( $n = 8$ ). The incision was closed and animals recovered as above. Animals were evaluated twice daily for any signs of deterioration in their condition and were killed immediately upon that finding. Survival curves were calculated and histological examination was completed for all brains.

#### *Local and Systemic Riluzole Efficacy Against Intracranial 9L Gliosarcoma*

The maximally tolerated local dose of riluzole was found to be 10% w/w; thus, this dose was used for intracranial efficacy studies. Following suction corticectomy, a 3-mm<sup>3</sup> 9L gliosarcoma explant was placed into the defect in female Fisher rats. Animals were divided into 3 groups and received either no treatment ( $n = 8$ ), a polymer wafer containing 10% riluzole ( $n = 8$ ), or daily intraperitoneal injections of riluzole (8 mg/kg) ( $n = 8$ ). A second efficacy experiment was conducted in which animals received a 9L intracranial tumor implant and then either no treatment ( $n = 10$ ), intraperitoneal injections of riluzole (8 mg/kg, twice daily) ( $n = 8$ ), radiation therapy (RT) (20 Gy using a Cesium 137 source) ( $n = 8$ ), or a combination of intraperitoneal riluzole and RT ( $n = 8$ ). Additionally, 1 day after RT, 2 of the animals treated with irradiation alone and 2 animals treated with irradiation and riluzole were anesthetized and perfused and the brains were removed and embedded in paraffin. Sections of 10 µm were prepared and TUNEL stained for apoptotic activity (Roche).

#### *Animal Surveillance*

Animals were evaluated daily. Any animals noted to be anorexic or in extremis or found to have significant neurological deficits were killed with carbon dioxide asphyxiation. All dead or killed animals with intracranial wafer and/or tumor underwent necropsy to confirm the presence of tumor and absence of other lethal intracranial mass lesions.

#### *Statistical Analysis*

A 2-tailed Student t-test was used in the analysis of tumor growth in organotypic cultures;  $p$  values  $< 0.05$  were considered significant. All survival analysis was carried out by the Kaplan-Meier method. Survival was the primary end point. Kaplan-Meier analysis was used to compare survival using GraphPad Prism 5.1. The log-rank (Mantel-Cox) test was used to compare groups and

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groups were considered statistically different at  $p < 0.05$ ; p value analyses were 2 sided.

## Results

### Riluzole and Memantine Cytotoxicity in Glioma and Gliosarcoma Cell Lines

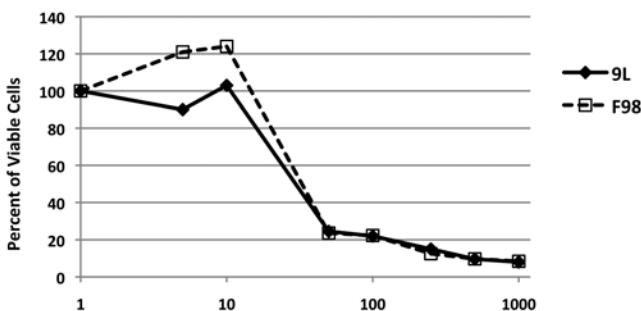
Riluzole administration to both 9L and F98 rodent gliosarcoma and glioma cell lines produced dose-dependent cytotoxic effects in both cell lines as demonstrated by the MTT assay. The  $LD_{50}$  for riluzole was approximately 25  $\mu\text{M}$  for both cell lines (Fig. 1). Both the F98 and 9L cell lines were less sensitive to memantine than to riluzole, with  $LD_{50}$  values of 200  $\mu\text{M}$  and 400  $\mu\text{M}$ , respectively (data not shown).

### Efficacy of Riluzole and Radiation Against F98 Glioma in an Organotypic Culture Model

Organotypic cultures of rat cerebellum were used to assess F98 glioma growth and response to treatment with radiation with and without riluzole. Tumor volume was measured prior to radiation treatment and expressed as percent change in area compared with pretreatment area at 1, 2, 3, 4, 5, and 8 days after treatment. Overall, there was a trend for decreased tumor growth in cultures treated with 5 Gy radiation or radiation + 10  $\mu\text{M}$  riluzole compared with untreated cultures, which reached significance at Days 2 and 5 for radiation + riluzole and Day 5 for radiation alone ( $p < 0.05$ ). At 8 days, there was significant decrease in tumor volume in cultures treated with radiation + riluzole compared with radiation alone ( $p < 0.05$ ) (Fig. 2A and B).

