Combination of Intracranial Temozolomide With Intracranial Carmustine Improves Survival When Compared With Either Treatment Alone in a Rodent Glioma Model

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BACKGROUND: Local delivery of temozolomide (TMZ) through polymers is superior to oral administration in a rodent glioma model.

OBJECTIVE: We hypothesized that the observed clinical synergy of orally administered TMZ and carmustine (BCNU) wafers would translate into even greater effectiveness with the local delivery of BCNU and TMZ and the addition of radiotherapy in animal models of malignant glioma.

METHODS: TMZ and BCNU were incorporated into biodegradable polymers that were implanted in F344 rats bearing established intracranial tumors. We used 2 different rodent glioma models: the 9L gliosarcoma and the F98 glioma.

RESULTS: In the 9L rodent glioma model, groups treated with the combination of local TMZ, local BCNU, and radiation therapy (XRT) had 75% long-term survivors (defined as animals alive 120 days after tumor implantation), which was superior to the combination of local TMZ and local BCNU (median survival, 95 days; long-term survival, 25%) and the combination of oral TMZ, local BCNU, and XRT (median survival, 62 days; long-term survival, 12.5%). To simulate the effect of this treatment in chemoresistant gliomas, a second rodent model was used with the F98 glioma, a cell line relatively resistant to alkylating agents. F98 glioma cells express high levels of alkyltransferase, an enzyme that deactivates alkylating agents and is the major mechanism of resistance of gliomas. The triple therapy showed a significant improvement in survival when compared with controls ($P = .0004$), BCNU ($P = .0043$), oral TMZ ($P = .0026$), local TMZ ($P = .0105$), and the combinations of either BCNU and XRT ($P = .0378$) or oral TMZ and BCNU ($P = .0154$).

CONCLUSION: The survival of tumor-bearing animals in the 9L and F98 glioma models was improved with the local delivery of BCNU and TMZ combined with XRT when compared with either treatment alone or oral TMZ, local BCNU, and XRT.

KEY WORDS: Carmustine, Glioma, Local delivery, Polymers, Temozolomide

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Recent clinical advances in glioma therapy, with multimodality treatments, have led to an improvement in expected survival for adult glioblastoma multiforme from 9 to 20 months.[1-4] In addition to surgical resection[5] and radiotherapy,[6] numerous chemotherapeutic agents have been used to treat this disease,[7] but limitations like poor central nervous system drug penetration and dose-limiting toxicities have restricted their use.[8] Novel strategies are needed to improve the outcome of patients with glioblastoma multiforme. Research efforts have been directed toward local delivery of agents to the site of the tumor to achieve maximal drug concentrations while limiting toxicity. Gliadel (Eisai, Inc, Woodcliff Lake, NJ), a biodegradable polymer containing the alkylating agent carmustine (BCNU), is implanted locally into the surgical bed at the time of high-grade glioma resection and has been shown to increase survival in both newly diagnosed[9-12]

ABBREVIATIONS: AGT, alkylguanine-DNA alkyltransferase; **BCNU**, carmustine; **LTS**, long-term survival; **TMZ**, temozolomide; **XRT**, radiation therapy

and recurrent[13] malignant gliomas. Temozolomide (TMZ), given orally as Temodar (Schering Corp, Kenilworth, NJ), has been shown in randomized, placebo-controlled, multi-institutional clinical trials to be effective in prolonging survival and has received United States Food and Drug Administration approval for the treatment of newly diagnosed[14,15] or recurrent[16] malignant glioma. TMZ is an imidazotetrazine second-generation alkylating agent, which, when given with radiation therapy (XRT), extends median survival by 2.5 months, compared with radiation alone[15] (Temodar dose of 150–200 mg/m^2). Higher doses of Temodar, which might increase efficacy, are associated with dose-limiting myelosuppression, including severe leukopenia and thrombocytopenia.[14-17] Recent clinical evidence suggests that treatment with a combination of modalities consisting of surgical excision, locally delivered BCNU, concurrent and adjuvant TMZ, and radiotherapy is safe and effective and provides improved survival compared with each treatment group alone.[1-3,18,19]

On the basis of the encouraging clinical data observed with orally administered TMZ, we previously developed a local delivery system for the drug, using biodegradable polymers.[20] In these earlier experiments, we showed that local delivery is superior to oral administration in a rodent glioma model. Given the potential synergistic effect seen clinically when combining intracranial BCNU with oral TMZ,[1-3,18,19] we hypothesized that there will be a significant survival benefit if the TMZ is also given locally. In this report, we investigate the possible additive effect of combined intracranial BCNU with intracranial TMZ and XRT in the treatment of 2 rat intracranial glioma models, the 9L gliosarcoma and the F98 glioma. We used the inherent resistance of the F98 glioma to alkylating agents to simulate the effect of this treatment in the common clinical scenario of chemoresistant gliomas.

