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Interstitial chemotherapy of experimental brain tumors: comparison of intratumoral injection versus polymeric controlled release

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Abstract

Interstitial chemotherapy with controlled release polymers is a clinical adjunct in the management of malignant gliomas. The need for polymer to release the chemotherapeutic drug rather than simply injecting the drug into the tumor warrants further investigation. Therefore, we compared the effects of direct intralesional injection of carmustine (BCNU) and 4-hydroperoxycyclophosphamide (4HC) into the rat brain tumor bed with those from the same agents delivered via controlled release polymers implanted intracranially. Treatment was initiated on the fifth day after intracranial implantation of 9L gliosarcoma into male rats; two doses of each drug were injected intratumorally, representing either the amount of drug typically released *in vivo* from polymer during the first 24 h, or the maximal drug loaded on each polymer. Control rats were treated with empty polymers. We found that the median lifespan was extended in the groups of rats treated with intratumoral injection of BCNU (23% and 36% for 1 mg and 2 mg doses), and 271% with BCNU-impregnated polymer. Similar results were found with intratumoral 4HC (21% and 36% for 0.1 mg and 2 mg injection doses), and 121% with 4HC-impregnated polymer. Overall survival after intraneoplastic injections, however, was not statistically significantly different from that of control rats ($p > 0.05$). Furthermore, improvement in survival was not consistent, and some animals subjected to 4HC injection died early in the course of treatment. Polymeric treatment resulted in statistically significant prolongation of survival, compared to control rats ($p < 0.001$ for both BCNU and 4HC). We conclude that direct intralesional injection of BCNU and 4HC is less effective than controlled release via polymers for the treatment of 9L gliosarcoma in the rat model.

Introduction

The failure of systemic chemotherapy to improve survival of brain tumor patients, unlike successes against neoplastic processes in other organ systems, has been attributed to the difficulty of transit from the intravascular space across the blood-brain barrier, which limits the capacity to attain therapeutic intracerebral drug levels without large increases in dosing and severe consequences of systemic toxicity [1].

To circumvent some of these adverse conditions, several different approaches have been devised to improve the exposure of intracerebral neoplastic cells to chemotherapeutic agents that are effective *in vitro* against brain tumors. Malignant gliomas tend to recur locally [2]. Therefore, a promising approach to improving drug delivery has been interstitial chemotherapy. Two methods have been used [3]. First, drug may simply be injected as a single dose intratumorally or in the cavity left after tumor resection, or injected chronically via implanted

catheters and/or pump systems. This method has been studied in the laboratory with varied results [3–15] and no proven clinical benefit was found [3, 14, 16–22]. A second method involves the slow, sustained delivery of high local drug concentrations over several days by the use of drug-impregnated, bioerodible polymers.

The advantages of the latter method of adjuvant interstitial chemotherapy of malignant gliomas with polymers have previously been established in the laboratory [23, 24], as well as in clinical trials [25]. Survival is prolonged in both rats and humans with malignant gliomas. Furthermore, release kinetic studies revealed controlled delivery of high local intracerebral drug concentrations, over approximately 3 weeks, and minimal systemic spillage of cytotoxic chemotherapeutic agents [26, 27].

To determine whether polymeric delivery is better than simply bathing the tumor bed with the chemotherapeutic agent, we used the rat 9L glioma model to evaluate the two modes of interstitial chemotherapy under the same experimental conditions. We chose to investigate BCNU and the preactivated cyclophosphamide derivative, 4-hydroperoxycyclophosphamide (4HC), because we have previously shown that these two cytotoxic agents, delivered via polymers, are effective against the 9L glioma [23, 24, 28–30].

Materials and methods

Experimental design

For all experiments, the 9L gliosarcoma was implanted intracranially into groups of rats as described below. Five days after implantation, the wound was reopened and treatment was initiated by either intratumoral injection of drug or placement of drug-impregnated polymer into the tumor bed. Control rats were treated with empty polymers, and a separate experiment tested the effect of injection of the drug vehicle.

Animals

The 117 male Fischer 344 rats used in the experiments weighed 200–300 g and were supplied by Harlan Sprague-Dawley, Inc. (Indianapolis, IN).

Anesthesia

A 100 ml stock solution for anesthesia consisted of a filter-sterilized mixture of 25 ml of ketamine, 2.5 ml of xylazine, 14.5 ml of 100% ethanol, and 58 ml of normal saline. Adequate anesthesia was provided by 0.5–0.8 ml of the solution for rats with weights in the range used in the present studies.