### Kinetics of Riluzole Release From Polymer

Riluzole was readily identified in supernatant, and a standard curve from the previously described HPLC setup was derived for further concentration determination (Fig. 3). Cumulative release of riluzole at 7 days from the 10%, 25%, and 40% w/w wafers was 22.8%, 11.4%, and 1.6% of the original riluzole load, respectively. This corresponds to mean total amounts of riluzole released per wafer of 0.228 mg (10%), 0.285 mg (25%), and 0.064 mg (40%). Both the 10% and 25% wafers demonstrated largely linear release following 72-hour incubation at 37°C, most



**FIG. 1.** Cytotoxicity of riluzole against 9L and F98 tumors in vitro. In vitro inhibition of cell growth results for F98 and 9L rat glioma cell lines treated with increasing concentrations ( $\mu\text{M}$ ) of riluzole. The  $LD_{50}$  for both cell lines was 25  $\mu\text{M}$ . Five wells were treated with each concentration.

likely due to the low water solubility of riluzole. Minimal release of the 40% formulation was demonstrated.

### Intracranial Riluzole Polymer Safety

Rats underwent intraparenchymal placement of one 10%, 25%, or 40% w/w riluzole/polymer wafer ( $n = 3$  each group). Animals receiving 10% and 40% wafers tolerated the implantation well and were observed for 120 days without untoward effects. Necropsy demonstrated small polymer remnants. However, 2 of 3 animals receiving 25% riluzole/polymer died within 24 hours of implantation. Necropsy demonstrated no lethal intracranial pathology such as hematoma. The remaining animal in the 25% riluzole/polymer group survived 120 days without signs of toxicity. The 10% and 25% w/w riluzole/polymer wafers demonstrated a similar cumulative release of riluzole (0.228 and 0.285 mg, respectively); however, given the unexplained apparent toxicity of the 25% wafer, the 10% dose was chosen for intracranial implantation throughout the efficacy studies.

### Intracranial Memantine Polymer Safety

Rats underwent intraparenchymal placement of a 10%, 20%, or 40% w/w memantine/polymer wafer ( $n = 3$  each group). All the animals receiving memantine wafers tolerated the implantation well (Fig. 4), being observed to 120 days without untoward effects. Necropsy demonstrated small polymer remnants. The 40% memantine polymer was used throughout the in vivo efficacy studies.

