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Interstitial Docetaxel (Taxotere®): a Novel Treatment for Experimental Malignant Glioma

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Abstract

Docetaxel (Taxotere®) is a hemisynthetic, anti-cancer compound with good preclinical and clinical activity in a variety of systemic neoplasms. We tested its activity against malignant gliomas using local delivery methods. Antitumor activity was assessed *in vitro* against human and rat brain-tumor cell lines. For *in vivo* evaluation, we incorporated docetaxel into a biodegradable polymer matrix, determined associated toxicity in the rat brain, and measured efficacy at extending survival in a rat model of malignant glioma. Also, we examined the combined local delivery of docetaxel with carmustine (BCNU) against the experimental intracranial glioma. Rats bearing intracranial 9L gliosarcomas were treated 5 days after tumor implantation with various polymers (placebo, 5% docetaxel, 3.8% BCNU, or 5% docetaxel and 3.8% BCNU combination). Animals receiving docetaxel polymers (n = 15, median survival 39.1 days) had significantly improved survival over control animals (n = 12, median survival 22.5 days, $P = 0.01$). Similarly, animals receiving BCNU polymers (n = 15, median survival 39.3 days, 13.3% long-term survivors) demonstrated an increase in survival compared to the controls ($P = 0.04$). Animals receiving the combination polymers demonstrated a modest increase in survival compared to either chemotherapeutic agent alone (n = 14, median survival 54.9 days, 28.6% long-term survivors) with markedly improved survival over controls ($P = 0.003$). We conclude that locally delivered docetaxel shows promise as a novel anti-glioma therapy and that the combination of drug regimens via biodegradable polymers may be a great therapeutic benefit to patients with malignant glioma.

Keywords

Biodegradable polymers; Carmustine (BCNU); Docetaxel (Taxotere®); Interstitial chemotherapy; Malignant glioma

Introduction

Docetaxel (Taxotere®) is a novel, hemisynthetic, anti-cancer agent, structurally related to paclitaxel (Taxol), that is derived from a precursor extracted from the needles of the European

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yew tree, *Taxus baccata* [1]. Its mechanism of action is through inhibition of tubulin depolymerization resulting in microtubule aggregation and cell death [33]. Docetaxel has shown efficacy in clinical trials against a variety of human tumors [4,6,7,10,12,20,30,31], as well as having been reported to act as a potent radiosensitizer against systemic malignancies [19,21,23,24]. In two Phase II trials, docetaxel showed no significant efficacy when given intravenously to patients with malignant glioma [9,29]. However, its potential role as an interstitial treatment of malignant brain tumors used either as monotherapy or in combination with other anti-glioma chemotherapeutic agents has hitherto not been investigated.

In order to avoid systemic toxicity associated with intravenous administration of docetaxel [11,22] and achieve very high local concentration of the drug [26], we incorporated docetaxel into biodegradable polymer matrices that could be implanted intratumorally into the cranial cavity. As the polymer matrix degrades, it releases the loaded drug interstitially directly to the tumor bed, bypassing limitations imposed by the blood-brain barrier and minimizing systemic exposure to the drug. Since most gliomas recur within 2 cm of the original tumor site [14,18], this anti-tumor strategy has the potential to control both local recurrence and improve overall survival. This approach has been evaluated in Phase III clinical trials with the nitrosourea, carmustine (BCNU) and has demonstrated significant improvement in survival in patients with malignant gliomas both at recurrence and at initial presentation [3,36,40].

To test the hypothesis that docetaxel delivered interstitially via biodegradable polymers could be an effective therapy for malignant glioma, we first assessed docetaxel cytotoxicity *in vitro* against a number of rat and human glioma cell lines. We developed docetaxel-impregnated polymers and studied the *in vitro* release kinetics of the drug. *In vivo* experiments were then performed to determine the toxicity associated with the polymer implant in the rat brain and to define the maximally tolerated dose. Finally, the rodent intracranial 9L gliosarcoma model was used to examine the efficacy of this implant at extending survival in rats. In addition, we evaluated whether interstitial docetaxel used in combination with local BCNU would exhibit synergism. In this report, we show that docetaxel holds promise as an effective anti-glioma agent. Furthermore, we demonstrate combination drug regimens via biodegradable polymers may be of great therapeutic benefit to patients with malignant glioma.

