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Efficacy of BCNU and paclitaxel loaded subcutaneous implants in the interstitial chemotherapy of U-87 MG human glioblastoma xenografts

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Dedicated to Prof. Dr Wolfgang Wiegrebe, University of Regensburg, on the occasion of his 70th birthday

Abstract

Nude mice were challenged with human U-87 MG glioblastoma tumors to assess the efficacy of different cytostatics and different application protocols. While the intraperitoneal application of BCNU solutions (3 times 20 mg BCNU/kg) had no effect on tumor growth, the application of polymer matrices made of a physical mixture of poly(1,3-bis[carboxyphenoxpropane]-co-sebacic acid) 20:80 with poly(D,L-lactic-co-glycolic acid) loaded with 0.25 mg BCNU, slowed down the growth of tumors significantly. When the animals were treated with implants carrying 0.25 mg BCNU they responded to the treatment whether the tumor had been inoculated recently (9 days ago) or whether it was fully established (after 20 days). After its sensitivity was proven, the xenograft model was used to further investigate the efficacy of anticancer drugs and some treatment regimens using polymer implants. Thus the tumor model allowed to discriminate between the efficacy of different doses of BCNU. Only implants loaded with 0.75 or 1 mg of BCNU led to a substantial suppression of tumor growth over approximately 2 months. While BCNU was only able to suppress the growth of the tumor, the combination of BCNU with paclitaxel led to a complete remission in some animals. These preliminary results suggest that combinations of cytostatics might improve local chemotherapy of malignant glioma substantially. Based on our data it will be worthwhile to investigate implants that release drugs such as BCNU and paclitaxel closer. Amongst other factors we will try to elucidate the effect of repetitive doses of drugs using programmable implants. © 2002 Published by Elsevier Science B.V.

Keywords: Malignant brain tumor; Local therapy; Biodegradable polymer; Polyanhydride; BCNU; Paclitaxel

1. Introduction

An analysis of data of the Surveillance, Epidemiology, and End Results Program of the Na-

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tional Cancer Institute from 1977 to 1995 clearly shows that the incidence of high-grade glioma in the age group of 45 years and older continues to rise (Legler et al., 1999). The standard treatment for patients diagnosed with malignant glioma includes currently a combination of surgery, irradiation and chemotherapy (Krauseneck and Muller, 1994). After maximum tumor resection patients are irradiated with a cumulative dose of 60 Gy (Roth and Weller, 1999) and, depending on their overall physical condition, they undergo chemotherapy. Nitrosoureas like BCNU (1,3-bis(2-chloroethy)-1-nitrosourea) are, thereby, usually administered systemically because they are among the few substances that are able to cross the blood brain barrier in significant amounts (Rubin et al., 1983; Mitsuki et al., 1991; Feher et al., 2000). However, this form of therapy leads to severe side effects including myelosuppression, hepatic toxicity and pulmonary fibrosis due to the toxicity of the drug (Kornblith and Walker, 1988). Furthermore, BCNU has a plasma half-life of only about 20 min, which further limits its efficacy after systemic application (Loo et al., 1966).

One promising approach to overcome the disadvantages of systemic chemotherapy is a local therapy using biodegradable polymer implants (Brem, 1990) and microspheres (Menei et al., 1999) that are implanted directly into the cavity of a resected brain tumor (Brem et al., 1991) or injected into the tumor tissue. Thereby, high local drug concentrations are established while the systemic toxicity is low due to low systemic concentrations (Tamargo et al., 1993; Sipos and Brem, 1995; Walter et al., 1995). This approach seems promising, since 80–90% of malignant glioma recur within a 2 cm margin around the primary tumor, an area into which most of the drug is released (Hochberg and Pruitt, 1980). Biodegradable BCNU loaded polyanhydride wafers consisting of poly(1,3-bis[carboxyphenoxpropane]-cosebacic acid) 20:80 (p(CPP-SA) 20:80) were extensively tested for this application and are already commercially available under the brand name Gliadel®. Compared to the systemic standard therapy, the local implantation of Gliadel® was reported to improve a patient's quality of life substantially (Brem et al., 1995).

Despite the advantages of local chemotherapy, the median time of survival of patients that undergo this type of treatment is still approximately one year and only slightly longer than after receiving the standard therapy (Brem et al., 1995; Valtonen et al., 1997). We became, therefore, interested in finding new ways to increase the efficacy of local chemotherapy. As malignant gliomas are known to be refractive to therapy it was important to use an animal tumor model, which adequately reflects this clinical situation and avoids false positive results. On the other hand such a model should be sensitive enough to allow the discrimination of the efficacy of different treatment protocols.