MATERIALS AND METHODS

Polymer Formation

TMZ, provided by the National Institute of Health/National Cancer Institute (Bethesda, MD), was incorporated into a polyanhydride poly(1,3-bis-[*p*-carboxyphenoxy propane]-*co*-[sebacic anhydride]) (CPP:SA) polymer at concentrations of 50% (w/w) by methods described previously.[21] BCNU (Bristol-Myers Squibb, Inc, Princeton, NJ) was purchased from the Johns Hopkins Hospital pharmacy, and polymers were made at concentrations of 3.8% (w/w) in a similar fashion. The polymers were then pressed into disks weighing approximately 10 mg. Blank polymers (100% CPP:SA) were made in an analogous manner. In vitro release kinetics, biodistribution studies, and studies to determine maximally tolerated dose of BCNU and TMZ polymers have been published.[20,22,23] Polymers were stored at –20°C until use.

Tumor Cells

The 9L gliosarcoma was obtained from Marvin Barker, MD, (University of California, San Francisco, Brain Tumor Research Center, San Francisco, CA). For tumor piece implantation, 9L tumor pieces measuring 2 mm^3 were passaged in the flank of F344 rats (female, 150–200 g) every 3 to 4 weeks. For intracranial implantation, the 9L gliosarcoma tumor was surgically excised from the carrier animal, cut into 1-mm^3 pieces, and placed in sterile 0.9% NaCl on ice. The F98 glioma was obtained from Rolf Barth, MD, (Ohio State University, Columbus, OH). Tumor cells were placed in humidified incubators and maintained in Dulbecco's minimal essential medium (Invivogen, San Diego, CA) containing 10% fetal bovine serum.

Animals

F344 female rats weighing 150 to 200 g, purchased from Harlan Bioproducts (Indianapolis, IN), were used. They were housed in standard facilities and given free access to food and water. All animals were treated in accordance with the policies and guidelines of the Johns Hopkins University Animal Care and Use Committee.

Anesthesia

Rats were anesthetized with an intraperitoneal injection of 0.6 mL of a stock solution containing ketamine HCl (75 mg/kg, 100 mg/mL), xylazine (7.5 mg/kg, 100 mg/mL), and ethanol (14.25%) in a sterile 0.9% NaCl solution.

Intracranial Glioma Model

For intracranial implantation of the 9L gliosarcoma, 127 F344 female rats (32 rats for the first experiment and 95 rats for the second experiment) were anesthetized. The head was shaved with clippers and prepared with alcohol and Prepodyne solution (West Penetone, Montreal, Canada). A midline scalp incision was made, exposing the sagittal and coronal sutures. With the use of an electric drill with a 2-mm round cutting burr, a small hole was made in the skull centered 3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture. Care was taken to avoid the sagittal sinus. Forceps were used to lift off the remaining bone. Under microscopic magnification, a dural opening and then cortical opening were made. A small area of cortex and white matter was resected, and, once hemostasis was achieved, a single tumor piece was placed in the resection cavity. The skin was then closed with surgical staples.

For intracranial tumor injection of the F98 glioma cells, 85 F344 female rats were anesthetized. The procedure was similar to that for the 9L implant; however, after the burr hole was drilled, the animals were placed in a stereotactic frame, and 1×10^5 F98 glioma cells were injected over 3 minutes via a 26-gauge needle inserted to a depth of 4 mm at the center of the burr hole. After tumor cell inoculation, the needle was removed, the site was irrigated with normal saline, and the incision was closed with surgical staples.

XRT

For XRT, animals were anesthetized, placed at a fixed distance from the radiation source, and shielded with a square primary collimator (1 cm in diameter) centered over the tumor implantation site. The radiated animals received external beam single-dose radiation treatment by using a Cs^{138} laboratory irradiator (Mark 1 Irradiator, model 68; J.L. Shepherd Associates, San Fernando, CA) at a dose of 20 Gy.

