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Laboratory Investigation

Local delivery of interleukin-2 and adriamycin is synergistic in the treatment of experimental malignant glioma

Wesley Hsu[1], Maciej S. Lesniak[2], Betty Tyler[1] and Henry Brem[1]
[1]*Departments of Neurosurgery and Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD;*
[2]*Division of Neurosurgery, The University of Chicago Pritzker School of Medicine, Chicago, IL*

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Summary

Introduction: Local delivery of adriamycin (ADR) via biodegradable polymers has been shown to improve survival in rats challenged intracranially with 9L gliosarcoma. Likewise, local delivery of interleukin-2 (IL-2) has been shown to extend survival in experimental brain tumor models. In the current study, we hypothesized that local delivery of ADR and IL-2 might act synergistically against experimental intracranial glioma.

Methods: Polyanhydride polymers (PCPP-SA) containing 5% ADR by weight were prepared using the mix-melt method. IL-2 polymer microspheres (IL-2 MS) were produced via the complex coacervation of gelatin and chondroitin sulfate in the presence of IL-2. Sixty male Fisher 344 rats received an intracranial challenge with a lethal dose of 9L gliosarcoma cells. In addition, a group of rats were injected with either IL-2 MS or empty microspheres. Five days later they received ADR or blank polymer. There were a total of four treatment groups: (1) empty microspheres, blank polymer; (2) empty microspheres, ADR polymer; (3) IL-2 MS, blank polymer; and (4) IL-2 MS, ADR polymer.

Results: Compared to control animals treated with empty microspheres and blank polymer, animals receiving empty microspheres and ADR polymer ($P < 0.0004$), IL-2 MS and blank polymer ($P < 0.0005$), and IL-2 MS combined with ADR polymer ($P < 0.0000002$) all showed statistically significant improvement in survival. In addition, animals receiving the IL-2/ADR combination had significantly extended survival compared to either ADR or IL-2 alone ($P < 0.000003$ and $P < 0.0004$, respectively).

Conclusions: Both ADR and IL-2, when delivered locally, are effective monotherapeutic agents against experimental intracranial gliosarcoma. The combination ADR and IL-2 therapy is more effective than either agent alone.

Introduction

Research in the field of biodegradable polymers and controlled drug delivery has led an improvement in the treatment of patients with malignant gliomas [1–5]. Local delivery of chemotherapeutic agents has the advantage of bypassing the blood brain barrier, thereby allowing a high concentration of a drug at the site of interest. This strategy also limits the toxicity associated with the systemic delivery of chemotherapeutic drugs. Indeed, BCNU-loaded biodegradable polymers (Gliadel®) have been shown efficacious in the treatment of malignant gliomas. Recent phase III clinical trials have confirmed an increased survival of patients receiving BCNU-loaded biodegradable polymers compared to placebo-treated patients at initial presentation [3,5] as well as at recurrence [2], with minimal, if any, reported side effects.

Despite the success shown with Gliadel, there is continued interest in developing new strategies to improve the efficacy of polymer-delivered chemotherapy against malignant gliomas. One important area of research is to test drugs other than BCNU for use in biodegradable polymers. Adriamycin (ADR), an anthracycline antibiotic currently used to treat a wide variety of cancers, represents one potential candidate drug. ADR blocks DNA and RNA synthesis by inhibiting topoisomerase II. ADR has potent antiglioma activity *in vitro* [6]. However, the efficacy of systemic ADR against intracranial malignancies has been limited. The blood-brain barrier, through tightly knit endothelial cells with possible contributions from p-glycoprotein [7], prevents a therapeutic concentration of ADR from reaching the CNS. The low lipophilicity and high molecular weight of ADR further prevent the drug's ability to cross the blood-brain barrier. Local delivery techniques can bypass the blood-brain barrier, and we have previously shown that ADR-loaded biodegradable polymers improve survival in an animal model of intracranial glioma (Lesniak et al., submitted).