We decided to investigate the efficacy of different implants in nude mice bearing subcutaneous human U-87 MG glioblastoma xenografts. As there is only limited in vivo data on U-87-MG tumors available from the literature (Pollack et al., 1996; Bradley et al., 1999; Joe et al., 1999; Hong et al., 2000) a first goal was to assess the sensitivity of these human brain tumor xenografts against BCNU, which is currently the 'gold standard' in local brain tumor chemotherapy. Once the sensitivity of the model had been proven we tested a number of approaches aiming at the improvement of the current local brain cancer therapy. First we were interested in assessing the efficacy of different treatment protocols starting not only immediately after tumor implantation but also at times when the tumor was fully established. Positive results would pave the way to using implants that are able to release several doses of drugs at different times. Second, as currently only low dosed BCNU implants are available for the clinical treatment of brain tumors, we investigated the efficacy of higher BCNU doses. Third, we tried to assess the efficacy of paclitaxel loaded implants. In addition to a monotherapy we also tested combinations with BCNU embedded into the polymer matrices. For all of these investigations we used only approved materials, that would allow immediately for clinical trials, provided that the preclinical data were promising.

2. Materials and methods

2.1. Materials

End-capped poly(D,L lactic-co-glycolic acid) 50:50 molecular weight 17 000 ($PLGA50_{17}$, Resomer RG502) was obtained from Boehringer Ingelheim (Ingelheim am Rhein, Germany). Sebacic acid and *p*-hydroxy benzoic acid were bought in analytical grade from Fluka Chemie AG (Buchs, Switzerland). 1,3-Dibromopropane, acetic acid, acetic anhydride, formaldehyde, picric acid, potassium sorbate and dichloromethane were purchased from Merck (Darmstadt, Germany). BCNU was obtained from Bristol-Myers-Squibb (Regensburg, Germany) and paclitaxel was bought from Synopharm (Barsbüttel, Germany). The human malignant glioblastoma-astrocytoma cell line U-87-MG was obtained from the American Type Culture Collection (Rockville, MD). Eagle's minimum essential medium (EMEM), $NaHCO_3$, sodium pyruvate and chloramphenicol were bought from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany). Foetal calf serum (south American origin) and Trypsin 0.05% (1:250)/0.02% EDTA was obtained from Life Technologies (Karlsruhe, Germany). T75 culture flasks (75 cm^2) were bought from Nunc (Wiesbaden, Germany). Ketamine (Ketanest®) was obtained from Parke-Davis (München, Germany) and Xylazin (Rompun®) was purchased from Bayer (Leverkusen, Germany). Eosin was bought from Chroma (Münster, Germany) and hematoxylin from Serva (Heidelberg, Germany). Breeding-maintenance diet 1434 was obtained from Altromin (Lage, Germany).

2.2. Methods

2.2.1. Polyanhydride synthesis

1,3-bis[*p*-carboxyphenoxpropane] was synthesized from 1,3-dibromopropane and *p*-hydroxy benzoic acid as described by Conix (Conix, 1966). Poly(1,3-bis[*p*-carboxyphenoxpropane]-co-sebacic acid) 20:80 [p(CPP-SA) 20:80] was synthesized as described by Domb and Langer 1987; Leong et al. 1987. In brief: 1,3-bis[*p*-carboxyphenoxpropane] and sebacic acid were activated with acetic anhydride. The resulting mixed anhydrides (prepolymers) were polymerized by melt polycondensation.

2.2.2. The incorporation of drug into the polymer

To enhance the mechanical stability of polyanhydrides that may suffer from high loading with cytostatics, a 1:1 (w/w) mixture of p(CPP-SA) 20:80 and $PLGA50_{17}$ was used for the manufacture of implants. The loading ranged from 3.57 to 14.29%. The drug and the polymer were dissolved in dichloromethane under vigorous vortex mixing resulting in a 10% (m/v) solution. The solvent was removed by vacuum drying using an RV5 two stage oil pump from Edwards, Crawley, Sussex, UK. After solidification the polymer was ground in a mortar to obtain a free flowing powder with a particle size of less than 400 μm.

2.2.3. Polymer matrix manufacturing

Drug loaded polymer matrix cylinders of 1.8 mm height and 2 mm diameter were manufactured by manual compression using a compression force of approximately 250 N for 10 s. For this purpose a set of 2 mm diameter cylindric punches and a die were machined from hardened steel and V4A steel, respectively. To obtain a maximum of uniformity, the polymer matrix cylinders were weighed after compression and cylinders with a mass in the range of 7 ± 0.05 mg were used for animal studies.