Materials and Methods

In Vitro Studies

Drugs and Chemicals—Docetaxel, obtained from Rhone-Poulenc Rhorer (Collegeville, PA), was stored at −20°C. Carmustine, [3-bis (2-chloroethyl)-1-nitrosourea] (BCNU), was purchased from Bristol Laboratories (Princeton, NJ) and stored at 4°C.

Tumor Cell Lines—Rat 9L gliosarcoma cells were obtained from Dr. M. Barker (San Francisco, CA). Rat F98 glioma was obtained from R. Barth (Ohio State University, Columbus, OH). Human glioma cell lines U87 and H80 were kindly provided by Dr. O. M. Colvin (Duke University Medical Center, Durham, NC). The cells were maintained in RPMI containing 10% FCS and penicillin/streptomycin in humidified incubators at 37°C, 5% CO_2. Cultured tumor monolayers were harvested with 0.025% trypsin, counted, and re-suspended in RPMI prior to use for *in vitro* and *in vivo* studies.

Growth Inhibition Assays—Glioma cell line sensitivities to docetaxel were determined using a modified clonogenic assay [27]. Briefly, at confluence the cells were trypsinized, re-suspended at a concentration of 400 cells/2 ml of medium and plated on Falcon 6-well tissue-culture plates. After 24 hours incubation, media was removed and fresh medium was added containing docetaxel at various concentrations. The docetaxel treatment solutions were then replaced with fresh docetaxel-free media after 1 hour (Group 1), 24 hours (Group 2), or a 6-

day incubation period (Group 3- continuous exposure). All plates were then fixed and stained with a solution containing 0.63 g Coomassie brilliant blue (BioRad, Richmond, CA), 125 ml methanol, 87 ml of H_2O, and 38 ml of acetic acid. Colonies were defined as groups containing more than 50 cells and were identified and counted. Survival was calculated as the ratio between the numbers of colonies formed by the treated cells over the number of colonies formed by the untreated cells. All tests were performed in triplicate and the entire experiment was performed twice. The percentage of cell kill values for the docetaxel was plotted as a function of drug concentration. The concentration of drug necessary to produce 1 log of cell kill was interpolated (LD_{90}) from the data.

Polymer Preparation—Poly[1,3-bis(carboxyphenoxy)propane-*co*-sebacic-acid] anhydride, (CPP:SA), with a 20:80 molar ratio was supplied by Guilford Pharmaceuticals Corp. (Baltimore, MD). Docetaxel was mixed with CPP:SA and synthesized according to the method of Domb and Langer [26] to result in polymers containing 0, 3, 5, 7, 10, 20, 30, 40, or 50% loading by weight. Polymer discs (3 mm in diameter, 1 mm in height, 10 mg in weight) were made by compression molding with a stainless steel mold under light pressure. BCNU polymers, 3.8% by weight, were prepared in the same manner. Polymers containing the combination of BCNU and docetaxel were prepared by co-dissolving docetaxel (5% by weight), BCNU (3.8% by weight), and CPP:SA in methylene chloride and solvent was evaporated. The resulting powder was then pressed into 10 mg discs.

Drug-release Kinetic Studies—Docetaxel-impregnated discs were placed in 1 ml 0.1 M phosphate buffer, pH 7.4 and incubated at 37°C. The releasing medium was replaced at specific times during the incubation period and the recovered solutions were analyzed using spectroscopy (wavelength =251 nm) to determine concentration of released drug.

In Vivo Animal Studies

Animals—Male Fischer 344 rats weighing 200 to 225 g were obtained from Harlan Sprague-Dawley (Indianapolis, IN). These animals were allowed free access to water and rodent chow. They were housed and treated in accordance with the policies and principles of laboratory care of the Johns Hopkins University School of Medicine Animal Care and Use Committee.

