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A thermal gel depot for local delivery of paclitaxel to treat experimental brain tumors in rats

Laboratory investigation

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Object. Paclitaxel, a cellular proliferation inhibitor/radiation sensitizer, while effective against gliomas in vitro, has poor CNS penetration and dose-limiting toxicities when administered systemically. OncoGel (paclitaxel in ReGel) provides controlled local paclitaxel release when placed into the CNS. The authors evaluated the safety and efficacy of OncoGel in rats with intracranial 9L gliosarcoma.

Methods. Safety studies included intracranial delivery of increasing volumes of ReGel and OncoGel containing 1.5 (OncoGel 1.5) or 6.3 (OncoGel 6.3) mg/ml paclitaxel. An in vivo radiolabeled biodistribution study was performed in 18 Fischer-344 rats to determine intracerebral distribution. Efficacy studies compared overall survival for controls, ReGel only, radiation therapy only, OncoGel 6.3, or OncoGel 6.3 in combination with radiation therapy. ReGel and OncoGel 6.3 were delivered either simultaneously with tumor implantation (Day 0) or 5 days later (Day 5). Radiation therapy was given on Day 5.

Results. Control and ReGel animals died of tumor within 17 days. Survival significantly increased in the OncoGel 6.3 group on Day 0 (median 31 days; $p = 0.0001$), in the OncoGel 6.3 group on Day 5 (median 17 days; $p = 0.02$), and in the radiation therapy–only group (median 26 days; $p = 0.0001$) compared with controls. Animals receiving both OncoGel and radiation therapy had the longest median survival: 83 days in the group with radiation therapy combined with OncoGel 6.3 on Day 0, and 32 days in the group combined with OncoGel 6.3 on Day 5 ($p = 0.0001$ vs controls). After 120 days, 37.5% of the animals in the OncoGel Day 0 group, 37.5% of animals in the OncoGel 6.3 Day 0 in combination with radiation therapy group, and 12.5% of the animals in the OncoGel 6.3 on Day 5 in combination with radiation therapy group were alive. In the biodistribution study, measurable radioactivity was observed throughout the ipsilateral hemisphere up to 3 weeks after the OncoGel injection, with the most radioactivity detected 3 hours after injection. The highest dose of radioactivity observed in the contralateral hemisphere was at the Day 3 time point.

Conclusions. OncoGel containing 6.3 mg/ml of paclitaxel is safe for intracranial injection in rats and effective when administered on Day 0. When combined with radiation therapy, the combination was more effective than either therapy alone and should be studied clinically for the treatment of malignant glioma.
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Key Words • **paclitaxel** • **OncoGel** • **9L gliosarcoma** • **radiotherapy** • **rat** • **radioactivity**

Paclitaxel (Samyang Genex), a cellular proliferation inhibitor, is effective in the treatment of a broad range of tumor types, and has been approved by the US FDA for ovarian, lung, and breast cancer and for AIDS-related Kaposi sarcoma. However, poor penetration of paclitaxel into the CNS following systemic administration precludes its use for the treatment of malignant gliomas.[13] Dose-limiting toxicities such as sensory neuropathies, gastrointestinal disturbances, and severe myelosuppression are noted with intravenous delivery of paclitaxel.[4,5,7] To limit the systemic toxicity and enhance the efficacy of paclitaxel, local intracranial delivery methods have been examined, including polymeric delivery in preclinical models[3,10,15,18] and most recently a Phase I/II study using convection-enhanced delivery.[11] Although the latter showed promising results, there were significant clinical and administration complications associated with this delivery method, including poor diffusion of the chemotherapeutic agent.

A gel depot delivery system (ReGel) was developed for delivery of paclitaxel (Protherics Salt Lake City, Inc.). ReGel is a thermosensitive, biodegradable triblock copolymer composed of poly(lactide-co-glycolide) and poly(ethylene glycol),[12] which can be tailored to deliver various agents at different rates.[9,16] ReGel loaded with paclitaxel (OncoGel; Protherics) provides an injectable,

controlled-release, biodegradable vehicle that releases paclitaxel for ~ 50 days and biodegrades within 4 to 6 weeks.[20] OncoGel has also been shown to reach therapeutic concentrations up to 3 to 5 cm from the injection site in normal pancreatic tissue in vivo.[14] Because gliomas recur at or near the primary tumor site in 80% of patients, placement of OncoGel into the tumor resection cavity is designed to deliver therapeutic concentrations to affected tissue for up to 6 weeks and may prevent or prolong time to tumor recurrence.