Efficacy Studies of Local TMZ Given in Combination With Local BCNU in 9L Gliosarcoma

To determine the efficacy of the combination of locally delivered TMZ with locally delivered BCNU, the tumor-bearing rats were randomized into groups of 8 for treatment on postoperative day 5. The animals received either no treatment (control), 3.8% BCNU polymer (total dose, 0.38 mg of BCNU), 50% TMZ polymer (total dose, 5 mg of TMZ), or both 3.8% BCNU and 50% TMZ polymers. Animals were observed for neurologic and systemic toxicity, and survival was

TABLE 1. Treatment of the 9L Experimental Malignant Glioma Model With Locally Delivered Temozolomide and Carmustine[a]

Group	Median Survival, d (Range)	Long-term Survivors (%)	*P* Value
Control (n = 8)	15 (15-20)	0	
3.8% BCNU polymer (n = 8)	20 (15-120)	12.5	.0435 vs controls; .1635 vs 50% TMZ
50% TMZ polymer (n = 8)	38 (15-120)	37.5	.0009 vs controls; .1635 vs 3.8% BCNU
3.8% BCNU polymer + 50% TMZ polymer (n = 8)	Median not reached (48-120)	87.5	<.0001 vs controls; .0018 vs 3.8% BCNU; .0433 vs 50% TMZ

[a] BCNU, carmustine; TMZ, temozolomide.

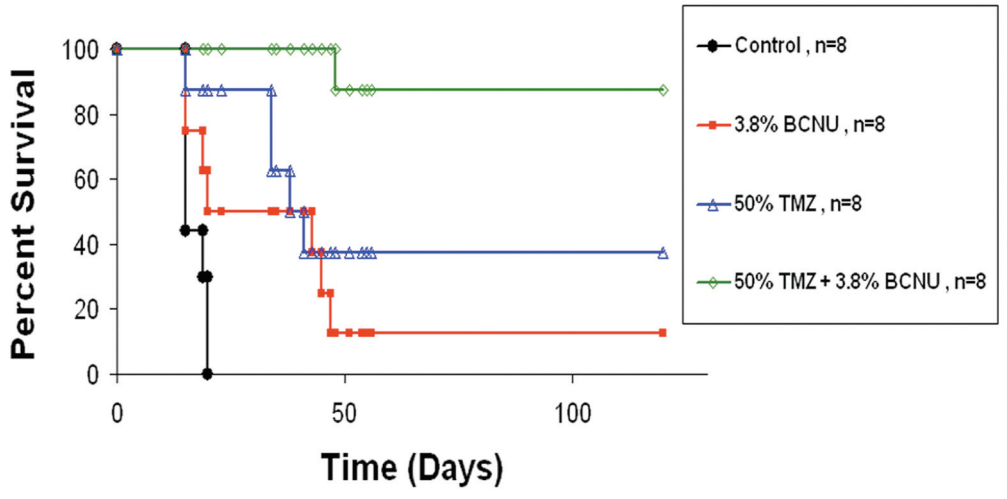

FIGURE 1. *Kaplan-Meier curve. The efficacy of the combination of local carmustine (BCNU) and temozolomide (TMZ) was tested in the rat 9L gliosarcoma using F344 rats. The animals were divided into 4 groups and received either no treatment, local BCNU, local TMZ, or a combination of local BCNU and local TMZ.*

recorded. Any animals appearing moribund were killed, and the date of death was recorded. At day 120, all surviving rats were deemed long-term survivors and were killed. Histopathologic studies of all animals' brains harvested at the time of death or killing were examined to confirm the presence or absence of tumor.

Efficacy Studies of Local TMZ Given in Combination With Local BCNU and XRT in 9L Gliosarcoma and F98 Glioma

To determine the efficacy of the combination of locally delivered TMZ with locally delivered BCNU and XRT, tumor-bearing rats were randomized into groups of 16 for treatment on postoperative day 5. Animals received either no treatment (controls), 3.8% BCNU polymer, 50% TMZ polymer, or both 3.8% BCNU and 50% TMZ polymers. On the same day, half of the animals in each group received XRT. Animals were observed for neurologic and systemic toxicity, and survival was recorded. Any animals appearing moribund were killed, and the date of death was recorded. At day 120, all surviving rats were deemed long-term survivors; however, the experiment was allowed to continue to 150 days because the treatment animals looked healthy. Histopathologic studies of all animals' brains harvested at the time of death or killing were examined to confirm the presence or absence of tumor.

Statistical Analysis

For all efficacy studies, death was the primary end point. The distribution of the intervals until death was determined by the method of Kaplan and Meier. Statistical analysis was completed using Prism 4 software (GraphPad Software, La Jolla, CA).