Drug preparation

4HC was generously provided by Dr. O. Michael Colvin of the Johns Hopkins Oncology Center (Baltimore, MD). 4HC (20 mg) was dissolved in 2 ml of sterile normal saline. BCNU (100 mg) was dissolved in 1 ml of ethanol diluent and mixed with 9 ml of sterile water. For the 2 mg, 1 mg, and 0.1 mg intratumoral injections, 0.2 ml, 0.1 ml, and 0.01 ml of the appropriate drug solution were used, respectively. The injection was delivered via a 30 gauge needle attached to a tuberculin syringe.

Polymer preparation

4HC was incorporated into FAD:SA (fatty acid dimer-sebacic acid) via melt casting [31]. The polymer and appropriate drug were heated to 70° C and mixed thoroughly by use of a spatula. The product was pressed between two metal sheets to the appropriate thickness (1 mm). The 2-mm discs were then punched out with a cork borer. PCPP:SA polymer(poly[bis(carboxyphenoxy-propane)-sebacic acid]) was fabricated by melt-polycondensation of the mixed anhydrides of dicarboxylic acids and acetic acid, followed by compression molding to incorporate drug, as described elsewhere [24, 32, 33]. Polymer discs were sterilized under UV light for 1–2 h prior to intracranial implantation. Each

drug-impregnated polymer weighed 10 mg and contained 2 mg of the drug.

Tumor preparation

The 9L gliosarcoma was propagated in the flanks of male Fischer 344 rats. Tumor grown to about 1–2 cm in diameter was enucleated from the flank of the anesthetized rat under sterile conditions and cut into pieces measuring 1 mm^3 in volume.

Tumor implantation

After anesthesia, each rat's head was shaved and prepared with 70% ethanol and povidone-iodine solutions. A midline sagittal incision was made down to the skull. The scalp and pericranium were fully retracted. A 3-mm left parasagittal burr hole was created 5 mm posterior to the bregma and 3 mm from the midline. By microsurgical techniques, brain tissue was carefully aspirated until the brainstem vasculature was visualized under the operating microscope. Hemostasis was attained via use of the Weck-Cel surgical spear (Edward Weck & Company, Inc., Research Triangle Park, NC). The tumor piece was inserted in the cavity and the skin was closed with staples. The rats were returned 4–4 to a cage and allowed free access to both water and Certified Rodent Chow (Agway, Inc., Syracuse, N.Y.).

Treatment protocol

Treatment was initiated on the fifth day after tumor implantation. The rats were anesthetized and their skin was prepared with 70% ethanol and povidone-iodine, as described for tumor implantation, and the wound was reopened. For polymer treatments, a niche was created in the middle of the tumor bed through an incision made with a Weck microsurgical knife (Edward Weck & Company, Inc., Research Triangle Park, NC). The polymer was placed in the cavity by using sterile jeweller's forceps. For intratumoral injections, the appropriate dose was injected at various points in the tumor bed, with care taken to prevent drug spillage. The skin was lifted up and stapled closed so as to trap any excess volume of injected drug that might otherwise have percolated outside the tumor bed.

Intratumoral 4HC injection

Sixty-six rats were randomized to the four groups defined in Table 1A. The 2-mg intratumoral injec-

Table 1. Experimental protocol and survival statistics for the three experiments in which groups of rats were treated with intraneoplastic injection of 4HC, BCNU or drug vehicle, or drug polymer implants, as described under Materials and methods

Treatment group	Number of rats	Median survival days (range)	Lifespan extension	Statistical significance (vs control)
A. 4HC Treatment				
Empty FAD/SA polymer	19	14 (10–22)		
Intratumoral, 0.1 mg	9	17 (12–27)	21%	NS
Intratumoral, 2 mg	19	19 (7–> 120)	36%	NS
20% 4HC/FAD:SA	19	31 (16–> 120)	121%	$p < 0.001$
B. BCNU Treatment				
Empty PCPP:SA polymer	10	15.5 (11–25)		
Intratumoral, 1 mg	6	19 (14–34)	23%	NS
Intratumoral, 2 mg	10	21 (14–> 120)	36%	NS
20% BCNU/PCPP:SA	10	57.5 (18–> 120)	271%	$p < 0.001$
C. BCNU Vehicle				
No treatment	10	17 (11–23)		
Intratumoral, 10% ethanol/water	5	13 (12–16)	–23.5%	$p = 0.07$

NS, not significant.

