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Local Delivery of a Synthetic Endostatin Fragment for the Treatment of Experimental Gliomas

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Abstract

OBJECTIVE: Endostatin is an anti-angiogenic agent that blocks matrix-metalloproteinase-2 and inhibits endothelial cell proliferation. Currently, endostatin is available through recombinant technology, which limits its broader use. In this study, a synthetic endostatin fragment (EF) was analyzed to determine its anti-angiogenic properties when locally delivered by controlled-release polymers and to establish its effect as a treatment for experimental gliomas.

METHODS: Cytotoxicity of EF against 9L gliosarcoma and F98 glioma was determined in vitro. EF was loaded into polyanhydride-poly-(bis-[carboxyphenoxy-propane]-sebacic-acid) (pCPP:SA) polymers at increasing concentrations. Pharmacokinetics of the EF/polymer formulations were defined in vitro. Anti-angiogenic properties of the EF/polymer formulations were evaluated in the rat-cornea micropocket assay. Toxicity and efficacy of locally delivered EF polymers either alone or combined with systemic bischloroethylnitrosourea (carmustine) were determined in rats intracranially challenged with 9L gliosarcoma.

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RESULTS: EF showed scarce cytotoxicity against 9L and F98 in vitro. EF/pCPP:SA formulations showed sustained release by day 19. Mean corneal angiogenesis index 20 days after tumor implantation was 4.5 ± 0.7 for corneas implanted with 40% EF/pCPP:SA compared with controls (8.5 ± 1.3, $P = 0.02$). Intracranial efficacy studies showed that EF polymers alone did not prolong animal survival. Combination of 40% EF/pCPP:SA polymers with systemic bischloroethylnitrosourea (carmustine) prolonged survival (median survival of 44 d, $P = 0.001$) and generated 33% long-term survivors.

CONCLUSION: Controlled-release polymers can effectively deliver a biologically active EF in a sustained fashion. EF inhibits angiogenesis in vitro and in vivo, and even though EF does not prolong survival as a single agent, it exhibits a synergistic effect when combined with systemic bischloroethylnitrosourea (carmustine) in the intracranial 9L gliosarcoma model.

Keywords

Brain tumor; Controlled-release; Endostatin; Gliomas; Local delivery polymer

Angiogenesis is an essential mechanism tumor progression and increasing evidence validates the role of specific inhibitors of this process as efficient antitumor agents (1,14). Endostatin, a protein derived from collagen XVIII, has been shown to inhibit endothelial cell proliferation (11), angiogenesis (1,14,34), tumor growth (35), and metastases (33). The cellular mechanisms by which endostatin exerts its anti-angiogenic activity have not been completely elucidated but include the inhibition of matrix-metalloproteinases (24) and arrest of endothelial and tumor cells in the G1 phase with induction of apoptosis seen in some tumor lines (10), among others (41,48).

Malignant gliomas constitutively express pro-angiogenic factors involved in several pathways of neovascularization and exhibit marked dependence on angiogenesis for growth (27). Endostatin has proven to be beneficial as an anti-angiogenic agent in experimental gliomas (22,40,42,48), specifically decreasing tumor size and vascularization without development of resistance or toxicity; however, endostatin has not been proven to prolong the survival of animals challenged intracranially with malignant gliomas when used locally as single therapy in an animal model (7). Currently, recombinant human endostatin is produced in limited amounts because of high production costs, which limits the availability of the protein. The endostatin obtained after recombinant synthesis encompasses 184aa, which makes it vulnerable to denaturation when placed in solution and limits its stability (8).

Our group has previously demonstrated that the antiangiogenic effects of endostatin are retained by fragments of the parent molecule (8,9). Fragments corresponding to sequences 6 to 49 and 134 to 178 have been isolated, characterized by circular dichroism spectroscopy and nuclear magnetic resonance spectroscopy, and evaluated for anti-angiogenic activity in vitro by using human umbilical vein endothelial cells for proliferation assays and Boyden chamber migration analysis. The anti-angiogenic activity of the 6 to 49 endostatin fragment (EF) has also been tested in vivo using the Matrigel assay in C57/b16 female mice (8,9). Furthermore, EF 6 to 49 has been shown to have better solubility and increased stability when compared with full-length endostatin.