RESULTS

In Vivo Efficacy of Locally Delivered TMZ and BCNU Against 9L Gliosarcoma

Intracranial delivery of combined TMZ and BCNU polymers increased median survival and produced more long-term survival (LTS) when compared with the control group and either treatment option given alone (Table 1; Fig. 1). Control animals had a median survival of 15 days. Animals treated with BCNU or TMZ alone had statistically improved survival as compared with controls, with a median survival of 20 days ($P = .0435$) and 38 days ($P = .0009$), respectively. The BCNU treatment group yielded 12.5% LTS, whereas the TMZ group had 37.5% LTS. There was no statistical difference between the 2 individual treatment groups ($P = .1635$). Animals treated with the combination of intracranial BCNU and TMZ had the longest prolongation of survival: 87.5% of the animals survived longer than 120 days (median survival was not reached). Survival was significantly greater in this combination group as compared with the control group ($P < .0001$), the group that received BCNU alone ($P = .0018$), and the group that received TMZ alone ($P = .0433$). Histopathologic review of the long-term survivors revealed no evidence of tumor burden on completion of the study. Histopathologic review of the animals that died earlier demonstrated the existence of tumor as the cause of death. No evidence of systemic toxicity was observed with our proposed treatment.

TABLE 2. Treatment of the 9L Experimental Glioma Model With Locally Delivered Temozolomide, Carmustine, and Radiation Therapy[a]

Group	Median Survival, d (Range)	Long-term Survivors (%)	*P* Value
Control (n = 8)	16 (9-17)	0	
50 mg/kg oral TMZ (n = 8)	24 (14-33)	0	.003 vs controls; .0322 vs local TMZ
3.8% BCNU polymer (n = 7)	27 (20-74)	0	.0002 vs controls; .0018 vs local TMZ and local BCNU
50% TMZ polymer (n = 8)	34 (11-95)	0	.0113 vs controls; .0158 vs local TMZ and local BCNU
XRT (20 Gy) (n = 8)	74 (27-120)	37.5	<.0001 vs controls
3.8% BCNU polymer + XRT (n = 8)	120 (33-120)	50	.0004 vs controls; .0004 vs local BCNU
50% TMZ polymer + XRT (n = 8)	120 (35-120)	25	<.0001 vs controls; .0091 vs local TMZ
3.8% BCNU polymer + 50 mg/kg oral TMZ (n = 8)	21 (9-28)	0	.0287 vs controls; .054 vs local BCNU; .3107 vs oral TMZ
3.8% BCNU polymer + 50 mg/kg oral TMZ + XRT (n = 8)	62 (20-120)	12.5	<.0001 vs controls; .1486 vs BCNU; .0234 vs oral TMZ; .1428 vs XRT
3.8% BCNU polymer + 50% TMZ polymer (n = 8)	95 (29-120)	25	<.0001 vs controls; .0018 vs local BCNU; .0158 vs local TMZ
3.8% BCNU polymer + 50% TMZ polymer + XRT (n = 8)	Median not reached (21-120)	75	<.0001 vs controls; .001 vs local BCNU; .0007 vs local TMZ; .1378 vs XRT

[a] TMZ, temozolomide; BCNU, carmustine; XRT, radiation therapy.

In Vivo Efficacy of Locally Delivered TMZ and BCNU With XRT Against 9L Gliosarcoma

Intracranial delivery of TMZ and BCNU polymers in combination with XRT increased median survival and produced more LTS when compared with the control group or either treatment option alone (Table 2; Fig. 2). Control animals had a median survival of 16 days. Animals treated with local BCNU and local TMZ had improved survival compared with controls ($P < .0001$) and either treatment alone (BCNU: $P = .0018$; TMZ: $P = .0158$), with a median survival of 95 days and 25% LTS. The addition of XRT in this treatment group resulted in the longest prolongation of survival, with median survival not reached and 75% of the animals surviving more than 120 days. Survival was greater in the combination group with XRT than in either the control group ($P < .0001$) or in the group receiving the combination of local BCNU and local TMZ ($P = .0652$). The combination of the 3 treatment modalities showed a clear trend toward superiority over the simultaneous local delivery of BCNU and TMZ but did not reach the level of statistical significance. However, the indices of survival with the latter option (median survival, 95 days; LTS, 25%) are clearly inferior to the triple therapy (median survival not reached; LTS, 75%). Interestingly, XRT did not prove statistically different from the triple combination treatment group, with a median survival of 74 days and 37.5% LTS. Median survival was not reached, and there were more long-term survivors in the triple combination therapy group. In addition, our proposed combination scheme appears to provide a greater survival benefit than the clinically used combination of local BCNU, oral TMZ, and XRT (median survival, 62 days; LTS, 12.5%; $P = .0033$). Histopathologic review of the long-term survivors revealed no evidence of tumor burden on completion of the study. Histopathologic review of the animals that died earlier demonstrated the existence of tumor as the cause of death. No evidence of systemic toxicity was observed with our proposed treatment.