In addition to finding new chemotherapeutic agents, there has been increasing interest in utilizing cytokines to enhance the local immune response directed against tumor cells. One such cytokine is interleukin-2 (IL-2), which is known to enhance T cell growth and activate T cells, monocytes, and natural killer cells [8]. IL-2 has been shown to promote inflammation in response to tumor antigens [9] and may bypass T-helper function in the generation of an antitumor response [10]. Because

IL-2 acts in a paracrine fashion, high concentrations of IL-2 localized near the tumor are necessary to promote an immune response [9]. A high systemic dose of IL-2 would be necessary to achieve an adequate concentration in the CNS, however, such doses have been associated with considerable toxicity [11]. As a result, IL-2 is an interesting candidate for local delivery strategies. Cells genetically engineered to release IL-2 or microspheres impregnated with IL-2 have demonstrated a significant antitumor response in experimental brain tumor models [12–16].

The success of local chemotherapeutic drug delivery or local immunotherapy has paved way for multimodal therapy. The rationale for combining these strategies is the possibility that cytotoxic drugs increase the immunogenicity of tumor cells [17,18]. We have shown that the co-administration of genetically engineered tumor cells that produce IL-2 with biodegradable polymers loaded with BCNU or carboplatin extends the survival of mice challenged with a lethal intracranial dose of glioma [19]. Recently, we have shown that IL-2 MS in combination with BCNU-loaded biodegradable polymer exhibit synergy in an animal glioma model [20]. In this paper, we report the initial animal study of the combined therapeutic efficacy of paracrine immunotherapy with IL-2 MS and interstitial chemotherapy with ADR-loaded polymer wafers for the treatment of intracranial 9L gliosarcoma.

Materials and methods

Study design

IL-2 was encapsulated into injectable microspheres composed of a biodegradable gelatin:chondroitin sulfate polymer (IL-2 MS) as previously described [16]. Release of IL-2 from microspheres *in vitro* was determined. After stereotactically implanting 9L glioma cells into the left parietal lobes of Fisher 344 rats, we tested the efficacy of local paracrine intracranial immunotherapy using IL-2 MS with and without subsequent locally delivered ADR.

Animals

Sixty 10-week-old, female Fisher 344 rats weighing 180 to 220 g were used as indicated below. These animals were purchased from Charles River Laboratories (Wilmington, MA), kept in standard animal facilities with 3 or 4 rats per cage, and given free access to rat chow and water. They were housed in accordance with the policies and principles of laboratory care of the Johns Hopkins University School of Medicine Animal Care and Use Committee.

Tumor line

The 9L wild type gliosarcoma cell lines (kindly provided by Dr. K. Plate, Freiburg University, Dept. Neuropathology, Germany) were maintained in tissue culture in Dulbecco's minimum essential medium with 10% fetal bovine serum, streptomycin (80.5 μg/ml), penicillin (base; 80.5 units/ml), and 1% L-glutamine (all products from GIBCO laboratories, Grand Island, NY). Cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C. The cells were grown to confluence, detached with 0.25% trypsin in Dulbecco's phosphate-buffered saline, and resuspended in medium.

IL-2 microspheres

Interleukin-2 obtained from Chiron Corp. (Emeryville, CA), was encapsulated into polymeric matrices by the complex coacervation of gelatin (Atlantic Gelatin, Woburn, MA) and chondroitin 6-sulfate (Sigma Chemical Corp, St. Louis, MO) in the presence of IL-2. A more detailed description of the encapsulation process is discussed elsewhere [16]. Briefly, 3 ml of a 4% gelatin solution in distilled water at 37 °C was mixed with 1 mg of lyophilized IL-2 dissolved in 3 ml of 0.2% chondroitin sulfate in phosphate buffered saline at room temperature. Coacervation was achieved by the addition of the gelatin solution to a rapidly mixed IL-2/chondroitin sulfate solution. Microspheres were then cross-linked with glutaraldehyde for 20 min and then poured into 10 ml of a 0.1 M aqueous glycine solution to stop the cross-linking reaction and quench the excess aldehyde groups. Cross-linked microspheres were collected by centrifugation and washed with phosphate buffered saline. Placebo microspheres were prepared identically but without IL-2.