2.2.4. Animals and housing conditions

NMRI (*nu/nu*) mice were randomly bred in the nude mouse laboratory at the University of Regensburg under pathogen-free conditions at 26 °C, 70% relative humidity and a 12 h light/dark cycle (Spruß et al., 1996). The animals were fed ad libitum with combined breeding-maintenance diet 1434 and water containing 1.3 g/l potassium sorbate and 2 g/l chloramphenicol; pH was adjusted to 2.5 by adding 0.1 M HCl. A maximum of eight animals were kept in one cage. The average animal weight prior to the treatment was 30.9 ± 3.3 g.

2.2.5. Tumor cell line and cell culture conditions

U-87 MG cells were grown in EMEM containing 2.2 g/l $NaHCO_3$, 110 mg/l sodium pyruvate and 10% FCS. The cells were cultured at 37 °C, 95% relative humidity in an atmosphere containing 5% CO_2. They were kept in T75 cell culture flasks and serially passaged following trypsinization using a trypsin/EDTA solution.

2.2.6. Tumor inoculation and transplantation

For tumor cell implantation in nude mice, U-87 cells were harvested by trypsinization using a trypsin/EDTA solution. They were washed two times with 5 ml serum-free medium and centrifuged at 1000 rpm for 7 min at room temperature using a Minifuge T centrifuge from Heraeus Instruments GmbH (Hanau, Germany). The resulting cell pellet was resuspended in serum-free EMEM and the cell number was adjusted to approximately 5×10^6 cells per ml. Solid U-87 MG tumors were established in 6 week old NMRI (*nu*/*nu*) mice by subcutaneous injection of approximately 5×10^5 cells per animal. For the serial passage from animal to animal, established tumors were cut into 2 mm^3 pieces and transplanted subcutaneously into the thoracic region with a trocar (Spruß et al., 1996).

2.2.7. In vivo pharmacology

Immunodeficient NMRI (*nu*/*nu*) mice underwent subcutaneous implantation of human U-87 MG glioblastoma tumors into the right flank. For i.p. application of BCNU an ethanolic stock solution containing 80 mg drug/ml was prepared, which was diluted 1:20 with sterile isotonic saline immediately before administration (100 μl per 20 g body weight) using a 1 ml disposable syringe. Animals in the corresponding control group were treated with the vehicle i.e. 5% ethanol in isotonic saline. Sham operated mice and mice receiving drug-free implants served as control groups. 6, 7, 9 or 20 days after tumor implantation the animals were anesthetized with a combination of 100 mg/kg ketamin with 4–6 mg/kg xylazin. Drug loaded matrices as well as drug free placebos were implanted next to the tumor. The wound was closed with surgical clips and the animals were returned to the housing facility where they had free access to food and water. Mice were randomly assigned to control or treatment groups. The number of animals per treatment group ranged from 4 to 9. The tumor size and the weight of the animals were measured twice a week until the animals were killed by cervical dislocation. Tumor growth kinetics were recorded by measuring tumor diameters with an electronic caliper. Tumor areas were calculated as the product of two perpendicular diameters, of which one represents the largest possible diameter of the tumor. The ratio T/C of the tumor area in a treatment group (T) and in the corresponding control group (C) served as a measure for the efficacy of a treatment regimen.

2.2.8. Histology

Tumors as well as surrounding connective tissue and adhering skin regions were fixed in Bouin's solution and prepared for routine paraffin histology. Sections were stained according to the Masson and Goldner method modified by Jerusalem (Romeis, 1989).

2.2.9. Statistical analysis

The experimental data were evaluated by one-way analysis of variance (ANOVA) ($\alpha = 0.05$) in conjunction with Scheffe's post hoc range test. The resulting P-values designate the level of significance. When they are given in the manuscript for a T/C ratio, they reflect the significance of the difference between a treatment group (T) and a control group (C).

3. Results and discussion

We carried out three different sets of in vivo experiments. In a first study we explored the sensitivity of the developed animal model. In a second and third tumor pharmacological study, we investigated the efficacy of different implants, loaded either with BCNU or paclitaxel alone or combinations thereof. Tables 1–3 give a survey on the individual groups in these studies.