Tumor Inoculation—Rats were anesthetized with an intraperitoneal injection of 0.6 ml of a stock solution containing ketamine hydrochloride (25 mg/kg), xylazine (2.5 mg/kg) and 14.25% ethyl alcohol in normal saline. The surgical site was shaved and prepared with 70% ethyl alcohol and iodine-containing solution. After a midline incision was made, a burr-hole 2 mm posterior to the coronal suture and 2 mm lateral to the sagittal suture was drilled. The animals were then placed in a stereotactic head-frame and tumor cells were delivered over a 3-minute period using a 26-gauge needle inserted to a depth of 3 mm. The site of injection was in the center of the burr-hole. Thus, subsequent local therapy with polymer through the burr-hole is centered on the tumor bed. The needle was then removed, and the site was irrigated with 0.9% NaCl solution and the incision was closed with staples.

Polymer Placement—After 5 days, the animals were again anesthetized and the surgical incision was opened. A single polymer was inserted through the original burr-hole and into the cortex below the level of the inner table of the parietal bone. This allowed for the polymer to be placed at the site of the previous tumor inoculation. After hemostasis was obtained, the incision was closed with staples.

Polymer Toxicity—To study toxicity in the rat brain and determine the maximal tolerated intracranial (IC) loading dose of the docetaxel-impregnated polymer implant, discs containing 0, 3, 5, 7, 10, 20, 30, 40, or 50% docetaxel, by weight, were implanted IC in non-tumor bearing

rats according to the procedure as described above. For each loading dose, 5 rats were implanted and examined daily for signs of neurotoxicity with respect to grooming, response to startle stimuli, weight loss, and gait. Animals exhibiting marked signs of toxicity were euthanized and histological analysis was performed. After 100 days, the 5 animals receiving 5% docetaxel by weight were sacrificed and their brains were removed and underwent histological analysis.

Monotherapy Efficacy Studies—Three separate experiments examining the efficacy of docetaxel-impregnated polymers against the 9L gliosarcoma were performed. For all experiments, animals underwent tumor inoculation as described above. Five days after the injection of tumor cells, animals were randomly divided into two groups. The control group (total n = 23) received an IC implantation of a blank CPP:SA polymer, which contained no drug. The animals in the treatment group (total n = 22) received a 5% docetaxel:CPP:SA polymer. All animals underwent complete autopsies with organ histopathology at the time of death.

Combination Drug Efficacy Study—All animals initially underwent tumor inoculation as described above. Treatment began on the 5^{th} post-operative day, allowing time for the tumor to become established. Group 1 (control group; n = 15) received an implantation of blank polymer. Group 2 (BCNU monotherapy; n = 15) received an implantation of 3.8% BCNU:CPP:SA polymer. Group 3 (docetaxel monotherapy; n = 15) had an implantation of 5% docetaxel:CPP:SA polymer. Group 4 (combination therapy group; n = 14) received an implantation of a polymer loaded with both 5% docetaxel and 3.8% BCNU.

Outcome and Statistical Analysis—For all efficacy studies, death was the primary endpoint. The distribution of the intervals until death was determined by the method of Kaplan and Meier. Two nonparametric statistical analyses, the Mann-Whitney *U* test and Kruskal-Wallis test, were used to compare survival between groups. Statview Version 4.51 (Abacus Concepts Inc., Berkeley, CA) software for Macintosh was used for statistical analyses.

Results

In Vitro Studies

Cytotoxicity—The effects of docetaxel on colony formation *in vitro* for two human glioma (U87, H80) and two rat glioma (9L, F98) cell lines are shown in Table 1. All of the cell lines were sensitive to docetaxel when exposed continuously to the drug for several days. As shown, the human cell lines were slightly more susceptible to docetaxel than were the rat cell lines.

The duration of exposure to the drug significantly affected docetaxel's potency *in vitro* (Table 1). After 1 hour of exposure to docetaxel, the LD_{90} values were increased by a factor of 7 (U87 cell line), 12 (H80), 13 (9L), and 13 (F98) when compared to values recorded for continuous exposure. Cells exposed for 24 hours yielded LD_{90} values ranging from only slightly higher than values for continuous exposure (U87, H80, 9L) to 2.9 times higher (F98).