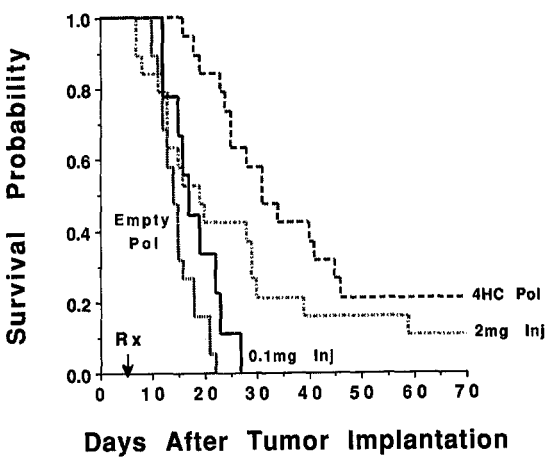

Fig. 1. Survival of four groups of rats randomly assigned for treatment with empty FAD:SA (1:1, wt/wt) copolymer (n = 19), intratumoral injection of 0.1 mg 4HC (n = 9), intratumoral injection of 2 mg 4HC (n = 19), or intratumoral placement of a 10 mg disc of 4HC-loaded FAD:SA copolymer (n = 19). All rats were treated on the fifth day following tectal implantation of pieces of 9L gliosarcoma. Pol, polymer; inj, injection.

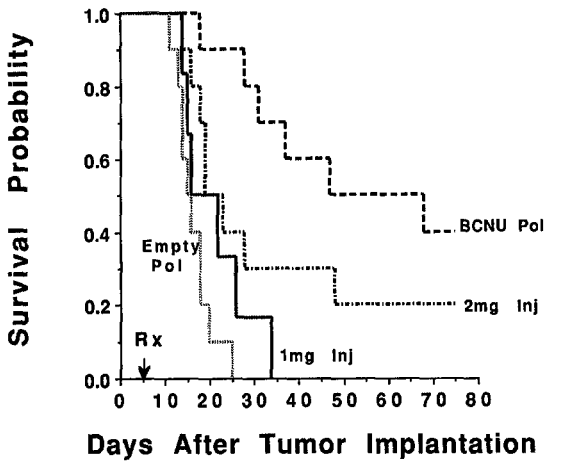

Fig. 2. Survival of four groups of rats randomly assigned for treatment with empty PCPP:SA (20:80, wt/wt) copolymer (n = 10), intratumoral injection of 1 mg BCNU (n = 6), intratumoral injection of 2 mg BCNU (n = 10), or intratumoral placement of a 10 mg disc of BCNU-loaded PCPP:SA copolymer (n = 10). All rats were treated on the fifth day following tectal implantation of pieces of 9L gliosarcoma.

tion dose was chosen because it was equivalent to the total drug contained in each 10 mg of 20% 4HC polymer disc used in the polymer treatment group. Therefore, it represented a bolus injection of the maximal amount of drug that could be delivered by the polymer over the estimated 3–4 weeks of sustained, controlled release. The 0.1-mg intratumoral injection, on the other hand, represented the total drug released in the first hour into an infinite sink as determined in kinetic studies *in vitro* [26].

Intratumoral BCNU injection

Thirty-six rats were randomized to the four treatment groups described in Table 1B. Again, the 1-mg dose was equivalent to the amount of drug released *in vivo* during the first hour after intracranial (ic) polymer implantation using ethylene vinyl acetate co-polymer [27], while 2 mg was the maximum drug potentially available from a 10-mg disc of 20% BCNU/PCPP:SA.

Intratumoral injection of BCNU vehicle

To control for possible extraneous effects of intratumoral injection of the chemotherapeutic agents, such as the mechanical disruption of the tumor bed and interference with tumor progression, we compared survival of untreated rats with that of rats treated with intratumoral injection of drug vehicle, a solution of 10% ethanol in sterile water. Fifteen rats were randomized to two groups and treated as described in Table 1C, 5 days after intracerebral 9L tumor implantation.

Statistical analysis

Survival after each treatment was analyzed with Kaplan-Meier plots. Statistical significance of differences in survival of any one or more groups from the others was estimated with the non-parametric Kruskal-Wallis statistic by using StatView software (Abacus Concepts, Inc., Berkeley CA). The non-parametric variable was the day the rat died following tumor implantation. When the null hypothesis of no intergroup difference was rejectable, we used the non-parametric modification of the Neuman-Keuls a posteriori procedure to evaluate pair-wise differences between groups. To analyze the results of the control experiment comparing effects of intratumoral injection of BCNU drug vehicle versus those of no treatment, the Mann-Whitney statistic was utilized.