The present series of studies were designed to determine whether the use of this unique delivery system would be safe and efficacious for the treatment of malignant gliomas. First we established the safety and biocompatibility of OncoGel injected into the rat brain. Next we demonstrated the efficacy of intracranial OncoGel administration in rats challenged with 9L gliosarcoma. Lastly, because glioblastoma multiforme is treated clinically with radiation therapy, we paired the treatment of OncoGel with radiotherapy to examine the safety and potential radiosensitizing effect of this combination therapy in rats challenged with 9L gliosarcoma.

Methods

Study Materials

ReGel (placebo) and OncoGel (paclitaxel in ReGel) were provided by Protherics and stored at –20°C. Two different concentrations of paclitaxel-loaded OncoGel were used in these studies: OncoGel 1.5, containing 1.5 mg paclitaxel per ml of OncoGel, and OncoGel 6.3, containing 6.3 mg paclitaxel per ml of OncoGel. The dose of 6.3 mg/ml of paclitaxel was determined to be the maximum amount of paclitaxel that could be incorporated into the OncoGel. OncoGel and ReGel were thawed at 4°C for 72 hours before each experiment, and were maintained on ice until use. The stereotactic injections were performed using a Hamilton syringe (Model 700, Hamilton Company) with a Luer lock tip and a modified shortened needle (~ 1.5 cm long).

Tumor Cells

The 9L gliosarcoma cells were obtained from the Brain Tumor Research Center at the University of California, San Francisco. Two intracranial 9L glioma tumor models were used in this study: cells were cultured in vitro for stereotactic injection of a cell suspension, and tumors were passed (transferred) in the flank of Fischer-344 rats for eventual intracranial implantation of a tumor piece. For culture, 9L cells were maintained in Dulbecco modified Eagle medium containing 10% fetal bovine serum (Gemini BioProducts) and 0.2% Plasmocin (InvivoGen) in humidified incubators at 37°C in 5% CO_2. Cultured tumor monolayers were harvested with 0.025% trypsin (Invitrogen), counted, resuspended in medium, and provided at the appropriate number and volume for stereotactic injection. For tumor piece implantation, 9L tumor pieces measuring 2 mm^3 were passed every 3 to 4 weeks in the flank of Fischer-344 female rats weighing 125–200 g each.

Study Animals

Female Fischer-344 rats, weighing 125–200 g each, purchased from Harlan Bioproducts were used for all studies. Rats were anesthetized with an intraperitoneal injection of 0.6 ml of a stock solution containing ketamine HCl (75 mg/kg), xylazine (7.5 mg/kg), and ethanol (14.25%) in a sterile 0.9% NaCl solution.

Safety Studies

We evaluated the safety of intracranial injections of ReGel (18 rats), OncoGel 1.5 (5 rats), or OncoGel 6.3 (21 rats) in 44 Fischer-344 rats. Five different injection volumes (10, 25, 50, 75, and 100 μl) were evaluated. Animals were prepared for intracranial implantation; after a midline scalp incision, the galea overlying the left cranium was swept laterally. With the aid of a Zeiss operating microscope (Carl Zeiss) and a high-speed TPS Universal Drill (Stryker Corp.), a 1-mm bur hole was made over the left parietal bone, with its center 3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture. When the dura was exposed, the animal was transferred to a stereotactic frame (also draped and equipped with a sterile needle). The stereotactic needle was loaded with either OncoGel or ReGel and placed 3 mm below the dura. The appropriate volume was injected over a period of 2 minutes and the animal was then removed from the frame. The scalp incision was closed with surgical staples. All animals were given an intraperitoneal injection of the analgesic buprenorphine (0.1 mg/kg) at 12 and 24 hours following each surgical procedure.

Animals receiving a 10-μl injection of ReGel, OncoGel 1.5, or OncoGel 6.3 were observed daily for any signs of neurotoxicity (such as ataxia or hemiparesis). Two animals from each group were killed on Day 10, while the remaining animals were observed through Day 120. After the animals were killed, their brains were placed in formalin (for more than 24 hours) and sectioned for histopathological analysis. Rats receiving ReGel or OncoGel 6.3 injections ranging from 25 to 100 μl were followed daily for 25 days (designated as study end), at which time the animals were killed. Rats were inspected daily for signs of pain and distress, including ruffled fur, weight loss, dehydration, hunched position, weakness, lethargy, immobility, lack of coordination, hypothermia, pale ears or feet, labored respiration, or cyanosis (bluish mucous membranes) according to the Johns Hopkins Animal Care and Use Guidelines. If these symptoms persisted, the animal was killed according to the protocol and the brain and organs were placed in formalin.