Polymer Release Kinetics—Release kinetics of docetaxel from 5% docetaxel:CPP:SA polymers showed an initial burst of drug release followed by a steady release rate over a 96-hour period (Figure 1). A standard curve was used to determine the percentage of drug released over time.

In Vivo Studies

Toxicity Studies—Docetaxel-loaded polymers containing 0, 3, 5, 7, 10, 20, 30, 40, and 50% docetaxel were implanted IC in non-tumor bearing animals. The maximally tolerated dose was found to be 5% docetaxel. These animals showed no signs of systemic or neurologic toxicity.

H & E staining of the 5 rat brains receiving 5% docetaxel polymer showed a mild reactive gliosis surrounding the site of polymer placement similar to that seen previously with higher loadings of BCNU [32]. Complete autopsies with organ histopathology of these same animals showed no signs of systemic toxicity. Animals receiving 7% and 10% docetaxel exhibited non-lethal signs of toxicity such as poor grooming and severe weight loss. Animals receiving the polymers containing 20–50% drug exhibited lethal toxicity.

Efficacy of 5% Docetaxel:CPP:SA Monotherapy—Animals with established intracranial malignant glioma that received polymers with 5% docetaxel showed significantly prolonged survival over control animals in all three efficacy experiments (Table 2).

Efficacy of 5% Docetaxel/3.8% BCNU-loaded Combination CPP:SA Polymer—Table 3 shows that monotherapy with either 5% docetaxel polymer or 3.8% BCNU polymer significantly improved survival over control animals. Combination 5% docetaxel/3.8% BCNU polymer also significantly improved survival over control animals and had survival advantage over either docetaxel or BCNU monotherapy (Table 3; Figure 2).

Discussion

Historically, malignant glioma has remained refractory to conventional intravenous chemotherapy. This is due to a number of reasons including limitations imposed by the blood-brain-barrier (BBB) and blood-tumor-barrier (BTB), difficulties in systemic drug dosing and delivery, tumor cell uptake, intrinsic tumor resistance, and the heterogeneous cell makeup of human gliomas. In recent years much effort has been placed on trying to improve and increase drug delivery to the central nervous system (CNS) [17,38]. Intravascular strategies such as chemical modifications of drugs that allow passage across the BBB and BTB, intra-arterial therapy with BBB disruption, and receptor-mediated transport have all proved promising but are associated with significant systemic toxicity and have poorly defined intrathecal pharmacokinetics [17]. Injection or infusion of anti-cancer agents directly into the brain or its cavities is an alternative strategy that has gained widespread popularity. Intrathecal or intraventricular infusion, intracavitary injection, microdialysis, biodegradable polymers, and convection-enhanced delivery are all relatively recent advances that have the principle advantage of administering the drug directly at the tumor site, bypassing the BBB, and limiting systemic exposure, thereby greatly reducing systemic toxicity [2,26]. As a result of these direct delivery modalities, many anti-neoplastic agents against malignant glioma that have previously been studied intravenously can be re-investigated using direct delivery to the CNS with attention paid to drug dosing, toxicity, and distribution.

In this paper, we investigate the novel anti-cancer agent, docetaxel, and its potential role in the treatment of malignant gliomas. We demonstrate that this agent has potent anti-glioma activity using *in vitro* analysis, and has a significant survival advantage over control animals when delivered directly to the brain using biodegradable polymers in a rat malignant gliosarcoma model. Furthermore, we show that combined local delivery of two chemotherapeutic agents (docetaxel and BCNU) has improved survival over either agent used alone. This treatment strategy, of using multiple locally delivered anti-cancer agents, may represent an important improvement in controlling local tumor growth and add to the armamentarium of malignant glioma treatment.