3.1. Characterization of the subcutaneous U-87 MG glioblastoma model with respect to BCNU chemosensitivity (study 1)

First, we compared the sensitivity of human brain tumor xenografts in a subcutaneous nude mouse model against BCNU. We administered an intraperitoneal dose of 20 mg/kg on day 1, 6, and 10 or implanted polymer matrices that were loaded with 3.6% (w/w) BCNU which is equivalent to a dose of 0.25 mg nitrosourea per implant.

In the control and the placebo group the U-87 MG glioblastoma grew progressively reaching on day 30 post tumor transplantation a tumor area of 132 ± 54 mm^2 and 161 ± 53 mm^2, respectively. Subcutaneous implantation of the BCNU loaded polymer matrices at the tumor site resulted in tumor regression with T/C-values of approximately $54.2 \pm 28.2\%$ on day 30 (average value obtained for group 4 and 5 in Table 1). By contrast, there was no statistically significant effect on tumor growth, when BCNU was administered intraperitoneally, although the dose of BCNU applied per individual via this route was approximately 8 times higher.

The results also showed that even though the tumor was large and, therefore, difficult to treat (DeVita, 1983) after 20 days, the treatment protocol applied to group 5 (Table 1) changed the exponential tumor growth kinetics obtained for the control group to a sigmoidal one (data not shown). Starting the treatment at day 9 after tumor inoculation (group 4) resulted in T/C values of $58.2 \pm 31.8\%$ ($P = 0.14$). With T/C = $50.1 \pm 25.5\%$ ($P = 0.09$) almost the same result was obtained when the matrices were implanted on day 20 (group 5).

Altogether these results suggested that the tumor was sensitive only against local administration of BCNU at the tumor site and that the sensitivity persisted with time. That the peritoneal application of BCNU is not potent enough to affect the tumor growth may have several reasons. From preliminary cell culture studies we knew, that BCNU was not active in a 2-dimensional culture of U87 MG cells unless it was applied via a polyanhydride release system such as p(CPP-SA) 20:80 (Altenschöpfer, 1998). There are two potential explanations for this phenomenon. The polyanhydride might stabilize BCNU by creating an almost anhydrous environment that protects BCNU inside the matrix from degradation (Brem and Gabikian, 2001). Alternatively it could stabilize BCNU via acidification since pH drops to values around 4 inside pores of the polymer matrix during erosion (Goepferich and Langer, 1995; Maeder et al., 1997), where BCNU is known to have a stability maximum. Either way the results illustrate that the activity of BCNU is highest when applied locally via an implant that provides additional stabilizing mechanisms.

However, despite the encouraging results that we obtained by local chemotherapy using BCNU loaded implants, the T/C values indicated that there is still a substantial need for higher efficacy. Human clinical trials with low doses of BCNU also suggest that higher doses may have higher antitumor activity. Polymer matrices loaded with 3.85% BCNU (Gliadel®) resulted only in a moderate increase of survival of glioma patients compared to systemic therapy (Brem et al., 1995). In accordance with the literature (Olivi et al., 1998) we, therefore, hypothesized that applying higher doses of BCNU might be more effective in local chemotherapy of malignant glioma.

Table 1
Survey on the design of the in vivo studies (study 1: characterization of the tumor model)

Group ($n = 9$)	Type of treatment	Dose	Dose per body weight $\pm$ SD (mg/kg)
Group 1	Control	–	–
Group 2	BCNU, i.p., on day 1, 6 and 10	20 mg/kg	20 ± 2.6
Group 3	Placebo implant	–	–
Group 4	BCNU implant on day 9	0.25 mg	8.7 ± 1.4
Group 5	BCNU implant on day 20	0.25 mg	8.9 ± 2.1

Table 2
Survey on the design of the in vivo studies (study 2: antitumor activity of high dose BCNU and paclitaxel implants)

Group	Type of treatment	Dose per implant (mg)	Dose per average body weight (mg/kg)
Group 1 ($n = 9$)	Control	–	–
Group 2 ($n = 8$)	Placebo	–	–
Group 3 ($n = 8$)	BCNU	1.00	30.1 ± 2.8
Group 4 ($n = 8$)	Paclitaxel	1.00	31.0 ± 1.4
Group 5 ($n = 8$)	BCNU	0.5	15.4 ± 1.5
	Paclitaxel	0.5	15.4 ± 1.5