Efficacy Studies

Monotherapy. We initially evaluated the efficacy of 10-μl intracranial injections of OncoGel 1.5 and OncoGel 6.3 against the experimental 9L gliosarcoma in Fischer-344 rats. This experiment used the 9L cells from culture. Fifty-one rats received a 2-μl injection of intracranial 9L gliosarcoma cells (100,000 cells).

Briefly, animals were anesthetized and the dura was exposed as described above. For stereotactic injection, a

needle containing 100,000 tumor cells in 2 μl was placed into the brain at a depth of 3.5 mm and 2 μl was delivered over 2 minutes. After injection of tumor cells, animals were randomized into the following 7 groups: untreated controls (8 rats); animals receiving simultaneous administration (Day 0) of 10 μL of ReGel (6 rats), OncoGel 1.5 (8 rats), or OncoGel 6.3 (8 rats); and groups that received 10 μl of ReGel (6 rats), OncoGel 1.5 (7 rats), or OncoGel 6.3 (8 rats) 5 days after tumor implantation (Day 5). For delayed administration (5 days after tumor injection), rats were anesthetized, the original incision was reopened, and animals were placed in the stereotactic frame. The original site was injected with the appropriate treatment and the animal was removed from the frame. After tumor implantation, the animals were closely monitored daily for any signs of neurotoxicity (such as ataxia or hemiparesis). Rats were inspected for signs of distress and if symptoms developed, the rat was killed as stated above. The initiation of each experiment (intracranial injection of gliosarcoma cells) was considered Day 0 and survival was counted from this point. The experiment was continued for 120 days and animals alive at Day 120 were considered long-term survivors.

Combination Therapy. To mimic clinical treatment of gliomas more closely, radiation therapy was used in combination with OncoGel 6.3. This experiment used the 9L tumor piece from the flank carrier model with tumor pieces implanted intracranially. Briefly, 9L tumor propagated as a solid tumor in the flank of a Fischer-344 rat was excised and fragments measuring 2 mm^3 were cut using the surgical microscope. The fragments were kept at 4°C before implantation. Sixty recipient animals were anesthetized and their dura was exposed as described above, except that a dull drill bit was used to generate a modest amount of heat to coagulate the underlying cortical vessels. The dura was opened and the cortex was aspirated to expose the sulcus on the dorsum of the brainstem between the thalamus and the superior colliculus. Hemostasis was achieved and the tumor piece was deposited into the defect.

Rats were then randomized into 8 groups. Animals randomized to receive Day 0 treatment were placed in the stereotactic frame and injected with 35 μl of either OncoGel 6.3 or ReGel. For delayed administration (5 days after tumor injection) rats were anesthetized, the original incision was reopened, and animals were placed in the stereotactic frame and injected with 35 μl of either OncoGel 6.3 or ReGel. Group 1 (control) received no further intervention (8 rats). Group 2 received a 35-μl intracranial injection of ReGel on Day 0 (8 rats). Group 3 received a 35-μl intracranial injection of ReGel on Day 5 (5 rats). Group 4 received a 35-μl intracranial injection of OncoGel 6.3 on Day 0 (8 rats). Group 5 received OncoGel 6.3 on Day 5 (8 rats). Group 6 received radiation therapy at a dose of 20 Gy on Day 5 (7 rats). Group 7 received a 35-μl intracranial injection of OncoGel 6.3 on Day 0, followed by radiation therapy on Day 5 (8 rats). Group 8 received a 35-μl intracranial injection of OncoGel 6.3 on Day 5 and radiation therapy on Day 5 (8 rats). For the radiation therapy procedure animals were anesthetized, placed individually into a Shepherd irradiator, and exposed to a single radiation dose of 20 Gy. Animals that received both OncoGel 6.3 and radiation therapy on Day 5 received both within 15 minutes. Upon death, the brain was removed and fixed in formalin. The experiment was continued for 120 days and animals alive at Day 120 were considered long-term survivors.