Polymer preparation

pCPP:SA, with a 20 : 80 molar ratio, was supplied by Guilford Pharmaceuticals Corp (Baltimore, MD). pCPP:SA polymers containing ADR (Sigma, St. Louis, MO) at 5% loading by weight were prepared as described previously [21,22]. The polymers for implantation were pressed into disc shapes weighing 10 mg each (1.5 mm in diameter, 0.5 mm in height).

Intracerebral implantation of microspheres and tumor cells

Rats were anesthetized with an intraperitoneal injection of a stock solution containing 25 mg/ml ketamine hydrochloride, 2.5 mg/ml xylazine, and 14.25% ethanol that was diluted with 0.9% NaCl solution. The surgical site was shaved and prepared with 70% ethanol and iodine-containing solution. After a midline incision, a 3 mm burr-hole was made 1 mm posterior to the coronal suture and 3 mm lateral to the sagittal suture. The animals were then placed in a stereotactic frame, and a mixture of the tumor cells and microspheres were delivered over 15 min by a 26-gauge needle inserted to a depth of 3.0 mm at the center of the burr hole. The needle was then removed, the site was irrigated with 0.9% NaCl solution, and the wound was closed with surgical clips.

Polymer implantation

The surgical incision used for inoculating the tumor was reopened 5 days later, and a single polymer was inserted in the cortex entirely below the level of the inner table of the parietal bone. After hemostasis was achieved, the placement site was irrigated and closed with surgical clips.

Efficacy studies

Sixty animals divided into four groups initially underwent intracranial injection of 10^4 9L gliosarcoma cells. Groups 1 and 2 received a co-injection of 11 µl of placebo microspheres followed by implantation on the fifth postoperative day of blank pCPP:SA polymer (control group: empty MS/blank polymer, $n = 16$) or 5% ADR polymer (empty MS/5% ADR, $n = 15$), respectively. Groups 3 and 4 received a co-injection of 11 µl of IL-2 MS followed by implantation on the fifth postoperative day of blank pCPP:SA polymer (IL-2 MS/blank polymer, $n = 14$) or 5% adriamycin polymer (IL-2 MS/5% ADR, $n = 15$), respectively.

Histological evaluation

A representative animal was set aside for the purpose of histopathological examination. The brains was removed, the tissue was fixed in 10% formalin, blocked in paraffin, sectioned in coronal plane in 10-μm sections, and stained with hematoxylin and eosin (H&E). Immunohistochemistry using the peroxidase anti-peroxidase technique was also used with the following primary antisera: CD4, or CD8. A lymph node was used as a positive control.

Outcome and statistical analysis

The primary statistical outcome for all efficacy studies was time to death measured from time of tumor implantation. All animals were monitored for any signs of neurotoxicity and autopsied, when possible, to confirm that death was due to intracranial tumor. Survival distributions were estimated by the product-limit method [23]. Differences between survival distributions were assessed in two stages. First, an overall test of heterogeneity among treatment groups was performed using the log-rank test [24]. This test rejected the groups, that is, if treatment differences were present, pair-wise comparisons between the treatment groups and the controls were performed using the log-rank statistic. All probability values reported are two-sided.

Results

IL-2 encapsulation and release kinetics from polymer microspheres

Polymer microspheres obtained by complex coacervation of positively charged gelatin with negatively charged chondroitin-sulfate in the presence of IL-2 were spherical and averaged 10 µm in diameter. Encapsulation efficiency was approximately 88.5%. The release kinetics of IL-2 from microspheres has been previously published [20].