3.2. Antitumor activity of high dosed BCNU and paclitaxel implants (study 2)

In this second study we investigated a number of approaches towards an improved local chemotherapy. A basic rule in cancer chemotherapy seems to be that a given dose of drug kills a constant fraction of cells, (Schabel, 1964). Chemotherapy can, therefore, be improved substantially when higher doses of drugs are applied to increase the fractional kill or when the treatment is started early during the development of a tumor (Schabel, 1964). Based on this hypothesis and on the results that we obtained with our first study, we applied higher doses of BCNU. However, since only 1/3 of patients with glioblastoma multiforme respond to such a BCNU treatment (Brem and Gabikian, 2001), the search for alternative drugs is deemed mandatory. Despite its low activity in systemic therapy (Chamberlain and Kormanik, 1995; Rosenthal et al., 2000), which is most likely related to its poor ability to cross the blood brain barrier (Glantz et al., 1995), paclitaxel was effective against glioma cells in vitro (Cahan et al., 1994) as well as malignant glioma in rats after local administration (Walter et al., 1994). Therefore, paclitaxel seemed to be a promising candidate. As combinations of drugs were found to be of substantial value for brain tumor chemotherapy (Brem and Gabikian, 2001) we also tested combinations of BCNU with paclitaxel.

The in vivo study outlined in Table 2 revealed that tumor growth was delayed substantially in all groups treated with drug loaded implants (groups 3–5) compared to the control or placebo group (group 1 and 2) (Fig. 1a). Like in the previous study, no significant difference in tumor growth existed between control group and placebo group (group 2) ($P = 0.967$). The U-87 MG glioblastoma grew again substantially, reaching a tumor area of 233 ± 118 and 208 ± 65 mm^2, respectively, on day 27 post tumor transplantation. The subcutaneous delivery of 1 mg BCNU (group 3), in contrast, resulted in an approximately 16-fold tumor size reduction compared to the sham operated mice (group 1) after 27 days ($T/C = 6.3\% \pm 4.0\%$, $P < 0.001$). This was a significant improvement over the therapy with low BCNU doses that showed a less pronounced tumor size reduction after the same time.

The study also revealed that the subcutaneous delivery of 1 mg paclitaxel (group 4) resulted only in a 3-fold tumor size reduction compared to the control group ($T/C = 31.1 \pm 27.7\%$, $P = 0.001$). The histological examination of excised tumor tissue gave evidence that paclitaxel was released in an active form. Histological sections revealed the presence of multinucleated tumor cells which is typical of paclitaxel (Fig. 2a and b) (Roytta et al., 1987).

The local delivery of paclitaxel in combination with BCNU (group 5) resulted in a 9-fold tumor size reduction after 27 days compared to sham operated animals ($T/C = 10.9 \pm 13.0\%$, $P < 0.001$). The fact that only 0.5 mg BCNU were delivered by these implants might explain why the tumor size reduction was less than under the monotherapy with 1 mg BCNU (group 3). However, paclitaxel seems to contribute to the overall tumor size reduction as well. Multinucleated cells could again be detected in tissue sections, although they were less frequent than in sections taken from mice treated with 1 mg of paclitaxel (group 4) alone (Fig. 2b and c).

To investigate the long term effect of implants on tumor growth, the tumor size of group 3 and 5 was followed until day 64 post implantation. Tumors treated with 1 mg BCNU had still not relapsed after 2 months and had still an average size that was not larger than at the beginning of the therapy. The implants loaded with a combination of both cytostatics resulted in a tumor area that was half the size of the tumors in the control group. Compared to the results with group 3 the less pronounced therapeutic effect can be explained by the lower dose of BCNU. To assess the long term effect of the therapy on the physical condition of the animals, we recorded their mean body weight (Fig. 1b). The constant values suggest that the therapy did not negatively affect the animals and can, therefore, be considered as well tolerated.

3.3. Antitumor activity of implants loaded with combinations of paclitaxel and BCNU (study 3)

The two previous studies showed that established tumors could be treated successfully with BCNU or paclitaxel alone or with a combination of both drugs. The focus in this third study was on the further investigation of the antitumor activity of implants loaded with a combination of paclitaxel and BCNU. Three groups of animals treated with 0.25 or 0.75 mg BCNU or 1 mg paclitaxel served as positive controls. The study design is summarized in Table 3.