Local delivery of anti-angiogenic agents has been advantageous in experimental models of malignant gliomas (22,40,45,50). Local delivery of drugs at the tumor bed allows bypass of the blood-brain barrier and results in higher concentrations in the targeted location while minimizing undesired systemic effects (25,26). Furthermore, polymeric formulations of chemotherapeutic agents have regulatory approval for clinical application (6,31,53), and the combination of this therapeutic strategy with localized anti-angiogenic drug delivery could potentially result in enhanced antitumor responses because of a synergistic effect. Indeed,

previous work by our group and others demonstrated the benefit of combining locally delivered anti-angiogenic agents such as the tetracycline derivative minocycline with chemotherapeutic agents such as bischloroethylnitrosourea (BCNU, carmustine). This combination prolonged survival in animals challenged with intracranial 9L gliosarcoma (15,52). Furthermore, the combination of endostatin with a protein kinase Cα DNA enzyme (an inductor of apoptosis) was successful in prolonging survival of rats with intracranial malignant glioma (47). This strategy has also been successful in preclinical studies when a combination of thalidomide (an anti-angiogenic agent) and either carmustine or temozolomide has been administered and is currently undergoing further clinical testing (3,13).

In the present study, we hypothesized that controlled-release polymers loaded with EF were capable of delivering bioactive concentrations of the fragment and would be effective to treat animals challenged intracranially with 9L gliosarcoma either alone or combined with systemic chemotherapy.

MATERIALS AND METHODS

Polymer Preparation

Polyanhydride poly (bis-[carboxyphenoxy-propane]-sebacic-acid) polymer (pCPP:SA) 20:80, by weight, was loaded with synthesized EF at 1%, 10%, 20%, 30%, and 40% concentrations (w:w), as previously described (16,51). pCPP:SA and 6 to 49 EF were dissolved in 0.5 ml of methylene chloride (Fisher Chemicals, Fair Lawn, NJ) and placed in a vacuum desiccator for 2 hours. The resultant polymer mixture was pressed into 10 mg wafers and stored at −20°C.

BCNU

BCNU was purchased as BiCNU for injection (Bristol-Myers Squibb Company, Princeton, NJ) and reconstituted as instructed by the manufacturer. Preparations were administered intraperitoneally (IP) at a dose of 10 mg/kg. BCNU was selected as the systemic agent for combined therapy to compare the effects of this treatment with those previously obtained with the combination of minocycline and BCNU (15).

In vitro Pharmacokinetics

Release kinetics were determined by using the bicinchoninic acid assay (Pierce, Chemical Co., Rockford, IL). Triplicate samples of pCPP:SA polymers (10 mg wafers) loaded with 6 to 49 EF at concentrations of 10%, 20%, and 40% were suspended in separate glass vials each containing 1 ml of phosphate-buffered saline at 37°C, following the methods previously described by Tamargo et al. for in vitro pharmacokinetic analysis of controlled-release polymers (51). Polymers were sequentially transferred to fresh 1 ml aliquots of phosphate-buffered saline at several time points up to 432 hours (19 d). The amount of EF was determined spectrophotometrically by comparison with a standard absorbance curve.

In vitro Cytotoxicity of 6-49aa EF in 9L Gliosarcoma and F98 Glioma Cells

Cytotoxicity of the EF was evaluated in vitro against 9L and F98 cells with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay (46). 9L and F98 cells were plated in a 96-well plate (3500 cells/well) and incubated for 24 hours at 37°C, with a total volume of 100 μl/well. EF was given at concentrations of 1, 10, 25, 50, and 100 μg/ml, and percentages of cell viability were determined. Absorbance was measured with an enzyme-linked immunoadsorbent assay plate reader (Beckman Coulter, model AD 340, Fullerton, CA) at 270 nm.

Toxicity of pCPP:SA/Endostatin Fragment Polymer in Fischer 344 Rats

Animals (n = 18) were intracranially implanted with 10 mg wafers of empty pCPP:SA and 5%, 10%, 20%, and 30% 6 to 49 EF-loaded pCPP:SA polymers. Rats were evaluated and weighed preoperatively daily for 2 weeks, biweekly for 3 weeks, and weekly for 6 weeks to determine signs of local and systemic toxicity. Animals were killed at 120 days, and their organs were harvested, fixed, and processed for histopathological analysis.