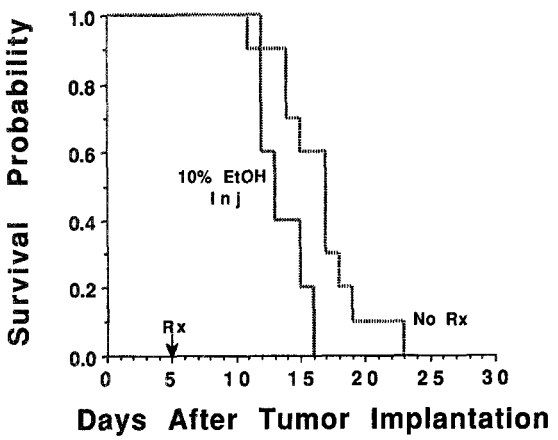

Fig. 3. Survival of two groups of rats randomly assigned for treatment with intraneoplastic injection of 0.2 ml BCNU drug constitution vehicle (10% ethanol, n = 5) or receiving no treatment (n = 10). All rats were treated 5 days after tectal implantation of pieces of 9L gliosarcoma.

Results

Intratumoral 4HC injection and polymer treatment

Rats treated with a single injection of the 4HC directly into the bed of an established tumor lived longer than control rats (Fig. 1, Table 1A). In the group treated with 2 mg injection, 10–20% of the rats were long-term (> 80 days) survivors, there was 36% average extension in lifespan, and a median survival of 19 days. However, this improved survival was balanced by frequent early deaths; e.g. 3/19 rats in this group died (days 7–8) before the first control rat (day 10) and 7/19 died in less than 14 days, the control median survival. These early deaths appeared to reflect drug toxicity of direct bolus injection. For the 0.1 mg intratumoral injection group, median survival was 17 days and increase in lifespan, 21%. There were no long-term survivors.

In contrast, rats treated with 20% 4HC polymer had longer median survival (31 days), greater extension in lifespan (121%), and a higher percentage of long term survivors (20–30%). The earliest death occurred on day 16. Thus, by releasing 4HC in a polymer, the drug benefits are maximal while toxicity is minimized.

Intratumoral BCNU injection and polymer treatment

Rats injected intratumorally with 1 mg of BCNU survived longer (median 19 days, lifespan extension 23%) than did control rats treated with empty polymers (15.5 days), whereas the group treated with 2-mg intratumoral injections lived longer (median 21 days, lifespan increase 36%) and had more long-term survivors (20%) than either control or 1-mg injection groups. The group treated with 20% BCNU-loaded PCPP:SA polymers, however, had the longest survival, with median survival 57.5 days, corresponding to a 271% increase in lifespan, and 40% long-term (> 120 days) survivors.

Injection of BCNU drug vehicle

Compared to control rats that received no treatment after tumor implantation, the group of rats treated with BCNU drug vehicle (0.2 ml of a 10% solution of ethanol in sterile water) had median survival of 13 days. This represented a lifespan diminution of 23.5%. However, the difference in survival was not statistically significant ($p = 0.07$).

Discussion

In the present study, we have demonstrated that injection of the chemotherapeutic drugs BCNU or 4HC into the tumor bed of an established rat intracranial 9L glioma provided dose-dependent improvement in survival. However, with the same total dose of BCNU or 4HC, and under the same experimental conditions, polymeric controlled release resulted in longer survival and less toxicity.

When median survival of rats in the various treatment groups was normalized to that of control rats by the use of the percent lifespan extension, the results for the two drugs BCNU and 4HC were remarkably similar (Fig. 4). When the two drugs were introduced by intratumoral injection at concentrations corresponding to the amount of drug released over the first hour after intracranial implantation of drug-loaded polymer, there was little more than 20% extension of lifespan. Even if the dose of drug injected were increased to the maximal amount of drug loaded on the polymer, lifespan was only ex-

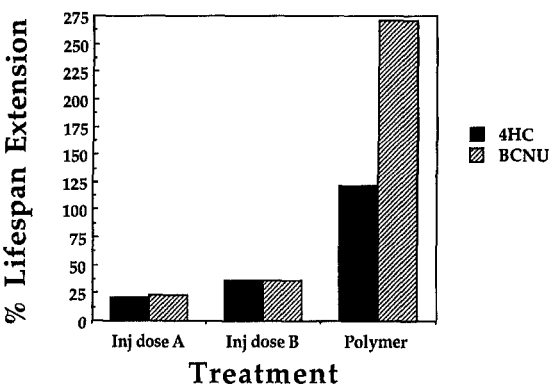

Fig. 4. Relationship between drug dose, treatment mode, and lifespan extension. Established intracranial tumor was treated with intralesional placement of 4HC- or BCNU-impregnated polymer, or injection of one of two doses of either agent. Injection dose A was equivalent to the amount of drug released *in situ* by either agent during the first hour after intracranial implantation of polymer, as estimated from release kinetic studies. Injection dose B was the total drug loaded on each polymer. Control rats were treated with empty polymers. Note the similarity in the magnitude of the normalized lifespan extensions produced by each agent at each level of intratumoral injection drug dose.

tended by 36%. These insignificant extensions are in marked contrast to the statistically significant increases in lifespan achieved by polymeric delivery of 4HC and BCNU (121% and 271%, respectively).