Histological Analyses

Safety Studies. Brains in formalin were sectioned for histological analysis to determine cytological pathology from the gel (both placebo [ReGel] and OncoGel). One coronal brain section centered through the polymer implant was obtained for each rat and stained with H & E. Sections were examined by the authors for cytological changes, inflammation, and necrosis.

In Vivo Biodistribution Studies. The ethyl acetate solvent was evaporated from the ^{14}C-labeled paclitaxel (Sigma) in a fume hood for 24 hours and ethanol (200 μl) was added to the vial. This was then mixed into thawed OncoGel (1 ml) and the mixture was stirred overnight and maintained at 4°C. Eighteen Fischer-344 female rats were anesthetized and implanted with 9L tumor pieces from flank carrier animals as described above. Hemostasis was achieved and the animal was transferred to a stereotactic frame (also draped and equipped with a sterile needle). At 4°C the mixture of OncoGel (35 μl) was administered over a period of 2 minutes and the skin was closed with surgical staples. Animals were observed as described above. Animals were then killed, 3 at each time point, including at 4 and 72 hours, and 1, 2, and 3 weeks postinjection. Serum levels of radiolabeled paclitaxel were counted, and brains were harvested. Each brain was divided into hemispheres and then cut into 1.5-mm coronal sections, both ipsilaterally and contralaterally. Each section was weighed and homogenized using the reagent Solvable (NEN Life Sciences) and examined for radioactivity to determine drug concentration. A standard curve was constructed from actual amounts of radiolabeled paclitaxel compared with scintillation counts. All counts were performed using a Beckman liquid scintillation counter.

Efficacy Studies. All brains were prepared as detailed in the safety studies. A coronal section obtained through the injection/implantation site was stained with H & E. The section was examined to confirm the presence or absence of tumor growth and to determine the effect of the gel intracranially. At the end of the experiments (Day 120) all remaining animals were killed and brains were examined as described above.

Data Analysis and Statistical Methods

No statistical analyses were performed on survival data from the safety studies. The date of killing or death was recorded for each animal, and the percentage of animals surviving for each group was plotted using Microsoft Excel.

The primary statistical outcome for all efficacy studies was time until death measured from the time of tumor

implantation (Day 0). Survival was plotted on a Kaplan-Meier survival curve and statistical significance was determined by a nonparametric Kruskal-Wallis analysis of variance, followed by a nonparametric Wilcoxon rank-sum test using GraphPad Prism (version 4.0). Probability values < 0.05 were considered statistically significant.

Results

Safety Studies

For intracranial safety studies, the injection volumes of ReGel, OncoGel 1.5, and OncoGel 6.3 were varied. All rats treated by injection of 10 μl of either ReGel (5 animals), OncoGel 1.5 (5 animals), or OncoGel 6.3 (5 animals) survived for the length of the study (120 days; data not shown). Two rats from each of these groups were killed on Day 10 and the remainder on Day 120 for histopathological analysis (data not shown). These analyses showed no toxic effects from the intracranial injection. Rats that received 25-μl injections of either ReGel (2 animals) or OncoGel 6.3 (2 animals) also survived throughout the duration of the study (25 days).

Rats that received intracranial injections of 50 μl (6 rats), 75 μl (2 rats), or 100 μl (3 rats) of ReGel or 50 μl (9 rats), 75 μl (2 rats), or 100 μl (3 rats) of OncoGel 6.3 demonstrated toxicity that appeared to be volume dependent (Fig. 1). Death occurred in < 5 days at volumes of 50 μl or greater. No difference in the maximally tolerated volume of ReGel or OncoGel injected was observed. Because deaths were observed in both the ReGel (placebo) group and the chemotherapeutic (OncoGel) groups at these injection volumes, deaths were attributed to volume as opposed to chemotoxicity. We therefore concluded that a volume < 50 μl was the maximum volume that could be used for rat intracranial injection, and a volume of 35 μl was selected for the subsequent combination efficacy study.