Rat Cornea Micropocket Angiogenesis Assay

Fischer 344 rats were used for this experiment (n = 8 rats, n = 16 corneas). The corneal micropocket assay was used in this study as previously described (38,43). Loaded polymers were implanted in the right eye of the animals, and empty polymers were implanted in the left eye; therefore, every animal had its own control. Animals were anesthetized as previously described (50), and topical anesthesia was administered with Proparacaine-HCL, (0.5% Bausch and Lomb Pharmaceuticals Inc, Tampa, FL). Under direct vision with a Zeiss operating microscope with use of a 14G needle and a #11 blade, a 2 × 2 mm pocket was made by blunt dissection of the corneal epithelium with a 2 mm spatula not penetrating the anterior chamber. A fresh tumor piece of 9L gliosarcoma grown in the flank of a carrier animal (rat) was harvested and inserted followed by the polymer implant. Angiogenesis was measured with a Zeiss slit lamp (Carl Zeiss, Thornwood , NY) at 0, 5, 8, 12, 15, and 21 days after implantation. An angiogenesis index (AI), previously described (50), was used to evaluate the corneas. The AI was obtained by multiplying the vessel length from the sclerocorneal junction to the leading edge of the new vessel by the vessel number represented in a four-level scale in which 0 = 0 vessels, 1 = 1 to 10 vessels, 2 = more than10 vessels loosely packed with the iris adequately visible through the gaps, and 3 = more than 10 vessels tightly packed without visualization of the iris.

Efficacy of EF/pCPP:SA Polymers in Rat 9L Gliosarcoma Model

Experimental design—Female Fischer 344 rats (n = 64, 8 per group) were housed in standard animal facilities, given free access to Baltimore city water and rodent chow, and randomized into eight experimental groups (Table 1). Animals in group 1 (controls) received 9L intracranial tumor only. Animals in group 2 received EF/pCPP:SA polymer only simultaneously with the tumor implant (day 0), whereas animals in group 3 received EF/pCPP:SA polymer only on day 3. Group 4 received EF/pCPP:SA polymer on day 5 only, and group 5 received IP BCNU only on day 5. Animals in group 6 received combination of EF/pCPP:SA polymers on day 3 and IP BCNU on day 5. Animals in group 7 received combination of EF/pCPP:SA polymer on day 5 and IP BCNU on day 5, whereas animals in group 8 received the combination EF/pCPP:SA polymer on day 0 and IP BCNU on day 5. All implanted polymers were 40% loaded with EF by weight.

Surgical procedure

Anesthesia was administered as previously described (50). The heads were shaved and prepared, and tumor and polymers were implanted (51). A midline scalp incision was made, the galea was dissected laterally, and a 3 mm burr hole was made over the left parietal bone with its center 5 mm posterior to the coronal suture and 3 mm lateral to the sagittal suture. A small section of parietal cortex was removed with suction, and a 40% EF/pCPP:SA polymer was placed into the brain parenchyma approximately 1 mm below the dura. After ensuring hemostasis, the skin was closed with surgical staples, and animals were returned to their cage and closely monitored daily. Median survival and long-term survivors were considered as end points. The Animal Care and Use Committee of the Johns Hopkins University approved all experimental protocols.

Statistical analysis

Statistical analysis was performed with SPSS version 8.0 for windows (Chicago, IL). Cytotoxicity determined in vitro by the MTT assay and angiogenic indexes calculated for the cornea angiogenesis assay were analyzed with one-way analysis of variance, followed by a Student-Newman-Keuls test, and values are expressed as mean ± standard error of the mean (SEM). Kaplan-Meier curves for the efficacy studies were analyzed on the basis of survival algorithms, and significance was determined by the log-rank and Kruskal-Wallis tests. All experimental groups were analyzed by comparing their median survival with the median survival of the control group. Further analysis was performed by comparing the median survival of the groups treated with combination therapies with the survival of the group treated with BCNU alone, using the same statistical methodology. A probability value of less than 0.05 was considered significant for all tests.

RESULTS

In vitro Pharmacokinetics

The release rate of the EF from pCPP:SA polymers 10, 20, and 40% loaded (w:w) was measured in vitro over a 19 day period. During this time, 10% loaded polymers released 12.8 ± 0.004% of the loaded EF (128 ± 0.04 μg), 20% polymers released 7.2 ± 0.005% (144 ± 0.1 μg), and 40% polymers released 3.8 ± 0.0007% (152 ± 0.28 μg) (Fig. 1). Taking into consideration the total amount of fragment released and the fact that biodegradable polymers theoretically improve their degradation kinetics in vivo, the 40% loaded polymer was chosen for subsequent experiments.