The effects of intratumoral injection in this model were variable, unpredictable, and not statistically significantly different from those in control rats. Depending on the dose, direct injection may also produce unpredictable toxic effects and lead to early death.

Grossman *et al.* [34, 35] previously reported on cerebral drug distribution after intracerebral injection and polymer implantation in the rabbit brain. [^{3}H]BCNU-impregnated polymer or intracerebral injection of a similar dose of labelled drug was utilized to evaluate *in vivo* kinetics. They found that, 72 h after polymeric treatment, 40% of the brain section transecting the polymer was exposed to drug in adequate concentrations for tumoricidal activity against glioblastomas. A decline to 15% exposure did not occur until 180–350 h after polymeric treatments. In contrast, only 15% of the brain surface was exposed to drug after 24 h in the rabbits treated by injection of the drug.

Zeller *et al.* [7] treated a subcutaneous model of the rat G616 glioma with methotrexate-impregnated, biodegradable polylactide polymer rods (1.5 mg methotrexate/8 mg of polymer rod) and compared the degree of reduction of tumor volume against that achieved in three other groups of rats treated with intraneoplastic injections of methotrexate (4 mg/kg, weekly, three doses), systemic injections (4 mg/kg weekly, four doses), and non-treated control rats. They reported decreased tumor volumes that were more pronounced with either mode of interstitial treatment than those achieved with systemic methotrexate. However, they could not demonstrate an advantage of delivering the drug with polylactide over intraneoplastic injections.

In another study of the rat 9L gliosarcoma model, Kimler *et al.* [6] demonstrated that both bleomycin and AZQ, delivered intratumorally as single injections, provided statistically significant, dose-dependent prolongation of median survival, while intravenous and intraperitoneal treatments with AZQ did not improve survival even at doses that were at the toxicity threshold. Tator [9] reported that a single intraneoplastic dose of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) extended median survival and produced a significant number of long-term survivors in mice harboring intracranial ependymoblastoma.

Furthermore, many laboratory-based studies have utilized multiple, chronic intraneoplastic injections via implanted catheters and/or pump systems, generally with promising results in animal models [3, 14]. Nevertheless, the expected benefits have not been evident clinically. Agents tested clinically have included BCNU, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU), CCNU, methyl 6-[3-(2-chloroethyl-3-nitrosoureido]-6-deoxy-alpha-D-glucopyranoside (MCNU), cis-platinum, methotrexate, bleomycin, and adriamycin [3]. The use of multiple intratumoral injections, as opposed to the single-dose injection used in the present study, should lead to increased concentrations of the administered chemotherapeutic agent and consequent increase in cytotoxic activity. However, a concomitant increased risk of infection and probable elevation in intracranial pressure may counteract the presumed benefits

of increased exposure to drug. A major advantage of biodegradable polymeric delivery is the ability to increase the exposure of neoplastic cells to chemotherapeutic agents without the risks associated with repeated intracranial invasion or permanent implants.

We conclude that polymeric drug delivery has advantages over intratumoral injection as a mode of interstitial intracranial drug administration. The improvement in survival achieved with polymeric delivery reflects exposure of the brain to a much smaller actual drug dose, but over a prolonged period of time, as has previously been established in release kinetics studies *in vitro* and *in vivo* [26, 27].

The efficacy of local delivery of drugs via polymers may lead to a new approach for treating brain tumors. In the future, a biopsy specimen of a tumor may first be characterized in the laboratory and its sensitivity determined, so that at the time of definitive resection, the appropriate polymer-delivered therapy could be administered. After surgery, adjunctive irradiation or systemic therapy could begin and the patient would be followed for recurrence of tumor. When this occurs, the treatment cycle could be repeated by biopsy of the tumor again, and following the original steps. While speculative at this time, the present study, which clearly shows that polymeric drug delivery has significant advantages over intratumoral injection for both BCNU and 4HC, provides definitive reasons for continuing to explore ways to maximize the advantages of polymeric delivery for antitumor agents.

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