The results (Fig. 3) showed again that applying high doses of BCNU (group 4) is significantly more effective than low doses of BCNU (group 3) ($P = 0.046$). The subcutaneous delivery of 0.75 mg BCNU alone (group 4) resulted in an approximately 9-fold reduction of tumor size compared to the control group ($T/C = 11.7 \pm 12.7\%$, $P < 0.001$). The tumor growth reduction that resulted from the delivery of paclitaxel or low doses of BCNU were similar to those seen in the previous studies. Implants loaded with a combination of 0.75 mg BCNU with 1 mg paclitaxel (group 7) proved to be most effective in reducing tumor size and were superior to monotherapy (group 4 and 5). Tumor growth could be reduced approximately 19-fold ($T/C = 5.3\% \pm 8.8\%$, $P < 0.001$). The tumor growth kinetics that resulted from the treatment with 0.25 mg BCNU in combination with 1 mg paclitaxel (group 6) suggests that the contribution of low BCNU doses to the overall tumor size reduction is moderate. The tumor growth kinetics and the 3.1-fold tumor size reduction ($T/C = 32.2 \pm 34.8\%$, $P = 0.003$) compared to the control group were similar to results obtained with 1 mg paclitaxel alone (group 5). Again no difference in tumor growth ($P = 1.0$) could be seen between the placebo (group 2) and the control group (group 1).

The mean body weight served again as a measure for the physical condition of the animals and the toxicity of the treatment and was recorded for the entire period of investigation. Fig. 3b reveals that an overall slight reduction in the mean body

Table 3
Survey on the design of the in vivo studies (study 3: antitumor activity of implants loaded with combinations of paclitaxel and BCNU)

Group	Type of treatment	Dose per implant (mg)	Dose per average body weight (mg/kg)
Group 1 ($n = 8$)	Control	–	–
Group 2 ($n = 4$)	Placebo	–	–
Group 3 ($n = 8$)	BCNU	0.25	8.4 ± 1.8
Group 4 ($n = 8$)	BCNU	0.75	24.2 ± 1.7
Group 5 ($n = 8$)	Paclitaxel	1.00	31.6 ± 4.6
Group 6 ($n = 8$)	Paclitaxel	1.00	32.7 ± 2.9
	BCNU	0.25	8.2 ± 1.5
Group 7 ($n = 8$)	Paclitaxel	1.00	32.5 ± 2.9
	BCNU	0.75	24.4 ± 2.6

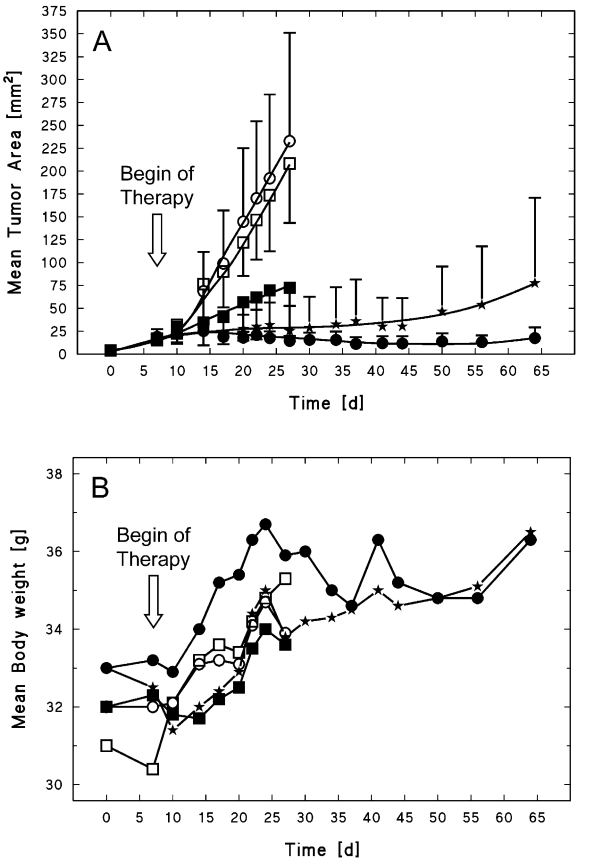

Fig. 1. Effect of high dosed BCNU and paclitaxel implants on human U-87 MG glioblastoma xenografts (bars refer to the standard error of the mean). (A) Tumor growth curves. (B) Change of mean body weight. Sham operation (○); placebo implant (□); homogeneous implant loaded with 1 mg paclitaxel (■); homogeneous implant loaded with 1 mg BCNU (●); homogeneous implant loaded with 0.5 mg BCNU plus 0.5 mg paclitaxel (★).

weight could be observed throughout all treatment groups. This was considered of being tolerable since the animals showed no changes in their normal behavior patterns.