In vitro Cytotoxicity in 9L Gliosarcoma and F98 Glioma

Cytotoxicity of the EF on 9L and F98 cells in vitro was determined with the MTT assay. Treatment with increasing concentrations of the EF showed growth inhibition of 9L gliosarcoma cells at all concentrations tested, including 1 μg/ml (lowest concentration) after 3 days of exposure ($P = 0.01$); treatment with 100, 50, 25, and 10 μg/ml decreased the percentage of cell viability to 71 ± 3%, 81 ± 2.4%, 91 ± 3%, and 91 ± 4%, respectively, compared with control (Fig. 2A). Similarly, treatment of F98 glioma cells with increasing concentrations of the EF showed a decrease in cell viability at concentrations of 100, 50, and 25 μg/ml, generating 65 ± 1.7%, 78 ± 2%, and 81 ± 2% cell viability, respectively, after 3 days of exposure ($P < 0.001$); treatment of F98 cells with 10 and 1 μg/ml (lowest concentrations) did not significantly decrease the percentage of cell viability when compared with control (Fig. 2B).

In vivo Toxicity in Fischer 344 Rats

Toxicity of the EF was evaluated by following neurological status and weight gain in animals implanted intracranially with 10 mg pCPP:SA polymer alone and pCPP:SA polymers loaded with EF 5%, 10%, 20%, and 30% (w:w). Animals in all groups continued to gain weight up to the day of euthanasia (120) without local or systemic toxicity (Fig. 3). After euthanasia, full necropsies were performed in all the animals. Analysis of histological specimens confirmed the absence of focal and systemic toxicity.

Rat Cornea Micropocket Angiogenesis Assay

The anti-angiogenic activity of 40% EF/pCPP:SA polymers was determined in the corneas of Fischer 344 rats implanted with 9L gliosarcoma. Angiogenesis was measured at 5, 8, 12, 15, and 20 days. At days 12, 15, and 20, animals implanted with 40% EF/pCPP:SA polymers had mean AI of 2.9 ± 0.58 compared with 4.6 ± 0.52 in the control at day 12 ($P = 0.038$), 4.0 ± 0.7 compared with 6.0 ± 0.64 in the control at day 15 ($P = 0.044$), and 4.5 ± 0.7 compared with 8.5 ± 1.3 at day 20 ($P = 0.02$) (Fig. 4A). Mean AI was not significant at both day 8 (1.5 ± 0.48

in the EF group compared with 2.1 ± 0.43 in the control, $P = 0.310$) and day 5 (0 compared with 0.2 ± 0.2 in the control, $P = 0.334$) (Fig. 4B)

Efficacy of Endostatin Fragment/pCPP:SA Polymers in the Rat 9L Gliosarcoma Model

Animals treated with single agents, either 40% EF/ pCPP:SA polymers implanted alone on day 0 (group 2), day 3 (group 3), or day 5 (group 4) and systemic BCNU administered on day 5 (group 5) had median survivals of 14, 12, 12, and 26 days, respectively (no statistical significance was found with EF polymers alone compared to controls). Animals treated with a combination of locally delivered EF and systemic BCNU had significantly higher survival rates when compared with either treatment alone and controls and produced long-term survivors (Fig. 6). Whereas combination therapy with intracranial 40% EF/pCPP:SA polymer on day 0 and systemic BCNU (group 8) significantly improved animal survival (median survival of 44 d, $P < 0.001$) and generated 33% long-term survivors (survival >120 d), animals in the control group (9L tumor no treatment, group 1) had a median survival of 11 days. Similarly, treatment with a combination of 40% EF/pCPP:SA polymer on day 3 and systemic BCNU on day 5 (group 6) had a median survival of 28 days ($P < 0.001$) and generated 12.5% long-term survivors, and animals treated with 40% EF/pCPP:SA polymer on day 5 and systemic BCNU on day 5 (group 7) resulted in a median survival of 24 days ($P < 0.001$) and generated 12.5% long-term survivors.

To further evaluate the benefit of combined therapy with EF and BCNU, the survival of group 5 (BCNU alone) was compared with that of groups 6, 7, and 8 combination groups. This analysis showed that treatment with EF day 0 + BCNU day 5 and EF day 3 + BCNU day 5 significantly prolonged animal survival when compared with BCNU alone ($P = 0.001$ and $P = .0368$, respectively). The survival of animals treated with EF day 5 + BCNU day 5, however, was not significantly greater than the survival of animals treated with BCNU alone ($P = 0.765$).