Efficacy sudies

Animals treated with empty MS followed by implantation of blank pCPP:SA polymer (Group 1) had a median survival time of 18 days (range: 6–24). (Figure 1, Table 1) Animals treated with empty MS/5% ADR (Group 2) demonstrated a statistically significant prolongation of survival compared to the control group (median: 30 days, range: 15–60, $P < 0.0004$). Animals treated with IL-2 MS/blank polymer (Group 3) also demonstrated a statistically significant prolonged survival compared to the control group (median: 39 days, range: 15–75, $P < 0.0005$). Animals treated with the combination IL-2 MS/5% ADR therapy (Group 4) survived significantly longer compared to control

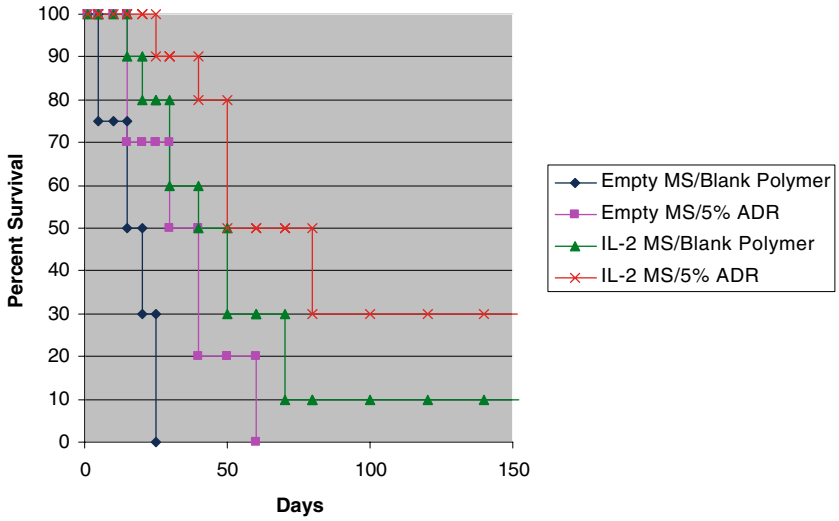

Figure 1. Kaplan-Meier survival curves for rats challenged with a lethal intracranial dose of 9L gliosarcoma cells and treated with empty microspheres and blank polymer, IL-2 MS and blank polymer, empty microspheres and 5% ADR polymer, or IL-2 MS and 5% ADR polymer.

Table 1. Efficacy of local interleukin-2 immunotherapy with or without interstitial adriamycin polymer chemotherapy[a]

Group	No. of animals	Median survival (days)	Survival range (days)	*P* value[b]
1: empty MS/blank polymer	16	18	6–24	–
2: empty MS/5% ADR	15	30	15–60	<0.01 vs. control
3: IL-2 MS/blank polymer	14	39	15+ +	<0.01 vs. empty MS/5% ADR <0.00002 vs. control
4: IL-2 MS/5% ADR	15	53	26+ +	<0.0005 vs. IL-2 MS/blank polymer <0.0001 vs. empty MS/5%ADR <0.0000001 vs. control

[a]MS, microsphere; IL-2, interleukin-2; ADR, adriamycin; d, days; BCNU, carmustine.
[b]Significance values were calculated by using the log-rank (Mantel–Cox) test.

group animals (median: 53 days, range: 26–154, $P < 0.0000002$) as well as to animals receiving either IL-2 MS/blank polymer ($P < 0.0004$) or empty MS/5% ADR ($P < 0.000003$).

We were also interested in comparing the efficacy of IL-2 MS/5% ADR treatment to the efficacy of IL-2 MS/BCNU therapy. Data demonstrating the efficacy of IL-2 MS/BCNU therapy in an experimental rat glioma model has been published elsewhere [20]. Both the IL-2 MS/5% ADR study and the IL-2 MS/BCNU study utilized the same set of controls. Thus, it is acceptable to compare the efficacy results directly (Table 1). IL-2 MS/5% ADR was significantly more effective than empty MS/4% BCNU ($P < 0.00003$) at extending survival. IL-2 MS/5% ADR was more effective than IL-2 MS/4% BCNU at extending survival, but this difference did not reach statistical significance ($P = 0.0596$). There was no significant difference between IL-2 MS/5% ADR and IL-2 MS/10% BCNU ($P = 0.83$).