Docetaxel is a hemisynthetic member of the taxane family of drugs, structurally related to paclitaxel [37], that exerts its anti-tumor effect by enhancing microtubule polymerization and inhibiting microtubule depolymerization [1,33]. This disruption of the normal equilibrium of tubulin function ultimately leads to cell death primarily during the G2/M-phase of the cell cycle [34]. As an inhibitor of microtubule depolymerization, docetaxel is approximately twice as

potent as paclitaxel, prompting many investigators to study its cytotoxicity in a variety of cell lines [25]. Further clinical studies have shown docetaxel to have potent anti-tumor activity against several human tumors, including ovarian [20], anthracycline-resistant breast [4,30], non-small cell lung [6,10,12] and head and neck squamous cell cancer [7,31]. However, its systemic use against CNS tumors has demonstrated no significant improvement in survival [9,29]. This is due primarily to evidence from clinical trials and animal studies that taxanes penetrate the BBB very poorly, if at all [33]. Previous clinical studies from Glantz *et al*. have demonstrated in the case of paclitaxel that drug levels were very low in the cerebrospinal fluid when given intravenously [15]. Similarly, paclitaxel does not appear in the rat brain when given systemically despite an otherwise wide biodistribution [15].

In an effort to overcome these restrictions we incorporated docetaxel into biodegradable polymers that could be directly implanted into the brain. The introduction of biodegradable polymers that release chemotherapeutic agents directly into the tumor has proved to be a promising strategy at increasing the local delivery of the drug. Nitrosoureas, including BCNU, are a class of chemotherapeutic agents that have been incorporated into biodegradable polymers and, upon intracranial implantation, have demonstrated efficacy in phase III clinical trials in patients with both recurrent and newly diagnosed malignant gliomas [3,36,40]. Importantly, in contrast to systemic administration of nitrosoureas, this treatment has not been associated with systemic side effects.

In addition to its poor penetration of the BBB, docetaxel may be suitable for local delivery against malignant glioma for several other reasons. First, we show that it is a far more potent anti-glioma agent than many other chemotherapeutic drugs including paclitaxel (Taxol) [5]. In cytotoxicity studies using a clonogenic assay, we found docetaxel to have an LD_{90} of 1000 times lower than BCNU or temozolamide [28], the two most frequently used agents against malignant gliomas. We demonstrate that log cell kill occurred at concentrations 2 to 9 times lower than paclitaxel (Taxol) for the same glioma cell lines. Also, previous studies with paclitaxel in systemic cancer lines have suggested prolonged exposure exhibits superior cytotoxicity to brief exposure whereas no such tendency is seen with docetaxel, indicating docetaxel to be a schedule-independent drug [37]. Our data contradicts this finding. We demonstrate prolonged exposure to docetaxel is far more potent than shortened exposure time. For example, continuous 6-day exposure to docetaxel yielded LD_{90} at doses 7 to 13 times lower than 1-hour exposure (Table 1). This suggests docetaxel may be an ideal candidate drug for polymeric local delivery where sustained-release over an extended period of time (1 month in the case of BCNU) is observed.

Another theoretical advantage of docetaxel as an anti-glioma agent is its known interaction with ionizing radiation [35]. Taxanes have been reported to be potent radiosensitizers in preclinical and clinical studies and have also been shown to sensitize rodent astrocytoma cells *in vitro* [8]. External beam whole brain radiation therapy remains one of the most important adjuvant therapies for patients with malignant glioma. Therefore, pursuing an agent with known radiosensitizing effect seems a logical strategy. Finally, since docetaxel exerts its anti-neoplastic effect predominantly during the M-phase of the cell cycle, it may be an excellent candidate drug to use in concert with other agents that have anti-tumor effect elsewhere in the cell cycle. BCNU, for instance, is an S-phase cytotoxic drug, therefore, theoretically the two drugs used in combination may exhibit synergism and may better overcome intrinsic tumor resistance.

We found 5% docetaxel polymers to exhibit no significant clinical or histologic toxicity. As with any direct delivery application, the potential of neurotoxicity must be addressed. Experience with other agents administered interstitially to the brain has shown a poor correlation between rodent studies and the potential of toxicity in humans. We have found

primate models to be a more accurate predictor for selecting drug dose prior to clinical trials. Notwithstanding this shortcoming in using a rat glioma model, we show that docetaxel-impregnated polymer may prove to be a safe and effective treatment against malignant glioma. *In vitro* release kinetics demonstrate sustained release of the drug from the CPP:SA polymer matrix over 96 hours (Figure 2) at concentrations that are theoretically tumoricidal. Furthermore, a previous *in vivo* biodistribution studies with paclitaxel polymers (which is structurally very similar) suggests excellent bioavailability of docetaxel when implanted intracranially [13].