DISCUSSION

In this study, a fragment of human endostatin corresponding to sequences 6 to 49 was synthesized and incorporated into controlled-release polymers to determine the anti-angiogenic and antitumor activity of the polymer formulations in an experimental model of malignant glioma. We found that the EF was released in a controlled fashion from pCPP:SA polymers in vitro and that it maintained its anti-angiogenic potential in the rat cornea micropocket assay. Furthermore, EF-loaded polymers implanted intracranially did not generate local or systemic toxicity. Treatment with EF polymers alone showed a trend toward prolonged animal survival, particularly when polymers where implanted on day 0 (simultaneously with the tumor implant) but did not reach statistical significance. When EF polymers were used in combination with systemic BCNU, however, a statistically significant synergistic effect was obtained that prolonged the survival of animals challenged intracranially with 9L gliosarcoma and generated long-term survivors.

Vascularization of malignant gliomas requires increased angiogenesis, which depends greatly on endothelial cell proliferation (21). Under physiological conditions, angiogenesis is stringently regulated by a delicate balance between enhancing and inhibitory modulators. Enhancers of angiogenesis such as vascular endothelial growth factor (VEGF) , basic fibroblast growth factor, and hepatocyte growth factor/scatter factor among others are counterbalanced by inhibitors of angiogenesis such as endostatin (21). Angiogenesis inhibitors modulate the interactions between endothelial cells and extracellular matrix components such as collagen and integrins (i.e., αvβ-3) (4), which are required for new vessel formation.

We have previously tested the anti-angiogenic activity of this EF using Matrigel assays in vitro as well as in vivo (9), and in the present study, we confirmed this activity against an

experimental glioma using the rat cornea-micropocket assay. Angiogenesis in the Matrigel assays previously performed was induced through stimulation with VEGF and fibroblast growth factor and the angiogenic stimuli in the rat cornea micropocket assay was provided by the implanted tumor (9L). Angiogenesis induced by 9L in particular has been shown to be largely dependent on the presence VEGF (36) and fibroblast growth factor (29) among other factors. Therefore, we believe that the anti-angiogenic activity of this EF must be maintained when the fragment is administered locally to the brain.

Endostatin is an endogenous antiangiogenic protein activated by proteolytic processing (12) that inhibits endothelial cell proliferation, migration, invasion, and microvessel formation (1). Its inhibitory activity involves binding to the $\alpha_5\beta_1$ receptor (49) and to glypican-1/4 among other mechanisms (23). Endostatin reduces endothelial cell survival, motility, and invasion possibly through blockade of VEGF/VEGF-receptor signaling (19), inhibition of metalloproteinases (24), and downregulation of c-*myc* and cyclin-D1 (20,44). The efficacy of endostatin as an anti-angiogenic/antitumor agent has been extensively evaluated in animal models (18,39); however, its effect in survival of animals challenged with malignant gliomas has shown variable results (7,33,40,48). Currently, endostatin is available through recombinant technology that generates the 184-amino acid parent molecule. To improve the solubility and stability of endostatin and to facilitate its synthesis the 6-49aa fragment of endostatin was synthesized by our group and tested to establish its pharmacological properties and to determine its anti-angiogenic and antitumor activity (8,9,30). When compared with full-length human endostatin and with other fragments of the parent molecule, 6 to 49 EF was found to be fully biologically active and in some instances, showed even greater potency and efficacy than full-length human endostatin itself (8).

To enhance the potential activity of the 6 to 49 EF, adequate bioactive concentrations must reach the tumor site. Because of the nature of the blood-brain barrier, penetration to the tumor site by systemic administration of the fragment is likely to be inadequate. Therefore, a biodegradable polymer system, currently approved by the Food and Drug Administration for direct delivery of BCNU to clinically treat malignant glioma, was used to determine the pharmacokinetic properties of the locally delivered EF. The pCPP:SA polymer system allows diffusion of the fragment toward the interstitium and further release through biodegradation of the polymer matrix.

Successful preclinical studies on anti-angiogenic agents have resulted in clinical trials that tested diverse strategies of anti-angiogenesis to treat several malignancies (2). Among these strategies, monoclonal antibodies against VEGF-receptor (5), EGF-receptor, and $\alpha_v\beta3$, as well as specific inhibitors of pro-angiogenic factors such as matrix metalloproteinase inhibitors and integrin blockers (2) have been used with varied results. In the present study, although the anti-angiogenic activity of endostatin was not sufficient to significantly prolong animal survival when used as a single agent, the combination of locally delivered endostatin and systemic chemotherapy was synergistic and greatly prolonged survival.