### Toxicity of Systemically Delivered Riluzole

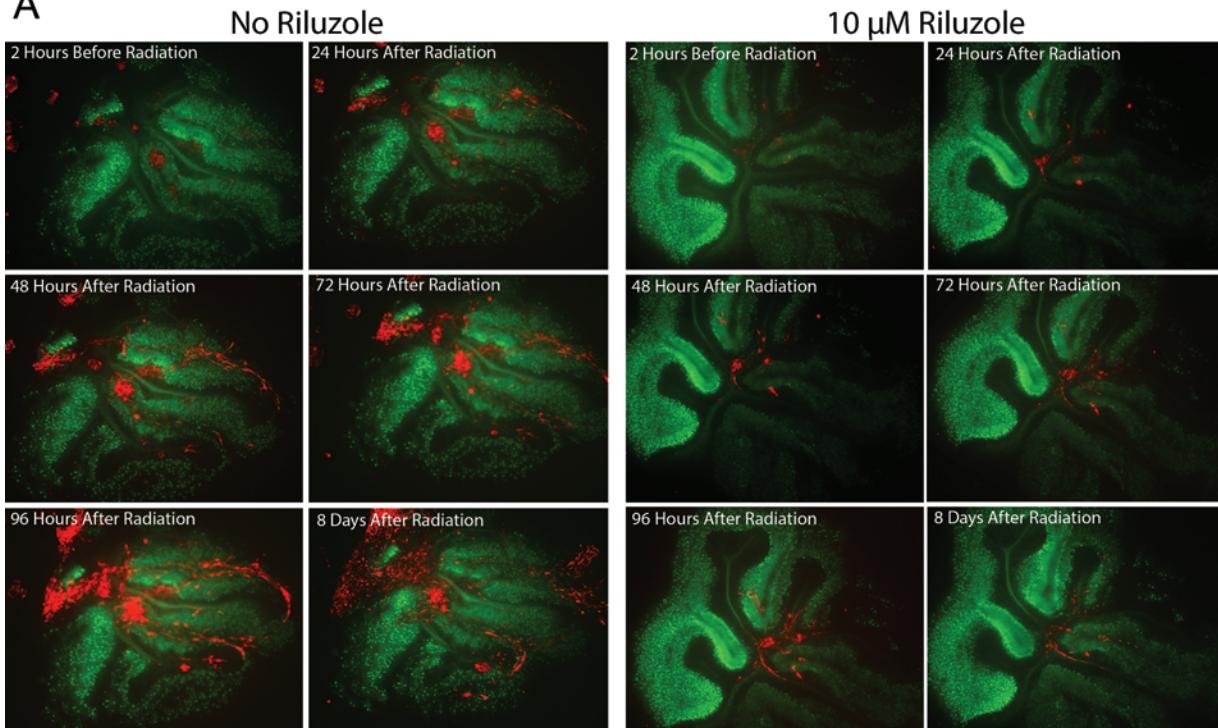
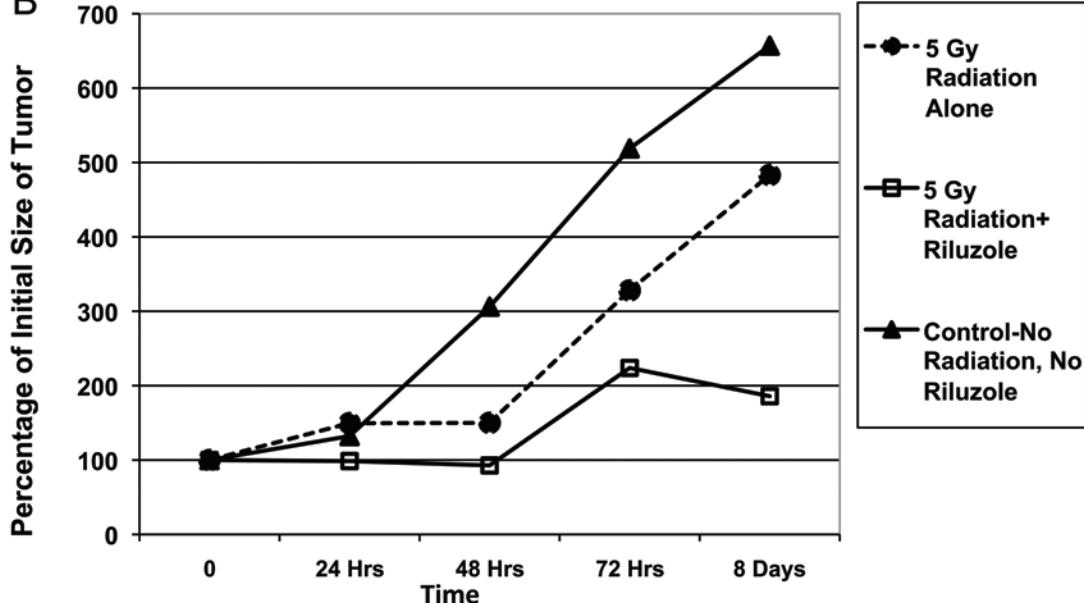
To establish a systemic treatment dose of riluzole for eventual comparison with local delivery of riluzole, F344 rats were administered injections of riluzole at concentrations ranging from 8 to 100 mg/kg twice per day (Fig. 5). All of the animals receiving 100 mg/kg and 80 mg/kg riluzole regimens died within 1 day of treatment. All of the animals in the 40 mg/kg group died by Day 2 of treatment. One animal from the 20 mg/kg group died on Day 9, at which the point the study was terminated. Due to this toxicity, we injected doses no higher than 10 mg/kg per day in the efficacy studies.

### Efficacy of Local Memantine Polymer Against Intracranial 9L Gliosarcoma

Intracranial delivery of memantine polymers increased median survival in comparison with the control group (Fig. 6). Control animals had a median survival of 14 days. Survival was significantly greater in the memantine polymer group, with a median survival of 27 days ( $p < 0.0001$  vs controls,  $p = 0.0004$  vs systemic memantine group). Systemic memantine had no effect on survival (median survival of 16.5 days,  $p = 0.5437$  vs controls). Significant intracranial tumor was demonstrated by necropsy in all available dead or moribund and killed animals.