At a first glance implants loaded with 1.0 mg BCNU or combinations of 0.75 mg BCNU with 1.0 mg paclitaxel resulted in similar tumor growth kinetics (Figs. 1 and 3). However, substantial discrepancies (Fig. 4a and b) became apparent when we compared the tumor growth in individual animals. When mice were treated with 1.0 mg of BCNU they showed similar tumor growth curves with little interindividual variation (Fig. 4a). For approximately 2 months individual tumors grew almost at identical velocity resulting in tumors that were of the same size or even smaller compared to the beginning of the therapy. After approximately 80 days however, the tumors started to grow again progressively. By contrast,

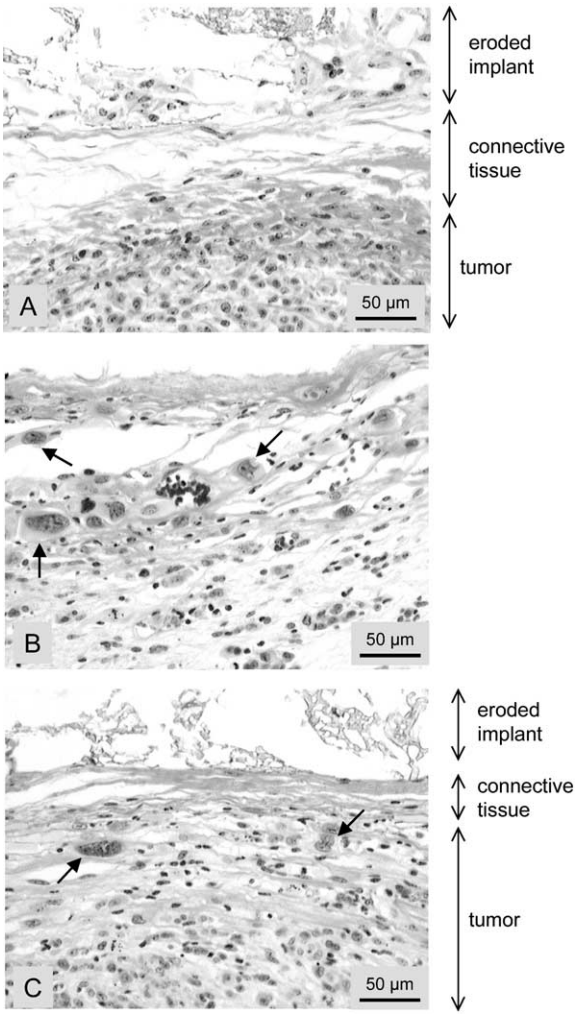

Fig. 2. Effect of local treatment with implants loaded with (A) BCNU, (B) paclitaxel or (C) a combination of paclitaxel/BCNU on the microarchitecture of subcutaneous U-87 MG tumors. To prepare the Mason–Goldner stained sections, the tissue samples were collected from individuals of the respective treatment groups during the course of study 2. The arrows indicate multinucleated tumor cells, with typical paclitaxel induced appearance (Roytta et al., 1987).

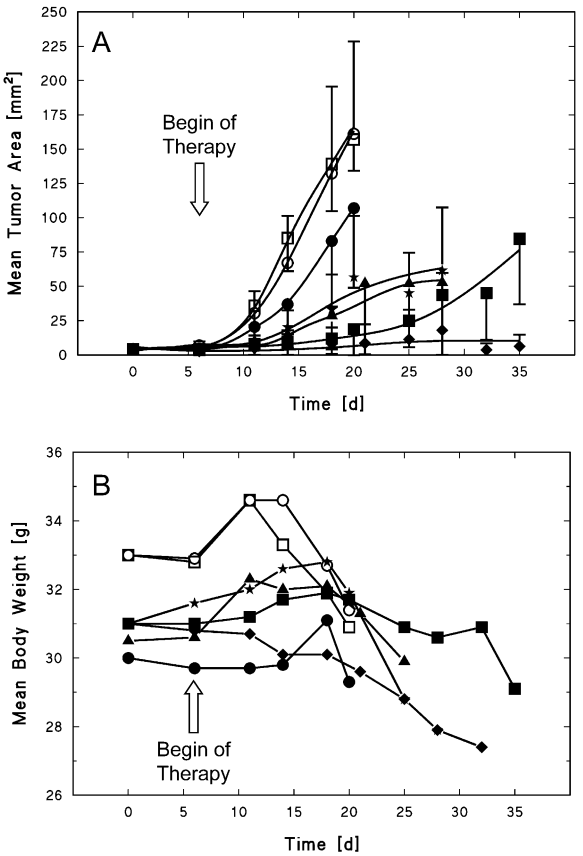

Fig. 3. Antitumor activity of implants loaded with combinations of paclitaxel and BCNU against U-87 MG glioblastoma xenografts (bars refer to the standard error of the mean). (A) Tumor growth curves. (B) Change of mean body weight. Sham operation (○); placebo implant (□); homogeneous implant loaded with 0.25 mg BCNU (●); homogeneous implant loaded with 0.75 mg BCNU (■); homogeneous implant loaded with 1 mg paclitaxel (★); implant loaded with 0.25 mg BCNU plus 1 mg paclitaxel (▲); implant loaded with 0.75 mg BCNU plus 1 mg paclitaxel (◆).