The response to endostatin observed in vitro, as well as the anti-angiogenic activity seen in the corneal assays, contrasts the limited response to monotherapy with the EF polymers in vivo. This phenomenon, however, has been observed with several antiangiogenic therapies in a variety of tumors including gliomas in experimental and clinical scenarios (17,28,32). Our findings and those of other groups suggests that angiogenesis inhibitors behave primarily as cytostatic agents that stabilize the disease, and therefore anti-angiogenesis alone is unlikely to significantly decrease glioma growth in vivo. Nevertheless, the temporal stasis in tumor growth provided by anti-angiogenic agents is likely to enhance the activity of conventional cytotoxic agents with proven anti-glioma activity.

On the basis of the results of our toxicity study, an increase in the dose of EF delivered can be pursued to enhance its efficacy in vivo. This can be achieved by either increasing the loading concentration of EF in the polymers, implanting more polymers (limited by the size of the rat brain), or selecting an alternative delivery system such as convection-enhanced delivery.

Experience gathered in preclinical studies by our group and others has shown that combined therapy with cytotoxic alkylating agents such as BCNU and locally delivered anti-angiogenic agents such as endostatin is an effective strategy for treating experimental malignant gliomas (15,37,52). This strategy is further supported by the results obtained in this study, which show that combination of systemic BCNU and locally delivered EF provides a synergistic and complementary approach that generates long-term survivors.

In conclusion, this synthetic EF demonstrates strong anti-angiogenic properties; it is not efficacious for treatment of intracranial experimental gliomas when used alone, but has a synergistic effect when combined with BCNU for treatment of experimental gliomas. When incorporated into controlled-release polymers, the fragment maintained its biological activity and exhibited no signs of local or systemic toxicity. Further studies are required to evaluate the potential for synergistic activity of this EF when administered simultaneously with other anti-neoplastic agents and radiotherapy to establish its potential translation into clinical scenarios.

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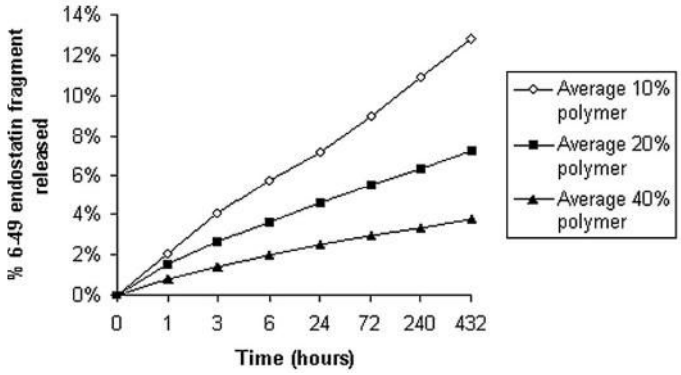

FIGURE 1.

Pharmacokinetics. The release rate of EF from pCPP:SA 10, 20, and 40% (w:w) measured over 19 days in vitro. Sustained release of EF was observed with all polymer formulations.

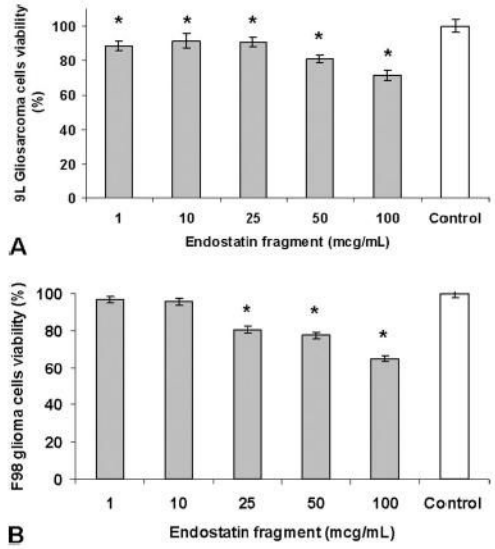

FIGURE 2.

Cytotoxicity of the endostatin fragment on 9L and F98 cells in vitro was determined with the MTT assay. (A) 9L gliosarcoma. Significant growth inhibition of 9L was achieved at all concentrations tested. (B) F98 glioma. EF showed significant decrease in F98 cell viability at 100, 50, and 25 μg/ml but not at 10 and 1 μg/ml when compared with control.