In Vivo Efficacy of BCNU and TMZ With XRT in F98 Glioma

Intracranial delivery of combined TMZ and BCNU polymers in combination with external radiation increased median survival when compared with the control group or either treatment option alone (Table 3; Fig. 3). Control animals had a median survival of 13 days. Animals treated with local BCNU combined with local TMZ and radiation had improved survival compared with controls ($P = .0004$) and with either local BCNU ($P = .0043$) or local TMZ ($P = .0105$), with a median survival of 21 days. At the same time, this scheme showed significantly better results when compared with the administration of local BCNU and radiation ($P = .0378$) or local BCNU and oral TMZ ($P = .0154$). The combined local delivery of BCNU and TMZ was superior to the administration of oral TMZ ($P = .0492$). There were no long-term survivors in any of the groups. No evidence of systemic toxicity was observed with our proposed treatment.

DISCUSSION

Recent advances in the local delivery of chemotherapeutic agents show encouraging results in the treatment of patients with malignant gliomas.[4,24,25] Although a number of therapeutic clinical trials are currently under way, there continues to be a limited number of agents in our armamentarium to effectively combat this disease.

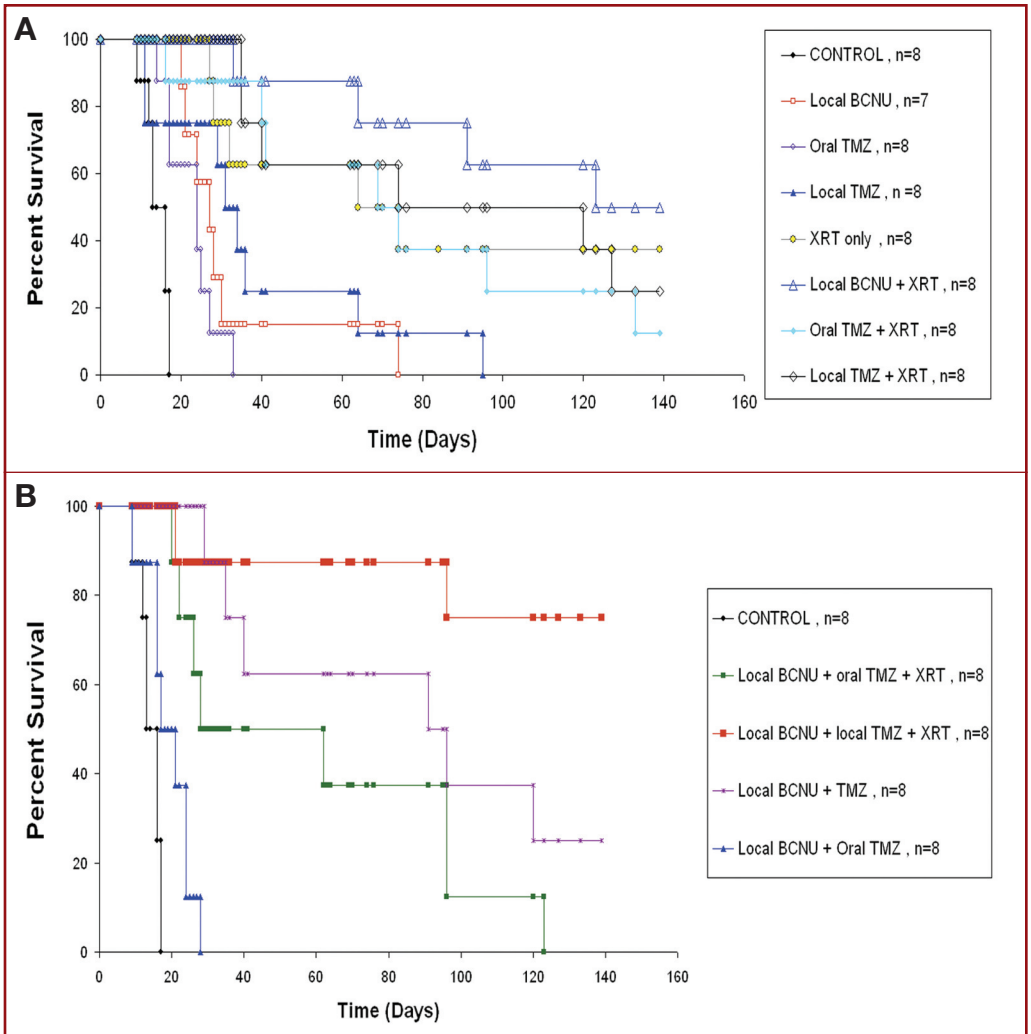

FIGURE 2. A *and* **B**, *Kaplan-Meier curves. The efficacy of the combination of local BCNU, local TMZ, and radiation therapy (XRT) was tested in the rat 9L gliosarcoma using F344 rats. The animals were divided into 12 groups and received either no treatment, local BCNU, local TMZ, local BCNU and local TMZ combined, oral TMZ, oral TMZ and local BCNU, XRT only, local BCNU and XRT, local TMZ and XRT, local BCNU and local TMZ combined with XRT, oral TMZ and XRT, or oral TMZ and local BCNU combined with XRT.*