### Efficacy of Local Riluzole Polymer Against Intracranial 9L Gliosarcoma

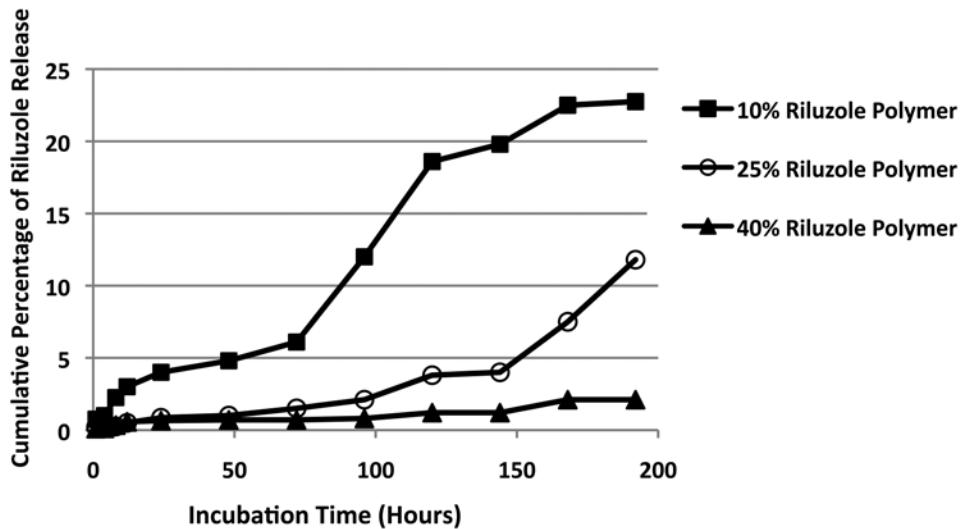
The median survival of the untreated control animals was 11.5 days (Fig. 7). The median survival for the group

**A****B**

**Fig. 2.** F98 glioma in rat cerebellar organotypic cultures with and without riluzole following radiation treatment. **A:** Rat cerebellar organotypic cultures (green) were implanted with F98 tumor (red) and then were left untreated ( $n = 4$ ), treated with 5 Gy radiation ( $n = 6$ ), or treated with both radiation and riluzole ( $n = 8$ ). **B:** Tumor volume was assessed prior to radiation and at 1, 2, 3, 4, and 8 days after radiation and is expressed as total percentage volume of pre-radiation tumor volume. There was a trend for decreased tumor growth in cultures treated with radiation or radiation + 10  $\mu$ M riluzole compared with untreated cultures, which reached significance at Days 2 and 5 for radiation + riluzole and Day 5 for radiation alone compared with untreated cultures ( $p < 0.05$ ). At 8 days, there was a significant decrease in tumor volume in cultures treated with radiation + riluzole compared with radiation alone ( $p < 0.05$ ).

that received systemic riluzole was 12 days, and for the group that received riluzole wafer, it was 17 days. Local delivery of riluzole resulted in a significant survival benefit versus both empty wafer ( $p = 0.0003$ ) and intraperito-

neal riluzole ( $p < 0.0001$ ). Intraperitoneal riluzole offered no survival benefit over empty wafer ( $p = 0.7935$ ). Significant intracranial tumor was demonstrated by necropsy in all available dead or moribund and killed animals.



**Fig. 3.** Riluzole release from CPP:SA polymer. Cumulative release kinetics of 10%, 25%, and 40% weight riluzole/weight CPP:SA wafer (total weight 10 mg per wafer; completed in triplicate) demonstrating superior release kinetics of 10% and 25% riluzole-loaded wafers.