In Vivo Biodistribution Study

There was variability in the distribution of paclitaxel throughout the brain. As expected, the highest radioactive counts were noted 3 hours after OncoGel injection in the ipsilateral hemisphere (Fig. 2 *upper*). Interestingly, the highest concentration of radioactivity was observed 6, 4.5, and 3 mm anterior to the injection site. There was also substantial radioactivity noted 1.5-mm posterior to the injection site at both the 3-hour and 7-day time points. Concentrations of paclitaxel during this time varied, and reached as high as 5 ng/mg in brain tissue. Measurable radioactivity was also noted throughout the ipsilateral hemisphere up to 3 weeks after the OncoGel injection. Concentrations measured in the ipsilateral hemisphere in the biodistribution study were higher than the 90% lethal dose concentrations in previously published studies of OncoGel in vitro.[3] Surprisingly there was an increase in radioactivity observed in the contralateral hemisphere 3 days following the OncoGel injection (Fig. 2 *lower*). Plasma samples showed little radioactivity following the OncoGel injections, which indicates that paclitaxel did not diffuse across the blood-brain barrier and confirms that paclitaxel delivered by OncoGel does not result in systemic toxicity in this model.

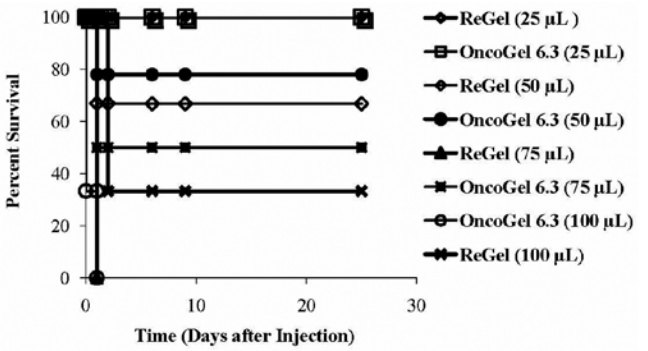

Fig. 1. Kaplan-Meier survival curves of animals intracranially injected with various volumes and concentrations of ReGel and OncoGel in the safety studies. Intracranial injections of 25 μl of either ReGel (2 rats; *solid diamonds*) or OncoGel 6.3 (2 rats; *open squares*) resulted in no deaths. Injections of 50 μl of ReGel (6 rats; *open diamonds*) or OncoGel 6.3 (9 rats; *solid circles*) produced deaths in 33 and 22%, respectively. Animals injected intracranially with 75 μl of ReGel (2 rats; *solid triangles*) or OncoGel 6.3 (2 rats; *solid squares*) showed deaths in 100 and 50%, respectively. Animals injected intracranially with 100 μl of either OncoGel 6.3 (3 rats; *open circles*) or ReGel (3 rats; ×) showed deaths in 100 and 67%, respectively. Based on these data, 10- and 35-μl injections were used for the subsequent efficacy studies.

Efficacy of Intracranially Injected OncoGel

Monotherapy. In animals receiving an injection of a 9L glioma suspension (100,000 cells), there was no difference in median survival time between the control group and the groups receiving ReGel on either Day 0 or Day 5 (median survival 25 days). Administration of OncoGel 1.5 or OncoGel 6.3 on Day 0 significantly increased survival as compared with animals receiving no further intervention and animals receiving a placebo injection of ReGel (Table 1). The groups that received 10 μl of either OncoGel 1.5 or OncoGel 6.3 on Day 0 showed a statistically significant increase in median survival compared with the group that received ReGel injected on Day 0. The OncoGel 1.5 group had a median survival of 29 days ($p = 0.0063$ vs ReGel Day 0 group), and the OncoGel 6.3 group had a median survival of 35 days with 12.5% long-term survivors ($p = 0.0017$ vs ReGel Day 0 group). On Day 120, all animals were killed and no tumor was detected in any of the long-term survivors.

No difference in median survival was noted between the control group (median survival 25 days) and the groups receiving either OncoGel 1.5 or OncoGel 6.3 on Day 5 (median survival 25 and 24 days, respectively; Table 1).

Combination Therapy. The second efficacy study used the more aggressive tumor implantation technique to test the efficacy of OncoGel, and this technique resulted in a decrease in the median survival for control animals. Also, to mimic possible clinical use of OncoGel more closely, additional groups were included that received both OncoGel 6.3 and radiation therapy. OncoGel 6.3 was given either simultaneously or 5 days after tumor