We show convincingly that interstitial docetaxel using biodegradable polymers significantly improves survival over control animals in the rat 9L gliosarcoma model (Table 2). In the same rodent model, interstitial paclitaxel had a lower median survival [39]. More importantly, we demonstrate combination IC treatment with interstitial docetaxel and BCNU improves survival over either chemotherapeutic agent used alone (Table 3; Figure 2), although synergism is not observed. With an eye towards clinical application, this finding may have several important ramifications for further investigation. First, we have shown the feasibility of developing polymers loaded with multiple drugs. As combination chemotherapy agents are used successfully for systemic cancers, it seems a natural extension that a similar strategy may prove beneficial in the CNS. This may be especially helpful when two drugs with different anti-neoplastic actions, such as docetaxel and BCNU, are used. Perhaps even more chemotherapeutic agents could be effectively delivered from a single polymer? Second, we have shown that combination treatment with different anti-tumor agents improves survival over monotherapy in experimental glioma models. Although synergy with combination therapy was not found in this study, it is possible that synergy may be exhibited with manipulation of the drug loading doses or of the experimental conditions.

This study provides the basis for the application of interstitial docetaxel as a novel anti-glioma agent [41]. In addition, it provides the first description of a multi-drug biodegradable polymer (docetaxel and BCNU), which demonstrates, improved efficacy over monotherapy. The potential of enhancing cytotoxicity with chemotherapeutic agents previously unable to penetrate the CNS and of using combination drug regimens via biodegradable polymers may prove to be of greater therapeutic benefit to patients with malignant glioma.

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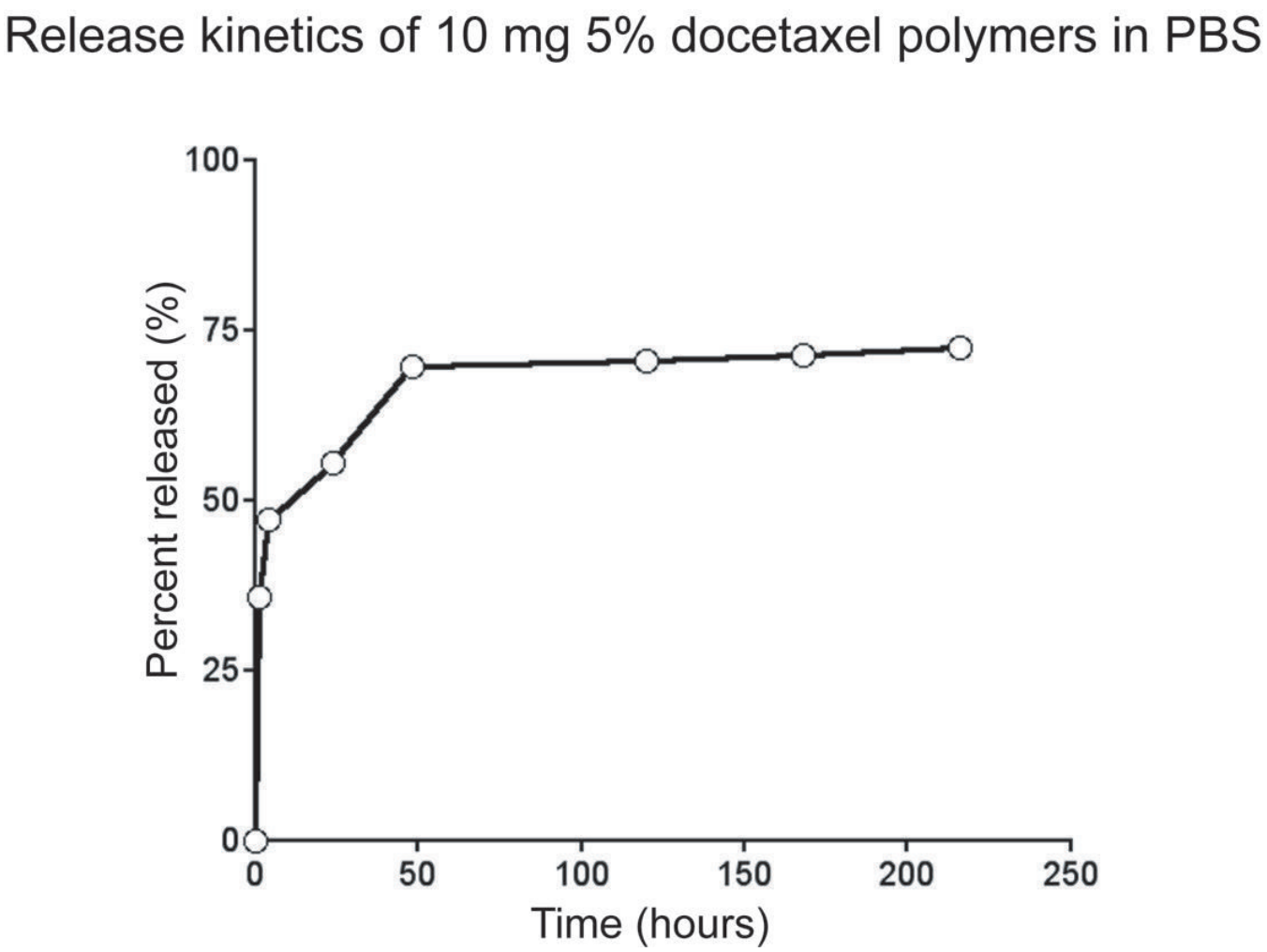