#### Efficacy of Riluzole and RT Against Intracranial 9L Gliosarcoma

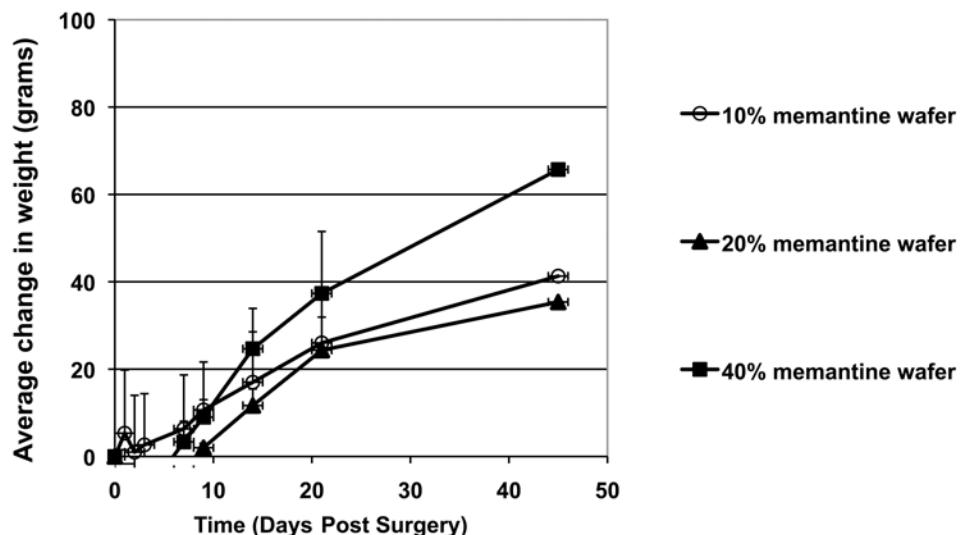
We then tested the combination of RT and systemic riluzole in our intracranial model (Fig. 8A). Fisher 344 rats received intracranial 9L tumor and then received no further treatment, 20 Gy RT on Day 5, systemic riluzole twice daily on Days 4–11, or both RT and systemic riluzole. The group that received RT alone showed a statistical improvement over the control group and the systemic riluzole group ( $p = 0.0026$  and  $p = 0.0011$ , respectively). The combination group that received both RT and systemic riluzole had a significant benefit over the 3 other groups ( $p < 0.0001$  vs control and systemic riluzole, and  $p = 0.0439$  vs RT alone).

Four additional animals (treated with RT alone [ $n = 2$ ] or a combination of RT and riluzole [ $n = 2$ ]) were treat-

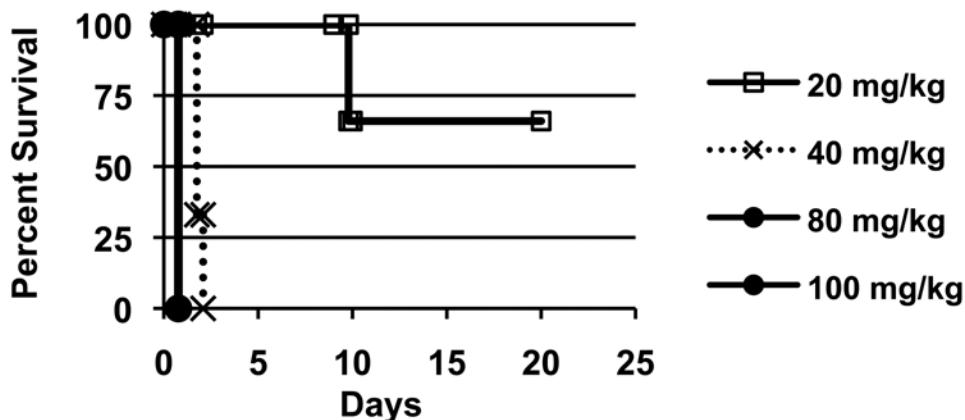
ed as described above but sacrificed 1 day after radiation treatment. Based on TUNEL staining, there was evidence of marked increase in apoptosis within the brain tumors of animals treated with the combination of RT and riluzole compared with those treated with RT alone (Fig. 8B).

#### Discussion

Alterations in glutamate regulation may aid growth and invasion of gliomas in the brain via excitotoxic mechanisms, and enhancement of tumor cell motility and inhibition of glutamate may inhibit glioma growth and invasion.<sup>26,27,31,34</sup> The goal of the current study was to evaluate the efficacy of the glutamate release inhibitor riluzole and the NMDA receptor antagonist memantine in animal models of intracranial glioma and gliosarcoma. Although



**Fig. 4.** Safety of intracranially implanted memantine wafers. F344 rats (3 each) were intracranially implanted with either a 10% memantine polymer, a 20% memantine polymer, or a 40% memantine polymer and were weighed consistently over 60 days. This plot shows the average change in weight over 45 days. There were no deaths in these groups.