animals treated with a combination of 1 mg paclitaxel with 0.75 mg BCNU (Fig. 4b) were subject to tremendous interindividual variations. While in some mice the tumor started to grow exponentially already after 10 and 30 days post tumor inoculation some animals showed complete remissions.

From these results we conclude that a treatment with 1.0 mg BCNU per animal is most effective. In the light of the remissions that we obtained when the animals were treated with a combination of BCNU with paclitaxel, this approach seems to be also very promising and will be the subject of further investigations. In future studies special attention will be paid to the treatment not only with monolithic drug loaded implants but also to the application of implants with programmable drug release that were recently developed (Vogelhuber et al., 2001). Such devices could be loaded with a combination of BCNU and paclitaxel, which could then not only be released simultaneously but according to a time schedule. Although the benefit of such an approach for an in vivo therapy has still to be explored, it would certainly broaden the range of potential treatment regimens for local chemotherapy of brain cancer.

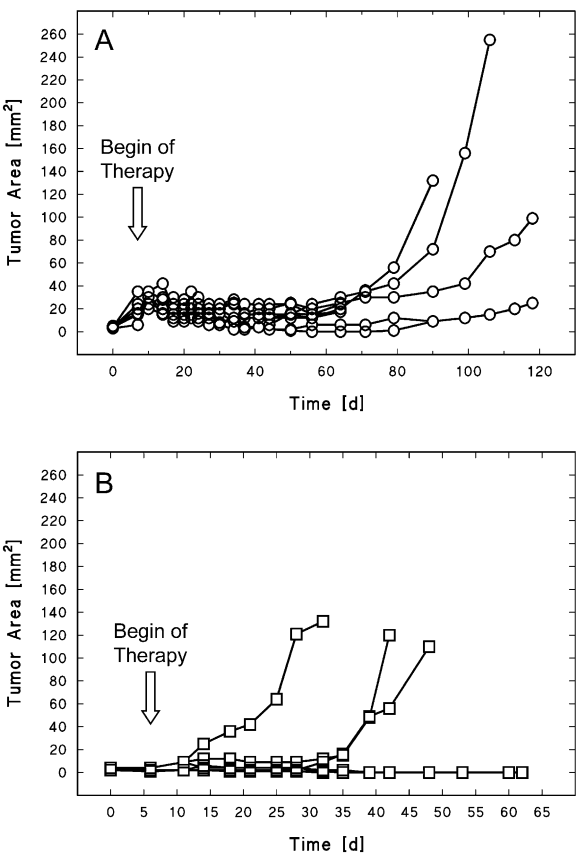

Fig. 4. Tumor growth curves of individual animals treated with implants loaded with 1 mg BCNU (A) and a combination of 1 mg paclitaxel with 0.75 mg BCNU (B).

4. Conclusions

We established successfully U87-MG tumors subcutaneously in nude mice. The xenograft model allowed to assess the efficacy of different drugs as well as different application protocols.

While there was no effect on tumor growth, when BCNU was administered intraperitoneally, biodegradable polymer implants loaded with BCNU affected the growth of the tumors significantly. We found that the tumors responded to a local therapy with BCNU implants even when the tumor had been fully established and reached a size that was several times larger than its original one. These results suggest that implants might be useful that can release several doses of cytostatics (Vogelhuber et al., 2001).

The model was very useful to investigate the effect of BCNU depending on the dose administered. Although increasing the dose of BCNU resulted in a more substantial regression of the tumor over a period of approximately 2 months, we did not observe that animals went into a complete remission with any BCNU implant. This is in an overall good agreement with clinical observations from the treatment of humans. Therefore, we conclude that our model was well balanced in a sense that it did not yield false positive results but was sensitive enough to discriminate results obtained from different treatment protocols. Individual tumor growth curves, for example, revealed that implants loaded with paclitaxel and BCNU led to a complete remission in some animals. More research will be necessary to elucidate the underlying mechanism and to confirm this encouraging result. In future work more attention will be paid to implants which release both drugs in a defined sequence.

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