To further understand the potential mechanism of an anti-tumor response, a brain from a representative animal was stained for the expression of both CD4 and CD8 T cells. Though the expression of both T cells was positive, a non-quantitative analysis suggests that the infiltration of CD8 cell was more pronounced than that of CD4 cells (Figure 2).

Discussion

In the present study, we have demonstrated that a combination of IL-2 MS and ADR-loaded biodegradable polymers improves the survival of rats injected with a lethal intracranial dose of gliosarcoma when compared to either therapy alone. This result is consistent with previous studies showing the efficacy of local immunotherapy with IL-2 in combination with biodegradable polymers loaded with various chemotherapeutic agents [19,20]. From our prior work, we know that IL-2 MS demonstrate a high and constant release of IL-2 during the first 7 days followed by a slower, sustained release for another 6 weeks [20]. Furthermore, IL-2 MS are able to elicit a strong inflammatory response characterized by polymorphonuclear and mononuclear leukocytes, while blank microspheres do not elicit any kind of inflammatory reaction [20]. Our histological examination of animal brain receiving treatment with IL-2 MS, ADR, or combination therapy has been consistent with our previously published results showing the infiltration of CD8+ T cells, presence of necrosis, and both necrosis and CD8+ within each group, respectively [19,20].

The poor prognosis of patients with malignant brain tumors and the high incidence of local recurrence after treatment have led efforts to control local disease. The unique environment of the CNS has made local control a significant challenge. The blood-brain barrier limits the ability of systemically administered drugs to enter the CNS. High doses of systemically administered drugs are necessary to achieve therapeutic drug levels in the CNS. This leads to significant side effects for patients. Thus, local drug delivery is an appealing alternative to the systemic administration of chemotherapy.

Despite advances in local delivery of chemotherapy, patients with malignant gliomas continue to have a poor prognosis. A search for drugs other than BCNU for polymer delivery continues to be an area of interest. One

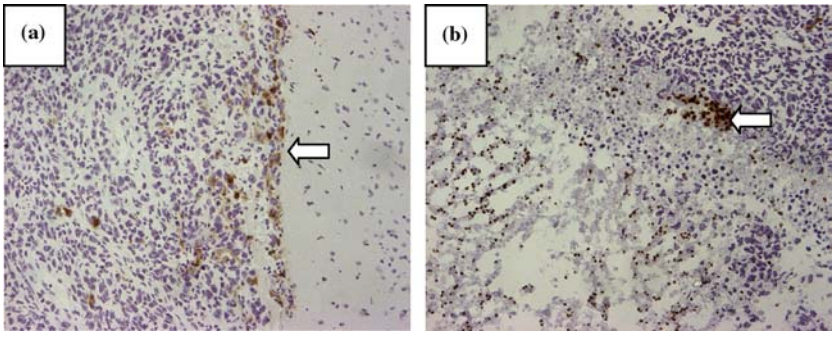

Figure 2. Immunohistochemical analysis of animal brains treated with both IL-2 and ADR shows (a) staining for CD4 and (b) CD8 cells (arrow).

study suggests that ADR is more cytotoxic to glioma cells compared to BCNU, at least *in vitro* [6]. Several others provide strong evidence that systemic delivery of adriamycin to high grade tumors results in significant survival advantage [25–27]. We have previously shown that ADR-loaded biodegradable polymers are effective in an animal model of intracranial glioma (Lesniak et al., submitted).

Immunotherapy of brain tumors is an area of active interest [12,13,28–30]. Initial efforts involved the isolation of elements of the host immune system, exposure of these elements to antigen and cytokine, and systemically readministering these immune effectors in order to generate an antitumor response. Such systemic approaches have been hindered by such obstacles as the blood-brain barrier, which restricts immune effector cells from entering the brain epithelium [31], poor target recognition within the central nervous system, immune resistance and other protective mechanisms intrinsic to brain tumors [32,33] and limited expression of major histocompatibilty antigens by tumor cells in the brain [34].