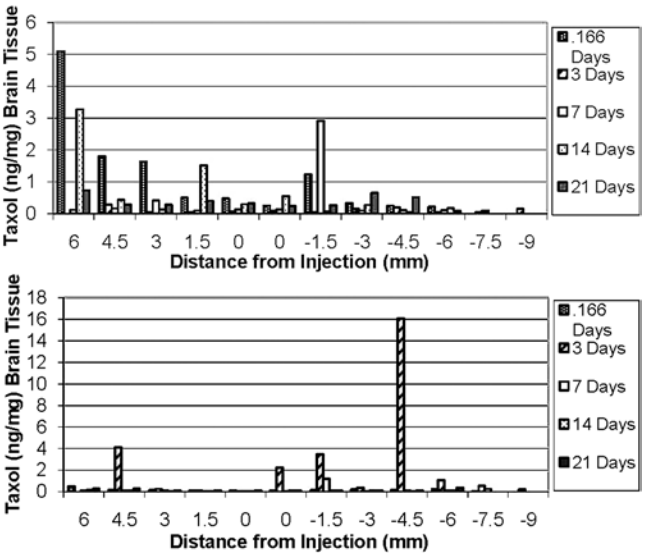

Fig. 2. Bar graphs comparing intracerebral distribution of paclitaxel (Taxol) in the rat ipsilateral (injection) hemisphere ***(upper)*** and contralateral hemisphere ***(lower)*** using OncoGel. The x-axis is the distance from the injection site. Positive numbers represent anterior distances and negative numbers represent posterior distances. Each bar represents 3 animals.

TABLE 1: Treatment of experimental glioma with intracranially injected OncoGel

Group*	No. of Animals	Median Survival (days)	p Value	Long-Term Survivors (%)
control (no Tx)	8	25		0
ReGel				
Day 0	6	25		0
Day 5	6	25		0
OncoGel 1.5				
Day 0	8	29	0.0063 vs ReGel Day 0	0
Day 5	7	25		0
OncoGel 6.3				
Day 0	8	35	0.0017 vs ReGel Day 0 0.01 vs control	12.5
Day 5	8	24		0

* All rats received 9L tumor cells (2 μL) injected at Day 0, in addition to 10 μL of treatment material (except controls). Therefore, total injection volumes were 2 μL for controls, and 12 μL for all other groups.

implantation. Radiation therapy, at a dose of 20 Gy, was given 5 days after tumor implantation (Table 2).

Animals implanted with 9L gliosarcoma alone (control group) had a median survival of 13 days and all died by Day 17 (Table 2; Fig. 3). There was no significant difference in median survival time between the control group and the groups that received the placebo ReGel. The ReGel Day 0 group had a median survival of 14 days ($p = 0.6394$ vs controls), and the ReGel Day 5 group had a median survival of 17 days ($p = 0.06$ vs controls). The animals that received an intracranial injection of OncoGel 6.3 on Day 5 had a median survival of 17 days, which was a statistically significant increase in survival as compared with controls ($p = 0.0255$), but not when compared with the ReGel Day 5 group ($p = 0.4691$). The animals receiving radiation therapy only (20 Gy) had a median survival of 26 days, and the OncoGel 6.3 Day 0 group had a median survival of 31 days, both of which were statistically significant compared with controls ($p < 0.0001$). In the group implanted with OncoGel on Day 0, 37.5% were long-term survivors.

The animals that received both OncoGel and radiation therapy on Day 5 had a median survival of 32 days and 12.5% were long-term survivors, which was significant ($p < 0.0001$ vs controls and vs the ReGel Day 5 group), but was not significant compared with the radiation therapy–alone group ($p = 0.1459$). The animals that received OncoGel 6.3 on Day 0 and radiation therapy on Day 5 experienced the most prolonged survival (median 83 days) and 37.5% were long-term survivors. This group had a significant increase in survival as compared with the control group ($p < 0.0001$), the ReGel Day 0 group ($p < 0.0001$), and the radiation therapy–alone group ($p = 0.0083$).

Histological Analysis

All animals that died during the experimental efficacy studies (both single and combination treatment) showed evidence of a large intracranial tumor. Hemispheric displacement was evident, typical of asymmetrical tumor implantation. At the end of the experiment (Day 120), all long-term survivors were killed and their brains were examined using histological analysis. These animals showed no signs of tumor and no evidence of inflammation. No necrosis was noted in the histological sections. There was evidence of surgery-related dystrophic calcification in some of the long-term survivors. In 2 of the 7 long-term survivors (1 animal in the OncoGel 6.3 Day 5 and radiation therapy group and 1 in the OncoGel 6.3 Day 0 group), encephalomalacia was present, but this debris was small and focused at the injection site.