Alkylating agents, such as BCNU and TMZ, have clearly shown effective dose-response cytotoxicity for many glioma cell lines in vitro.[2,26,27] The maximal doses for each drug, however, are limited, owing to dose-dependent systemic toxicity. To this end, Gliadel is used to maximize local concentrations of BCNU and minimize systemic exposure. On the basis of similar principles and the fact that systemic toxicity has been observed as a dose-limiting factor for TMZ,[15,17,28] we have previously shown that, in rodents, intracranial delivery of TMZ has improved efficacy when compared with systemic administration of TMZ.[2,20]

In our current experiments, we build on the observed clinical benefit of the combined treatment of BCNU and TMZ.[1-3,18,19] We hypothesized that the addition of locally delivered TMZ to locally delivered BCNU will prolong survival in rodent glioma models compared with use of either agent alone. Intracranial TMZ was administered at the maximal loading dose of the polymer (5 mg), and no signs of systemic toxicity or hematologic dysfunction were observed, as compared with oral administration. Thus, local TMZ treatment increased intracranial TMZ concentrations in the tumor bed while minimizing systemic exposure to TMZ.

Clinically, both BCNU and TMZ prolong the survival of patients with glioblastoma multiforme. While each has a similar benefit alone, we hypothesized that combining these agents would produce an additive benefit. To test this hypothesis, rats implanted with brain tumors were treated with TMZ, BCNU, or a combination of both treatments. During this first experiment, we confirmed our hypothesis that concurrent local delivery of both BCNU and TMZ is superior to the use of either agent alone. No signs of systemic toxicity were observed.

Most patients with malignant gliomas are treated with some form of XRT,[6,25,29] making it crucial to determine the interaction of any novel strategies with XRT. The positive results of our first experiment led to its expansion with the introduction of XRT after local treatment with TMZ and BCNU. The treatment groups received combinations including oral or local TMZ, local BCNU, and XRT. We tested our hypothesis that, in rats bearing 9L gliosarcoma, the combination of local TMZ, local BCNU, and XRT would be superior to the local administration of the 2 chemotherapeutic agents alone. We also hypothesized that the administration of the clinically used protocol of local BCNU and oral TMZ combined with XRT would be improved with the use of local BCNU and local TMZ in combination with XRT.

Our results confirmed previously published data[20] indicating the superiority of locally delivered TMZ polymers to orally administered TMZ (P = .03). We also demonstrated the superiority of the combination of local BCNU and local TMZ compared with either treatment alone. These results were confirmed in both 9L

TABLE 3. Treatment of the F98 Experimental Glioma Model With Locally Delivered Temozolomide, Carmustine, and Radiation Therapy[a]

Group	Median Survival, d (Range)	Long-term Survivors (%)	*P* Value
Control (n = 8)	13 (13-14)	0	
50 mg/kg oral TMZ (n = 8)	15 (13-18)	0	.013 vs controls; .2339 vs local TMZ
3.8% BCNU polymer (n = 7)	13 (13-19)	0	.0949 vs controls; .1269 vs local TMZ and local BCNU
50% TMZ polymer (n = 6)	15 (15-19)	0	.0002 vs controls; .0492 vs local TMZ and local BCNU
XRT (20 Gy) (n = 7)	19 (13-48)	0	.0062 vs controls
3.8% BCNU polymer + XRT (n = 8)	19 (14-21)	0	.0002 vs controls; .05 vs local BCNU; .0218 vs oral TMZ
50% TMZ polymer + XRT (n = 7)	19 (15-25)	0	.0001 vs controls; .05 vs local TMZ; .0093 vs oral TMZ
3.8% BCNU polymer + 50 mg/kg oral TMZ (n = 7)	18 (15-19)	0	.0001 vs controls; .1843 vs local BCNU; .0168 vs oral TMZ
3.8% BCNU polymer + 50 mg/kg oral TMZ + XRT (n = 7)	18 (18-26)	0	.0001 vs controls; .05 vs BCNU; .0087 vs oral TMZ; .9639 vs XRT
3.8% BCNU polymer + 50% TMZ polymer (n = 7)	18 (13-120)	14.29	.001 vs controls; .1269 vs local BCNU; .0492 vs oral TMZ; .2124 vs local TMZ
3.8% BCNU polymer + 50% TMZ polymer + XRT (n = 7)	21 (14-25)	0	.0004 vs controls; .0043 vs local BCNU; .0105 vs local TMZ; .0026 vs oral TMZ; .4116 vs XRT

[a] TMZ, temozolomide; BCNU, carmustine; XRT, radiation therapy.