Local immunotherapeutic techniques can be used to bypass the blood-brain barrier and achieve high concentrations of drug to the tumor bed and surrounding tissue. Local delivery systems distribute a drug in a paracrine fashion, thus mimicking how cytokines normally exert their immunomodulatory effect. Our initial efforts with local immunotherapeutic delivery systems were based on the stereotactic injection of autologous tumor cells transduced with IL-2 or IL-12 into tumor. This approach was successful at treating gliomas in animal models [13,35]. In addition, the combination of genetically engineered tumor cells that produce IL-2 and locally delivered chemotherapy was significantly more effective at improving survival compared to either treatment alone in a murine model of brain tumor [19,20]. One possible mechanism for this observation is that the presence of chemotherapy induces cell death which then may result in an increased exposure to potential tumor antigens and therefore augments the response of the immune system. While a similar mechanism of action is proposed to account for the observations reported in these sets of experiments, further studies will need to be performed to confirm this hypothesis.

In conclusion, we have shown that IL-2 delivered by microspheres in conjunction with adriamycin-loaded polymers is more efficacious at prolonging survival in an animal model of gliosarcoma compared to either therapy alone. The experiments presented in this paper provide another example of the synergistic effect of combining local chemoimmunotherapy and deserve further attention in future clinical studies.

Disclosure

Henry Brem is a consultant to Guilford Pharmaceuticals, Inc., and Aventis Pharmaceuticals, Inc. The Johns Hopkins University and Henry Brem own Guilford stock, the sale of which is subject to certain restrictions under university policy. The terms of this arrangement are managed by the university in accordance with its conflict of interest policies.

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References

1. Brem H et al.: Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas. J Neurosurg 74: 441–446, 1991
2. Brem H et al.: Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. Lancet 345: 1008–1012, 1995
3. Valtonen S et al.: Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas: a randomized double-blind study. Neurosurgery 41: 44–48, discussion 48–49, 1997
4. Walter KA, Tamargo RJ, Olivi A, Burger PC, Brem H: Intratumoral chemotherapy. Neurosurgery 37: 1128–1145, 1995
5. Westphal M et al.: A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. Neuro-oncol 5: 79–88, 2003.
6. Wolff JE, Trilling T, Molenkamp G, Egeler RM, Jurgens H: Chemosensitivity of glioma cells *in vitro*: a meta analysis. J Cancer Res Clin Oncol 125: 481–486, 1999
7. Ohnishi T. et al.: *In vivo* and *in vitro* evidence for ATP-dependency of P-glycoprotein-mediated efflux of doxorubicin at the blood-brain barrier. Biochem Pharmacol 49: 1541–1544, 1995
8. Tushinksi RJ, J.J., M: Biology of cytokines: the interleukins. In: Devita VT, Hellman S, Rosenberg SA (eds) Biologic Therapy of Cancer. J.B.Lippincott Company, Philadelphia, 1991, pp 87–94
9. Pardoll DM: Paracrine cytokine adjuvants in cancer immunotherapy. Annu Rev Immunol 13: 399–415, 1995
10. Fearon ER et al.: Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. Cell 60: 397–403, 1990
11. Rosenberg SA et al.: Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. Ann Surg 210: 474–484, discussion 484–485, 1989
12. Lichtor T, Glick RP, Kim TS, Hand R, Cohen EP: Prolonged survival of mice with glioma injected intracerebrally with double cytokine-secreting cells. J Neurosurg 83: 1038–1044, 1995
13. Thompson RC et al.: Systemic and local paracrine cytokine therapies using transduced tumor cells are synergistic in treating intracranial tumors. J Immunother Emphasis Tumor Immunol 19: 405–413, 1996
14. Glick RP, Lichtor T, de Zoeten E, Deshmukh P, Cohen EP: Prolongation of survival of mice with glioma treated with semiallogeneic fibroblasts secreting interleukin-2. Neurosurgery 45: 867–874, 1999
15. Lichtor T et al.: Application of interleukin-2-secreting syngeneic/allogeneic fibroblasts in the treatment of primary and metastatic brain tumors. Cancer Gene Ther 9: 464–469, 2002
16. Hanes J et al.: Controlled local delivery of interleukin-2 by biodegradable polymers protects animals from experimental brain tumors and liver tumors. Pharm Res 18: 899–906, 2001