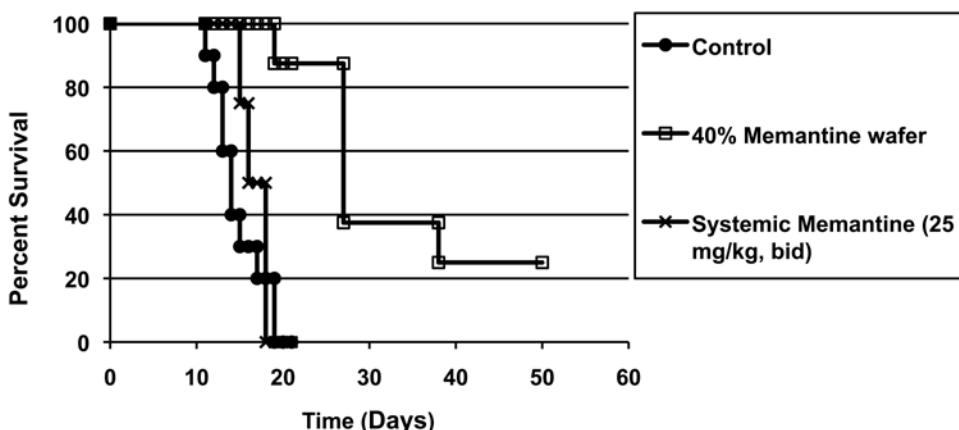


**Fig. 5.** Systemic toxicity of riluzole in F344 rats. Twelve female Fischer 344 rats were assigned to one of the 4 following groups ( $n = 3$  per group): 100 mg/kg riluzole; 80 mg/kg riluzole; 40 mg/kg riluzole; or 20 mg/kg riluzole. Each group was given twice daily intraperitoneal injections of their respective dosage of riluzole dissolved in 200  $\mu$ l DMSO.

it is well known that riluzole and memantine cross the blood-brain barrier and reach appropriate concentrations for the therapy of neurodegenerative disorders such as ALS and Alzheimer's disease, therapy with systemic riluzole or memantine in our models was ineffective. We hypothesized that local polymer-based delivery of these agents might provide improved local delivery and efficacy. We demonstrate that local polymer-based delivery of riluzole or memantine significantly improved survival in a rat intracranial glioma model when the polymer wafers were placed at the time of tumor engraftment.

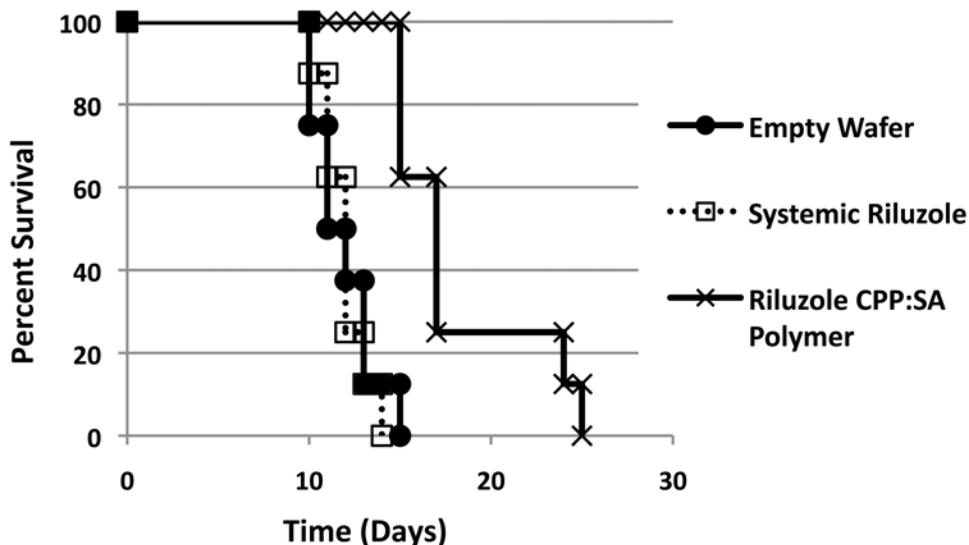
Additionally, our data suggest that riluzole may act synergistically with RT. Despite the lack of survival benefit seen in animals treated with systemic riluzole alone, when administered in conjunction with RT, systemic riluzole demonstrated increased survival and cell killing in a 9L in vivo model and reduced tumor growth compared with untreated tumor and tumor treated with radiation alone in a mouse cerebellar organotypic model utilizing F98 glioma.