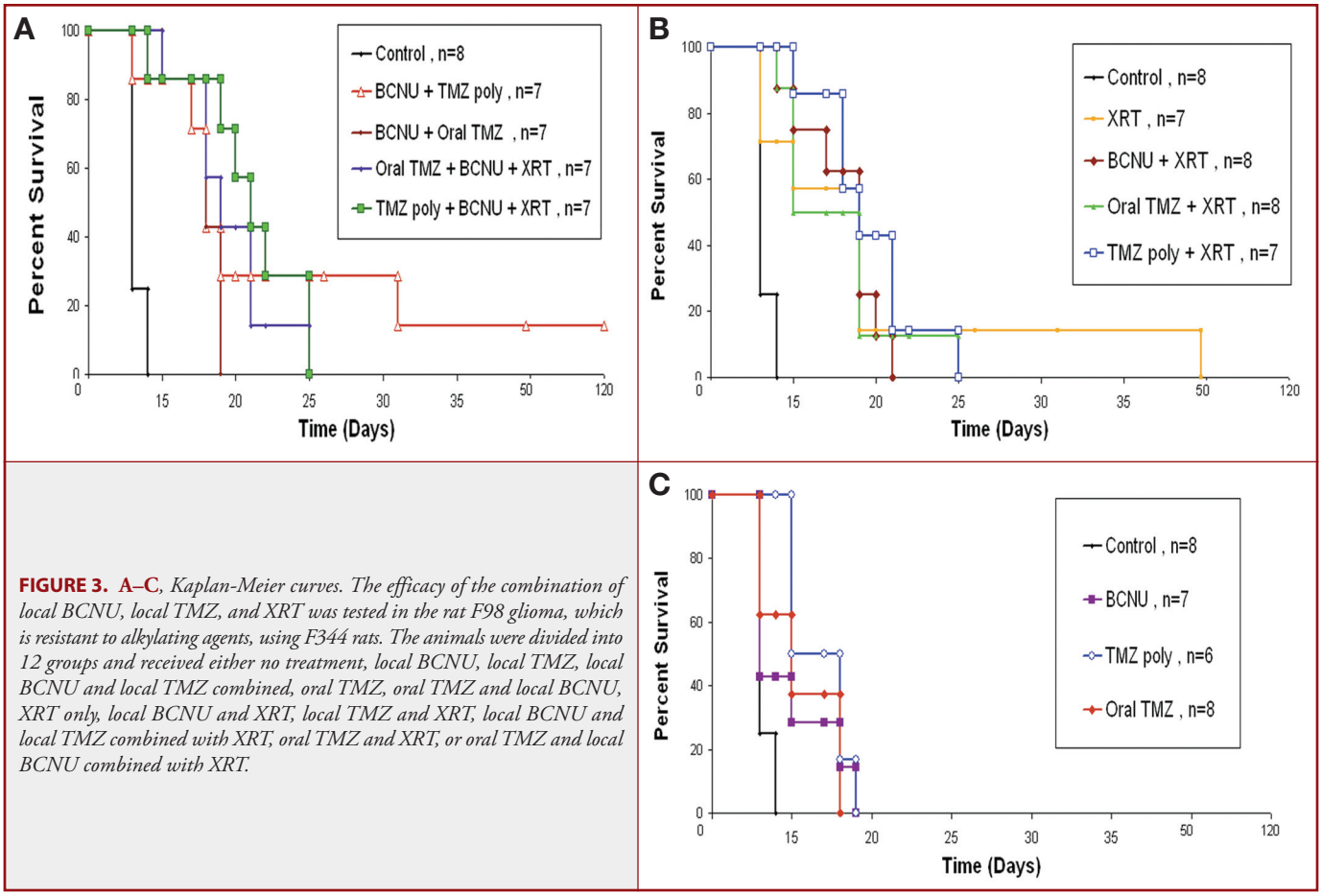

FIGURE 3. A–C, *Kaplan-Meier curves. The efficacy of the combination of local BCNU, local TMZ, and XRT was tested in the rat F98 glioma, which is resistant to alkylating agents, using F344 rats. The animals were divided into 12 groups and received either no treatment, local BCNU, local TMZ, local BCNU and local TMZ combined, oral TMZ, oral TMZ and local BCNU, XRT only, local BCNU and XRT, local TMZ and XRT, local BCNU and local TMZ combined with XRT, oral TMZ and XRT, or oral TMZ and local BCNU combined with XRT.*