17. Nigam A et al.: Immunomodulatory properties of antineoplastic drugs administered in conjunction with GM-CSF-secreting cancer cell vaccines. Int J Oncol 12: 161–170, 1998
18. Tsung K, Meko JB, Tsung YL, Peplinski GR, Norton JA: Immune response against large tumors eradicated by treatment with cyclophosphamide and IL-12. J Immunol 160: 1369–1377, 1998
19. Sampath P et al.: Paracrine immunotherapy with interleukin-2 and local chemotherapy is synergistic in the treatment of experimental brain tumors. Cancer Res 59: 2107–2114, 1999
20. Rhines LD et al.: Local immunotherapy with interleukin-2 delivered from biodegradable polymer microspheres combined with interstitial chemotherapy: a novel treatment for experimental malignant glioma. Neurosurgery 52: 872–879, discussion 879–880, 2003
21. Sipos EP, Tyler B, Piantadosi S, Burger PC, Brem H: Optimizing interstitial delivery of BCNU from controlled release polymers for the treatment of brain tumors. Cancer Chemother Pharmacol 39: 383–389, 1997
22. Olivi A et al.: Interstitial delivery of carboplatin via biodegradable polymers is effective against experimental glioma in the rat. Cancer Chemother Pharmacol 39: 90–96, 1996
23. Kaplan E, Meier P: Non-parametric estimation from incomplete observations. J Am Stat Associ 53: 457–481, 1958
24. Mantel N, Haenszel W: Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22: 719–748, 1959
25. Hau P et al.: Pegylated liposomal doxorubicin-efficacy in patients with recurrent high-grade glioma. Cancer 100: 1199–1207, 2004
26. Steiniger SC et al.: Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. Int J Cancer 109: 759–767, 2004
27. Rittierodt M, Harada K: Repetitive doxorubicin treatment of glioblastoma enhances the PGP expression – a special role for endothelial cells. Exp Toxicol Pathol 55: 39–44, 2003
28. Glick RP, Lichtor T, Kim TS, Ilangovan S, Cohen EP: Fibroblasts genetically engineered to secrete cytokines suppress tumor growth and induce antitumor immunity to a murine glioma *in vivo*. Neurosurgery 36: 548–555, 1995
29. Griffitt W, Glick RP, Lichtor T, Cohen EP: Survival and toxicity of an allogeneic cytokine-secreting fibroblast vaccine in the central nervous system. Neurosurgery 42: 335–340, 1998
30. Staib L. Harel W, Mitchell MS: Protection against experimental cerebral metastases of murine melanoma B16 by active immunization. Cancer Res 53: 1113–1121, 1993
31. Hickey WF: Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. Brain Pathol 1: 97–105, 1991
32. Fontana A, Hengartner H, de Tribolet N, Weber E: Glioblastoma cells release interleukin 1 and factors inhibiting interleukin 2-mediated effects. J Immunol 132: 1837–1844, 1984
33. Saris SC et al.: Treatment of murine primary brain tumors with systemic interleukin-2 and tumor-infiltrating lymphocytes. J Neurosurg 76: 513–519, 1992
34. Akbasak A, Oldfield EH, Saris SC: Expression and modulation of major histocompatibility antigens on murine primary brain tumor in vitro. J Neurosurg 75: 922–992, 1991
35. DiMeco F et al.: Paracrine delivery of IL-12 against intracranial 9L gliosarcoma in rats. J Neurosurg 92: 419–427, 2000
36. Weber CE: Cytokine-modified tumor vaccines: an antitumor strategy revisited in the age of molecular medicine. Cancer Nurs 21: 167–177, 1998

Address for offprints: M.S. Lesniak, The university of Chicago, Division of Neurosurgery, 5841 South Maryland Avenue, MC 3026, Chicago, Illinois 60637, USA; E-mail: mlesniak@surgery.bsd.uchicago.edu