Glutamate is the primary excitatory neurotransmitter in the mammalian CNS, but it also plays a significant role in neuroembryogenesis and in neural and astrocytic proliferation, migration, and differentiation. These non-neurotransmitter effects of glutamate are lost in the fully developed brain. Recent evidence suggests that glioma cells may recover their promigratory sensitivity to glutamate.<sup>22</sup> Normal astrocytes serve as a glutamate "sump," participating in glutamate reuptake and clearance from the extracellular milieu.<sup>25,26</sup> This function is reversed in gliomas, with a decreased reuptake and frequently increased secretion of glutamate. Human biopsy studies have demonstrated markedly elevated glutamate levels in peritumoral normal brain, in some instances well above that found in the tumor itself and at concentrations toxic to astrocytes as well as neurons in vitro.<sup>24</sup> This may provide a paracrine-type promigratory stimulus for the glioma cell as well as injure peritumoral normal astrocytes and neurons via glutamate-mediated excitotoxic mechanisms. This excitotoxic remodeling of the cellular geometry may also facilitate the infiltration of



**Fig. 6.** Efficacy of memantine, systemically delivered and locally delivered, against intracranial 9L gliosarcoma. Twenty-four female Fisher 344 rats were intracranially implanted with 9L tumor and then were either untreated, received a 40% memantine:CPP:SA polymer wafer, or received twice daily injections of memantine (25 mg/kg). Survival of rats treated with 40% memantine polymer was significantly improved as compared with both the control group ( $p < 0.0001$ ) and the systemic memantine group ( $p = 0.0004$ ). There was no statistically significant difference between the control group and the group that received systemic memantine ( $p = 0.5437$ ).

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**Fig. 7.** Kaplan-Meier survival curve for animals treated with intracranially implanted riluzole polymer. Fisher 344 rats received intracranial co-implantation of 10% riluzole:CPP:SA polymer and 9L glioma. Placement of riluzole wafer resulted in a significant survival advantage over animals that received the empty wafer ( $p = 0.0003$ ) or systemic riluzole ( $p < 0.0001$ ).

glutamate-stimulated glioma cells by minimizing spatial constraints. Many of these effects can be blocked in vitro with glutamate receptor antagonists<sup>14,28</sup> as well as by direct blockade of glutamate-mediated calcium channels with other agents.<sup>34</sup>

Well over 80% of high-grade glioma recurrences occur within 2 cm of the original resection site, almost certainly due to viable tumor cells remaining within grossly normal-appearing brain.<sup>18,30</sup> An effective local therapy targeted against these residual tumor cells has long been sought. However, this goal has evaded conventional DNA- and cell cycle-based therapeutic approaches. Anti-glutamatergic therapy with agents such as riluzole or memantine offers promise in a duality of both conventional and novel mechanisms. In vitro data presented here and supported by the literature demonstrate a direct cytotoxic effect against glioma cell lines. More unconventional, but of equal importance, is the novel concept of improved outcome via pharmacological neuroprotection in neuro-oncology. Toxic levels of glutamate associated with high-grade gliomas have been demonstrated both in vitro and in vivo.<sup>4,27</sup> Protection of the peritumoral functional, but possibly infiltrated, brain may indeed be beneficial for multiple reasons. Glutamate-mediated excitotoxicity to peritumoral astrocytes as well as neurons may contribute to decreased patient function due to the facilitation of peritumoral edema, direct cellular toxicity, and seizure activity.<sup>2</sup> Preservation of the normal peritumoral cytoarchitecture may maintain 3D migratory constraints on glioma cell migration. Decreased glutamate may also minimize deformability due to ion flux and remove a powerful promigratory factor, all favoring a less invasive phenotype.<sup>16,17</sup>

A significant effective therapy for malignant glioma remains elusive largely due to the highly infiltrative nature of these tumors and the inability to identify tumor cells in regions of functional brain. Anti-glutamatergic therapy with riluzole, memantine, and similar agents offer a multiplicity of potential therapeutic mechanisms directed

against both the tumor cell itself as well as indirectly protecting vulnerable peritumoral functional brain. Our data suggest that use of anti-glutamatergic strategies may have treatment benefit alone or in combination with traditional therapies such as RT and, in fact, anti-glutamatergic therapy with riluzole may offer synergistic tumor control when used in conjunction with RT.

### Conclusions

Modulation of glutamate may provide therapeutic benefit in the treatment of gliomas. Our results suggest that localized treatment of the 9L intracranial gliosarcoma model with the glutamate-release inhibitor riluzole or the NMDA receptor antagonist memantine shows significant survival benefit over treatment with systemic riluzole or memantine and/or no treatment. Additionally, data from both in vivo and in vitro studies show that riluzole may act synergistically with radiation to improve tumor cell killing and that systemic administration of riluzole in conjunction with RT increases tumor cell death and promotes increased animal survival.