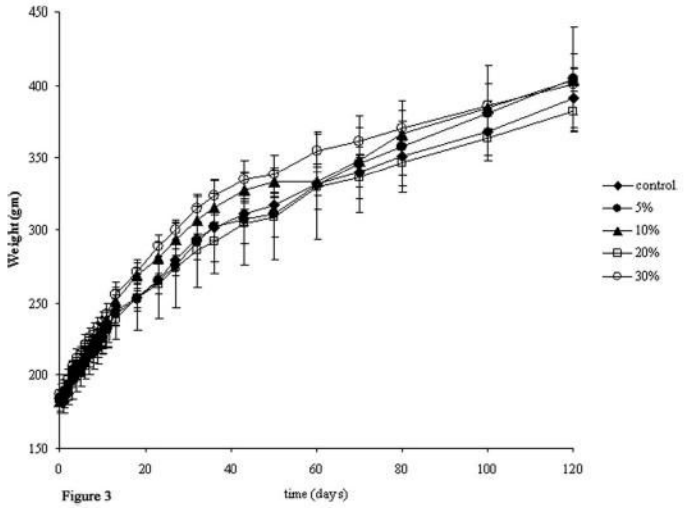

FIGURE 3.

Toxicity-weight gain correlation. Animals were treated with different formulations of EF polymers. All animals showed weight gain up to the day of euthanasia without local or systemic toxicity; no differences were found between groups.

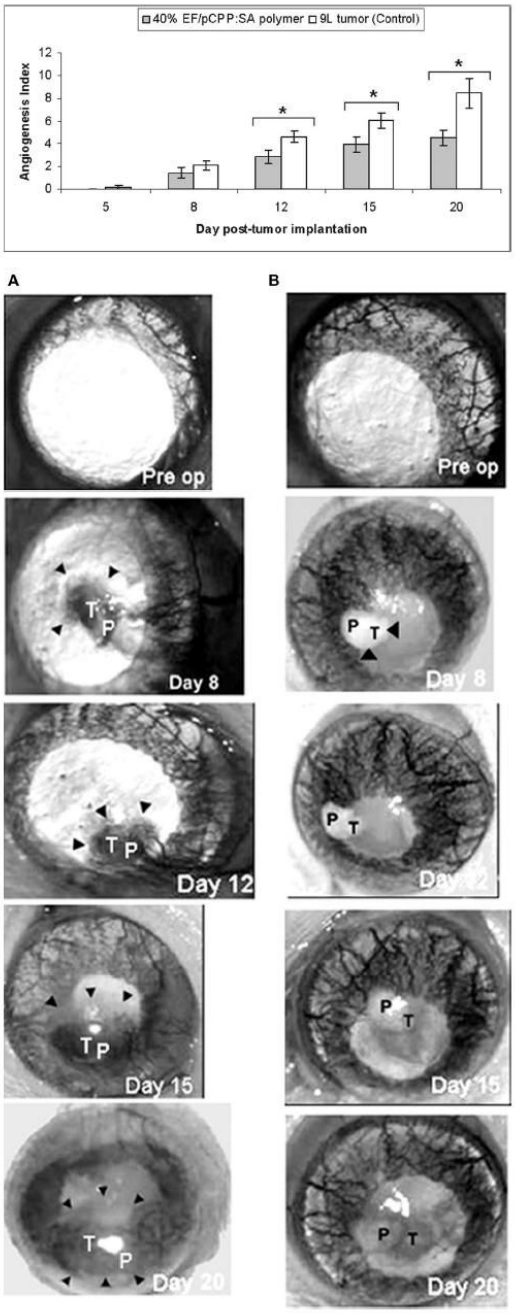

FIGURE 4.

Corneal angiogenesis: corneal angiogenesis index. The anti-angiogenic activity of 40% EF/pCPP:SA polymers was determined in corneas of rats implanted with 9L. Corneas treated with EF/pCPP:SA showed significantly lower AI values compared with control corneas. (A) Progression of angiogenesis and tumor size in a cornea implanted with 9L and treated with empty polymer. Arrows indicate the periphery of the tumor. (B) Progression of angiogenesis and tumor size in a cornea implanted with 9L and treated with 40% EF/pCPP:SA. P, polymer implant; T, 9L tumor piece.