Figure 1.
Docetaxel (Taxotere®) release kinetics from poly[1,3-bis(carboxyphenoxy)propane-*co*-sebacic-acid] anhydride (CPP:SA) polymers at 5% loading by weight as determined by spectroscopy (λ = 251 nm).

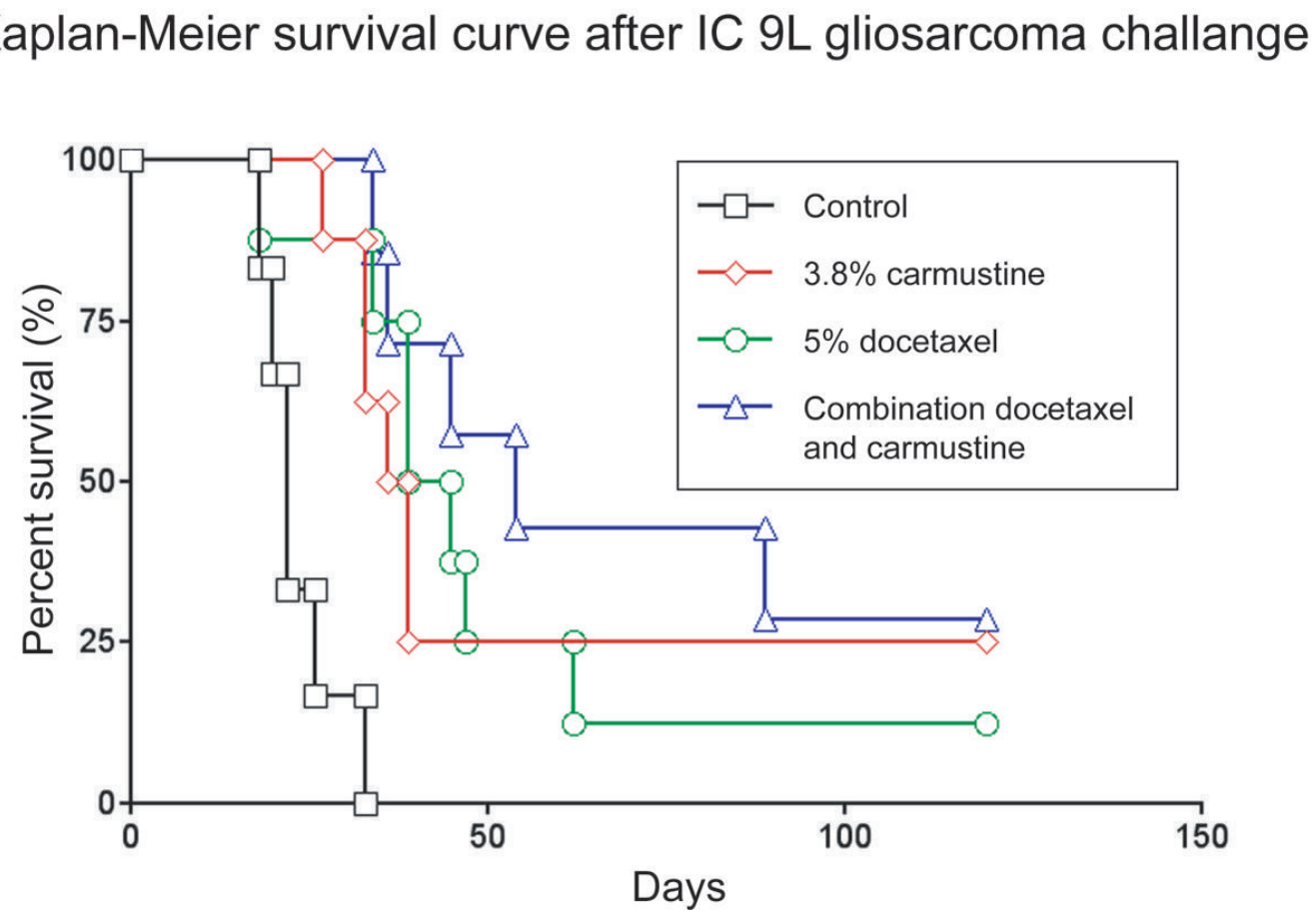

Figure 2.
Kaplan-Meier survival curve showing survival for animals after intracranial 9L gliosarcoma challenge treated with empty polymers (control), 3.8% carmustine (BCNU) polymers, 5% docetaxel (Taxotere®) polymers, or combination (5% docetaxel and 3.8% carmustine) polymers.

TABLE 1

Growth inhibition (LD_{90}) (nM) derived from clonogenic assay for rat glioma cell lines F98 and 9L and human glioma cell lines H80 and U87 with docetaxel

	LD_{90} concentration (nM)			
	Rat glioma cell lines		Human glioma cell lines	
Docetaxel treatment duration	**F98**	**9L**	**H80**	**U87**
1 hour	96	59	48	30
24 hours	21	4.8	4.7	4.7
6 days-continuous exposure	7.2	4.5	4.0	4.3

TABLE 2

Docetaxel-loaded polymers against established 9L malignant glioma efficacy studies[a]

	Median survival (days)	Range (days)	Long-term survivors	*P*-value[b]