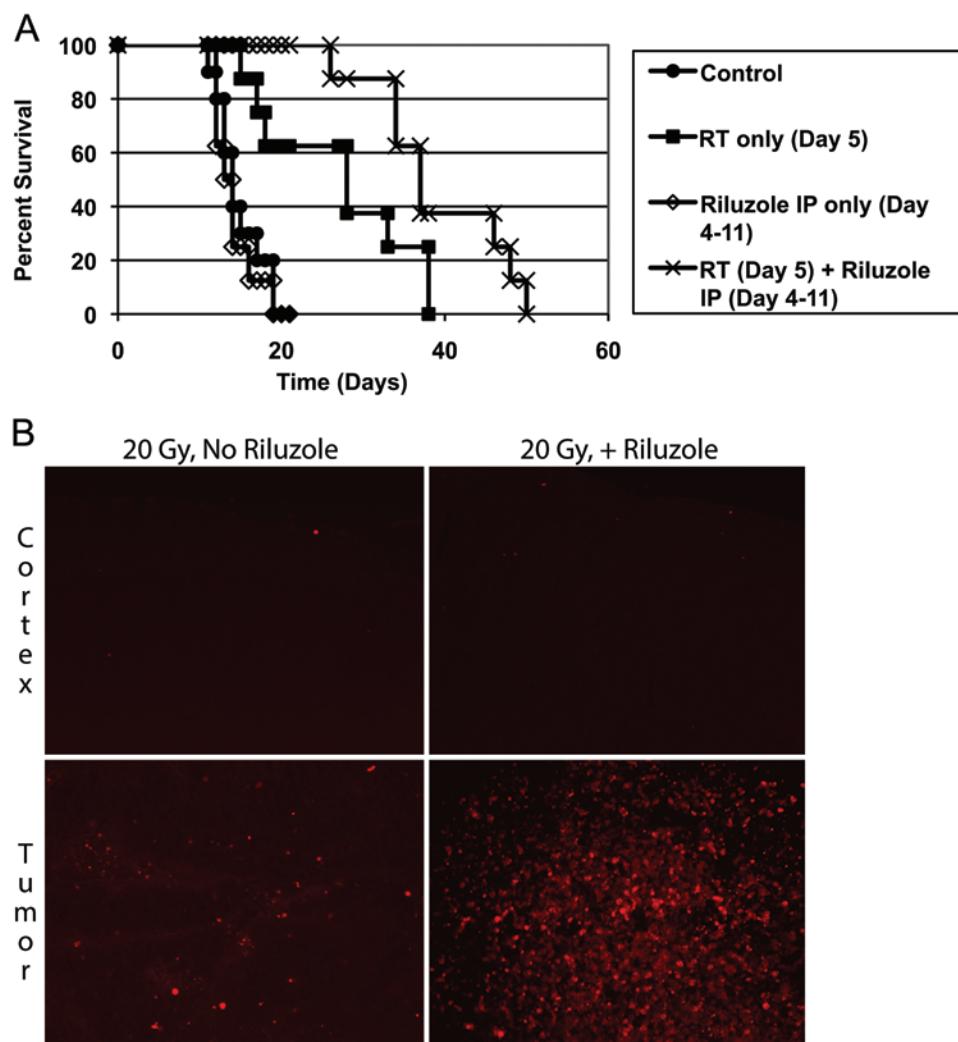
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**Fig. 8.** Efficacy of systemically delivered riluzole and RT. **A:** Fisher 344 rats received intracranial 9L tumor and then no further treatment, 20 Gy RT on Day 5, systemic (intraperitoneal [IP]) riluzole twice daily on Days 4–11, or both RT and systemic riluzole. The group that received RT showed a statistically significant improvement over the control group and the systemic riluzole group ( $p = 0.0026$  and  $p = 0.0011$ , respectively). The combination group that received both RT and systemic riluzole had a significant benefit over the 3 other groups ( $p < 0.0001$  vs control and systemic riluzole, and  $p = 0.0439$  vs RT alone). **B:** Additional animals were treated as above but were killed 21 hours after irradiation. The brains were stained with TUNEL to assess for cell death. Comparison was made between animals receiving 20 Gy RT alone ( $n = 2$ ) and animals treated with systemic riluzole twice daily from Day 4 until they were killed. Representative sections from 1 animal of each group are shown, demonstrating increased apoptosis in animals treated with riluzole and RT.

Weaver. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Rothstein. Statistical analysis: Yohay, Tyler, Weaver, Gincel. Administrative/technical/material support: Rothstein, Yohay, Tyler, Brem. Study supervision: Rothstein, Yohay, Brem.

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