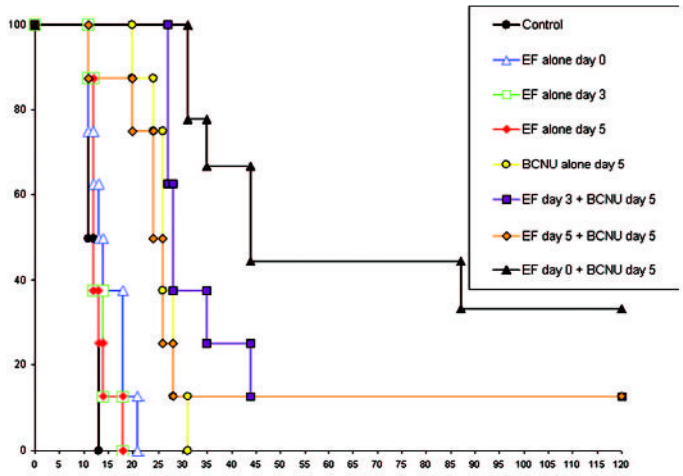

FIGURE 5.

Kaplan-Meier animal survival curve. Efficacy of 40% EF/pCPP:SA polymers was tested in the rat 9L model. Animals treated with a combination of locally delivered EF and systemic BCNU had significantly higher survival rates when compared with either treatment alone and controls and produced long-term survivors.

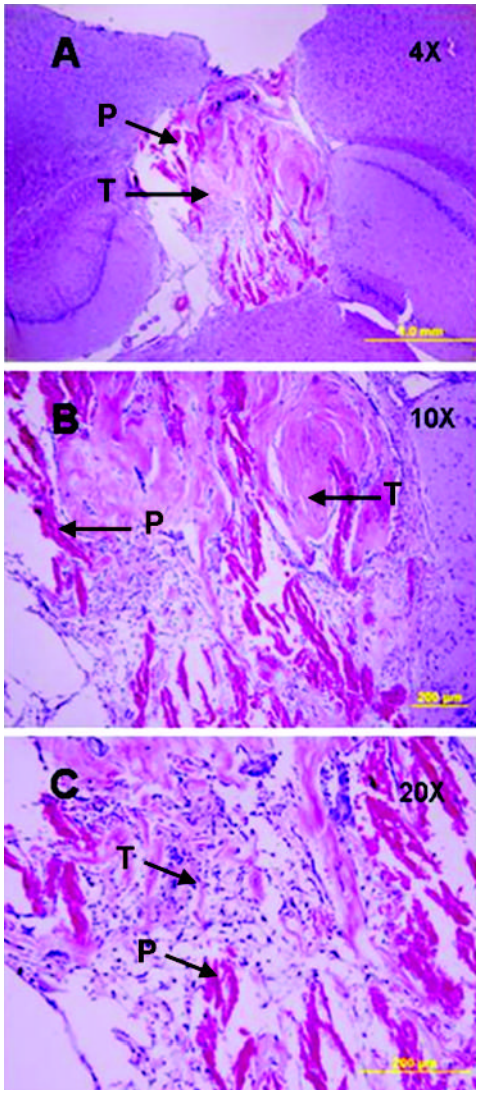

FIGURE 6.

Coronal section from the brain of a rat implanted with 9L and treated with EF/pCPP:SA on day 0 and systemic BCNU on day 5 (H&E). A ×4 magnification (A) shows the EF/polymer (P) matrix in its biodegradation process, and the 9L gliosarcoma tumor (T) surrounding the implant. Higher magnification is observed in B and C.

TABLE 1

Experimental groups: intracranial efficacy study in rats[a]

Group	Treatment groups	Median survival	Mean survival	Range
1	(Control) 9L intracranial tumor only	11	12	11–13
2	Endostatin fragment/polymer (Day 0)	14	15	11–21
3	Endostatin fragment/polymer (Day 3)	12	13	11–18
4	Endostatin fragment/polymer (Day 5)	12	13	11–18
5	IP BCNU (Day 5)	26	26	20–31
6	Endostatin fragment/polymer (Day 3) + IP BCNU (Day 5)	28	40	27–100
7	Endostatin fragment/polymer (Day 5) + IP BCNU (Day 5)	24	32	11–100
8	Endostatin fragment/polymer (Day 0) + IP BCNU (Day 5)	44	70	31–120

[a] IP, intraperitoneally; BCNU, bischloroethylnitrosourea (carmustine).