Experiment #1				
Placebo polymers (n = 7)	17.1	15–21		
5% docetaxel:CPP:SA (n = 6)	42.8	21–120	16.7%	0.033
Experiment #2				
Placebo polymers (n = 6)	28.5	18–49		
5% docetaxel:CPP:SA (n = 6)	58.1	22–120	16.7%	0.05
Experiment #3				
Placebo polymers (n = 10)	18.4	15–25		
5% docetaxel:CPP:SA (n = 10)	43.8	24–120	10%	0.002

[a] CPP:SA, Poly[1,3-bis(carboxyphenoxy)propane-*co*-sebacic-acid] anhydride

[b] Based on nonparametric Kruskal-Wallis statistical analyses

TABLE 3

Efficacy of docetaxel-loaded polymers or BCNU-loaded polymers used alone (monotherapy) or in combination

Groups	Median survival (days)	Range (days)	Long-term survivors	*P*-value[b]
Placebo polymers (control) (n = 12)	22.5	18–33	0%	--
3.8% BCNU alone (n = 15)	39.3	21–120	13.33%	0.04 vs. control
5% docetaxel alone (n = 15)	39.1	18–120	6.67%	0.01 vs. control
Combination of 5% docetaxel and 3.8% BCNU (n = 14)	54.9	18–120	28.6%	0.003 vs. control 0.14 vs. 5% docetaxel 0.18 vs. 3.8% BCNU

[a] BCNU, carmustine

[b] Based on nonparametric Kruskal-Wallis statistical analyses