Discussion

The poor penetration of drugs into the CNS contributes to the high mortality rate for patients with malignant glioma. Bypassing the blood-brain barrier by locally delivering potent chemotherapeutic agents is one way to both reduce systemic toxicity and increase therapeutic benefit at the tumor site. This bypass has been accomplished clinically by locally delivering polymeric wafers containing chemotherapy, such as Gliadel,[2,17,19] or through convection-enhanced delivery methods.[11] A recent Phase I/II study in which paclitaxel was administered intracranially by convection-enhanced delivery to patients with malignant glioma showed promising results, but also resulted in complications that included infection, low convection rates, leakage of the convected drug, and dose-limiting toxicity.[11] Additionally, with convection-

TABLE 2: Intracranial efficacy of OncoGel in a rodent glioma model with and without radiation therapy

Group*	No. of Animals	Median Survival (days)	p Value	Long-Term Survivors (%)
control (no further Tx)	8	13		0
ReGel				
Day 0	8	14	0.6394 vs controls	0
Day 5	5	17	0.06 vs controls	0
OncoGel 6.3				
Day 0	8	31	<0.0001 vs controls	37.5
Day 5	8	17	0.02 vs controls, 0.4691 vs ReGel Day 5	0
radiation therapy (Day 5)†				
only	7	26	<0.0001 vs controls	0
& OncoGel 6.3 (Day 0)	8	83	<0.0001 vs controls & ReGel Day 0, <0.0083 vs radiation therapy, <0.55 vs OncoGel Day 0	37.5
& OncoGel 6.3 (Day 5)	8	32	<0.0001 vs controls & ReGel Day 5, 0.1459 vs radiation therapy	12.5

* All rats received a 9L tumor (2 mm^3) implanted at Day 0. Except for the control and radiation therapy only–groups, all groups also received injection volumes of 35 μl.

† All radiation therapy administered in a single dose (20 Gy).

enhanced delivery paclitaxel could only be released over a 5-day period.[11]

ReGel, a water-soluble biodegradable compound that forms an insoluble gel at body temperature, was developed to locally deliver drugs such as paclitaxel. ReGel loaded with paclitaxel (OncoGel) is biodegradable and releases paclitaxel at a constant rate for ~ 50 days.[20] OncoGel implanted in a porcine pancreatic tumor model produced high tissue concentrations of paclitaxel 14 days after initial injection (a single time point was evaluated) and therapeutic levels of paclitaxel were detected 3–5 cm from the injection site.[14] This degree of diffusion would be beneficial in malignant glioma in which it has been shown that 80% of tumors recur within 2 cm of the original tumor bed.[8] Histological changes indicative of paclitaxel exposure have been shown to be present in the brains of rats in a glioma model up to 12 weeks after implantation of paclitaxel intracranially delivered from polilactofate:polyethylene glycol 1000 microspheres, indicating that locally delivered paclitaxel remains in the parenchyma.[10] Additionally, OncoGel was recently shown to delay the onset of paralysis in a rodent metastatic spinal tumor model.[1]