experiments. The concomitant use of local BCNU, local TMZ, and XRT proved better than all other treatment modalities, with median survival not reached and 75% LTS. Although the superiority of the triple combination against the local administration of the 2 chemotherapeutic agents did not reach the level of statistical significance, it is possible that the small population studied did not give us enough data to demonstrate this. Several studies have supported a synergistic effect of BCNU and TMZ in the clinical treatment of gliomas.[1-3,18,19] In addition, there is strong evidence from preclinical trials in favor of a synergistic effect of BCNU and TMZ.[30,31] It is hypothesized that this effect is probably mediated by the combined action of BCNU and TMZ that would maximize depletion of alkylguanine-DNA alkyltransferase (AGT), a DNA repair protein found in the majority of human brain tumors,[32] and would eliminate the resistant tumor cells surrounding the resection cavity in the immediate postoperative period, during which early tumor repopulation can occur.[24] In fact, Hammond et al[33] have shown a threefold decrease in AGT activity by the combination of BCNU and TMZ. Finally, this strategy takes advantage of the synergy between TMZ, BCNU, and XRT to inhibit sublethal damage induced by each modality alone.[24]

This potential synergistic mechanism has resulted in clinical studies showing that administration of oral TMZ, local BCNU, and XRT is superior to any of the treatments separately.[1-3,18,19] Our results promote this idea even further by proving that, in a rat glioma model, local TMZ, local BCNU, and XRT are better than the triple regimen containing oral TMZ, local BCNU, and XRT ($P = .0033$). The effectiveness of combined local administration is underlined further when considering that this option provided 75% LTS and consequently did not reach median survival, whereas the oral option resulted in a median survival of 62 days with 12.5% LTS.

We have also tested the effect of BCNU and TMZ, delivered locally together with XRT, on the survival of rats bearing F98 glioma tumor, which is known[34] to be resistant to alkylating agents. As expected, our intervention with local BCNU, local TMZ, and XRT demonstrated only a modest benefit. Although the survival indices of this modality were marginally higher than those of the other treatments, there was no LTS in any of the treatment groups.

It is documented in the literature[34] that F98 cells show the highest expression of AGT among all human and rodent glial tumor cell lines. This enzyme is responsible for the main mechanism of glioma resistance to alkylating agents[35] by removing DNA adducts formed by the action of alkylating agents before cytotoxic interstrand cross-linking can occur. An experimental study showed that the administration of O^6-benzylguanine, which inhibits AGT, reversed the resistance to the action of local BCNU in the F98 rodent glioma model.[34] Meanwhile, a phase I clinical study[36] showed enhanced effectiveness of oral TMZ when combined with O^6-benzylguanine. Further research on the use of O^6-benzylguanine and other inhibitors of AGT is warranted in cell lines showing high levels of resistance to alkylating agents, which imitates the clinical situation of a chemoresistant glioma.

With regard to the clinical applicability of this regimen, further research is warranted in developing new delivery vehicles that would carry both agents simultaneously. This investigation could take advantage of the newly developed intracranially implantable microchips[37] that could allow for sequential local delivery of BCNU and TMZ.

CONCLUSIONS

The combination of intracranial TMZ polymer with intracranial BCNU polymer and radiation is safe and effective in the treatment of the experimental rodent 9L gliosarcoma and F98 glioma model. This combination significantly prolongs survival compared with either treatment alone and with the current clinical investigative treatment of oral TMZ, local BCNU, and radiation in the 9L gliosarcoma model. Likewise, the proposed triple combination significantly prolongs survival compared with either treatment alone in the F98 glioma model. Further investigation into overcoming the resistance to alkylating agents of cell lines or tumors with high expression of AGT is warranted. Nonetheless, the local delivery of TMZ from a biodegradable polymer in combination with BCNU wafers appears to be a promising approach to treating malignant brain tumors and warrants further investigation.

Disclosures

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COMMENT

The authors investigated the potential synergistic effect of local intracranial delivery of temozolomide (TMZ) and carmustine (BCNU) via biodegradable polymers and found that the combination of local delivery of TMZ and BCNU with radiation therapy prolonged survival, compared with either treatment alone. They also found that intracranial delivery of TMZ in this combination was more effective than oral delivery. These findings are interesting and may help explain why, to date, combination approaches of intracranial Gliadel (BCNU) wafers and oral TMZ have shown little advantage over either agent alone.

The fact that this in vivo study was done with 2 glioma cell lines, 1 cell line (F98) with a high expression of the chemoresistance marker alkyltransferase, is a strength of this nicely performed study. It suggests that the route of administration of chemotherapy drugs (such as TMZ) may overcome some factors that traditionally confer chemoresistance in glioblastoma. The mechanisms behind possible differential chemoresistance of glioblastoma based on the route and/or timing of drug administration warrant further study.

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