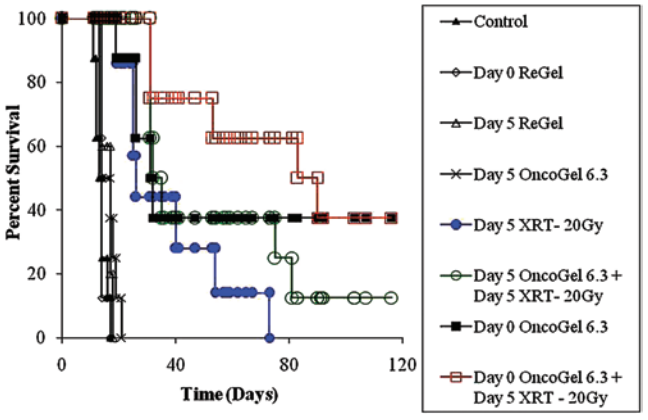

Fig. 3. Intracranial efficacy of OncoGel 6.3 with and without radiation therapy for the treatment of experimental malignant glioma. Fischer-344 rats were implanted with 9L glioma tumor. Controls (8 rats; *black solid triangles*) received no further treatment and had a median survival of 13 days. All treatment and placebo injections were delivered in 35 μl. Animals receiving ReGel on either Day 0 (8 rats; *open diamonds*) or Day 5 (5 rats; *open triangles*) had median survivals of 14 and 17 days, respectively. Animals that received OncoGel 6.3 on Day 5 (8 rats; ×) had a median survival of 17 days. Animals receiving 20 Gy radiation therapy only (7 rats; *blue solid circles*) had a median survival of 26 days with a mortality of 100%. An injection of OncoGel on Day 5 with radiation therapy (8 rats; *green open circles*) resulted in a median survival of 32 days with 12.5% long-term survivors. Animals receiving either OncoGel 6.3 on Day 0 alone (8 rats; *black solid squares*) or on Day 0 in combination with radiation therapy (8 rats; *red open squares*) had median survival times of 31 and 83 days, respectively, with 37.5% long-term survivors in both groups.

To determine the potential utility of OncoGel for the treatment of brain tumors, we first tested the safety of delivering OncoGel intracranially in rats.[20] There were no signs of toxicity in any rats that received intracranial injections of up to 35 μl of ReGel, OncoGel 1.5, or OncoGel 6.3. Neither inflammation nor cellular infiltrate was observed at the site of injection for these groups when analyzed at the end of each study. We believe that toxicity noted at higher injection volumes (50–100 μl) was due to the volume of the injection, and was not specifically due to the delivery system (ReGel) or the drug. Similar or greater doses of intracranial paclitaxel have been shown to be nontoxic with other delivery systems in similar animal models.[6,18] Therefore, the toxicity associated with doses of paclitaxel in the 300–600 μg range after injection of OncoGel 6.3 (50–100 μl) was probably the result of the volume of the injection. This is additionally supported by the deaths observed in rats in the control groups that were

injected with large volumes (50–100 μl) of the placebo ReGel and represents a limitation of the rodent glioma model. Therefore, an injection volume < 50 μl was used for subsequent studies and 35 μl was chosen for the efficacy study in combination with radiation therapy.

The intracerebral biodistribution study demonstrated that the highest dose of paclitaxel was measured in the ipsilateral hemisphere 3 hours after the OncoGel was injected, but paclitaxel was measurable throughout the 3-week study period. The highest concentrations of paclitaxel were found up to 6 mm away from the site of injection at the first time point. In the contralateral hemisphere the highest dose of paclitaxel was observed 3 days following injection and was measurable up to 9 mm from the injection site, suggesting that the OncoGel remains in the brain and diffuses from the initial site of injection. This study demonstrated that an OncoGel injection results in elevated levels of paclitaxel throughout the rodent brain, including the contralateral hemisphere, and can be detected up to 3 weeks after initial introduction. Also, although these levels of paclitaxel are within the 1–16 ng/mg brain tissue range, they are still orders of magnitude higher than lethal dose concentrations for in vitro brain tumor cell lines.[3]

We attribute the lack of efficacy of OncoGel delivered on Day 5 in the monotherapy study to the limited drug injection volume (10 μl) in this study. The tested volume may not have delivered enough paclitaxel to arrest the growth of the established tumor. In a larger animal model in which the resected cavity would have the capacity to allow for a greater volume of OncoGel, results might be more encouraging. In contrast, both OncoGel formulations (6.3 and 1.5 mg paclitaxel per ml OncoGel) were found to be efficacious in the rodent intracranial glioma model when delivered on Day 0 as compared with controls. This encouraging result led us to further investigate the efficacy of OncoGel in combination with the standard clinical treatment regimen of radiation therapy, in which a higher volume of OncoGel was administered (35 μl).

In the combination therapy study, animals that received an intracranial injection of OncoGel 6.3 on either Day 0 or Day 5 showed statistically significant increases in survival as compared with control groups, with the OncoGel 6.3 Day 0 group resulting in 37.5% long-term survivors. The animals that received OncoGel 6.3 on Day 0 in combination with radiation therapy had a median survival of 83 days (37.5% long-term survivors). This group had a significant increase in survival as compared with all control groups (untreated animals, ReGel-only group, and the radiation therapy–alone group).

Conclusions

OncoGel is a novel delivery system for paclitaxel, which once injected forms an insoluble gel at body temperature. OncoGel can be delivered safely to the rodent brain. Treatment with OncoGel improves survival in a rodent glioma model when sufficient volume is injected. Survival is improved further with the addition of radiation therapy. Based on the results of this study, a Phase I/II clinical trial of OncoGel for the treatment of recurrent malignant glioma has been initiated.

Disclosure

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