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Original Paper

Enhancement of ACNU Treatment of the BT_4An Rat Glioma by Local Brain Hyperthermia and Intra-arterial Drug Administration

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To evaluate the role of intra-arterial (i.a.) chemotherapy, intravenous (i.v.) chemotherapy, and local brain hyperthermia in the treatment of gliomas, the effect of i.v. versus i.a. drug delivery, with or without local brain hyperthermia, was evaluated in BD IX rats with BT_4An gliomas implanted in the right frontal lobe. The rats were given ACNU 18 mg/kg i.a. in the right carotid artery or i.v. in the inferior cava with or without local microwave hyperthermia at 42.4°C for 45 min. ACNU i.v. alone had no notable effect on survival. Survival was prolonged when ACNU without hyperthermia was given i.a. instead of i.v. ($P < 0.05$). Thermochemotherapy with ACNU i.a. was more effective than with ACNU i.v. ($P < 0.01$). Survival improved as hyperthermia enhanced the i.v. drug effect ($P < 0.01$), and hyperthermia also improved the i.a. ACNU effect ($P < 0.01$). Post-treatment survival was more than doubled for the group given combined i.a. ACNU and hyperthermia, compared to controls. Thermochemotherapy, particularly with i.a. drug administration, seems to be a promising new approach for the treatment of primary brain tumours. However, more knowledge about tolerance of human brain tissue to hyperthermia is necessary before this treatment modality is used in patients with a reasonable life expectancy.

Key words: ACNU, brain tumour, glioma, hyperthermia, intra-arterial, nimustine
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INTRODUCTION

LACK OF tumour control within the skull is the principal reason for fatal outcome in patients with brain gliomas. Surgery and radiotherapy are in most cases insufficient to achieve permanent local tumour control. Chemotherapy has, therefore, been investigated as adjuvant treatment [1–5]. Primary brain tumours generally show moderate drug sensitivity, and high levels of cytotoxic drugs outside the tumour area may contribute to severe side-effects [6, 7]. Attempts to reduce the systemic and contralateral brain exposure have been pursued by intra-arterial (i.a.) infusion of cytotoxic drugs, especially with drugs having a high first pass uptake, such as the nitrosoureas [4, 5, 8–10]. However, brain and eye toxicity limit the drug doses that can be given [11–14].

Treatment of human primary brain tumours with hyperthermia and interstitial radiotherapy has already been attempted [15, 16], while experience with combined hyperthermia and chemotherapy is scarce [17]. Hyperthermia has been shown to enhance the cytotoxic effect of several chemotherapeutic drugs [18], among them the nitrosoureas used in the treatment of primary brain tumours [19, 20]. A combination of these two modalities could induce an enhanced local effect in the tumour area, with reduced cytotoxicity in the rest of the brain and body.

To investigate treatment with combined i.a. chemotherapy and local hyperthermia, a method for i.a. treatment was developed in an already established rat brain tumour model [21], suitable for treatment with local microwave hyperthermia [22]. The catheterisation procedure alone has been found to induce a weight loss of about 7%, and was without procedure related mortality [23]. Animals without tumour, treated with ACNU i.a. regained pretreatment weight after 20–30 days, and behaved normally for several months. However, 2–3 months after treatment, the rats developed partial alopecia and whitening of the remaining hairs in the area on the right side of the scalp, supplied by branches of the pterygopalatine artery, a branch of the right internal carotid artery. This area had been exposed to the same first pass effect of i.a. ACNU as the frontal part of the right cerebral hemisphere, supplied by the other branches of the right internal carotid artery.

ACNU has been reported to have few eye complications [2], probably due to its water solubility, making ethanol-containing vehicle unnecessary. It was effective when combined with heat in the BT_4An subcutaneous tumour model [20]. ACNU was

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superior to BCNU in the treatment of xenografts of astrocytomas and oligodendroglioma in a nude mice model [3]. Studies comparing intravenous (i.v.) and i.a. ACNU treatment of rats with brain tumours, showed increased drug uptake in the tumour area after i.a., compared to i.v., administration [10]. Because of these factors, ACNU was chosen instead of BCNU [22] for the present study.

Previous experiments have shown a substantial effect of combined i.a. ACNU and hyperthermia, but no significant effect of each modality when used alone [23]. Although there are good indications of a drug dose-dependent thermochemotherapy effect in other settings [24, 25], there are practically no data comparing i.v. and i.a. administration of the same drug dose. The role of i.v. versus i.a. infusion of ACNU with or without hyperthermia was, therefore, studied in experiments using the treatment model described above.

MATERIALS AND METHODS

Animals and tumour

Male BD IX rats with an average weight of 381 g (range 293–441 g) were used. The tumour implantation procedure has been previously described [21]. Briefly, exponentially growing BT_4An-cells were trypsinised, counted, and 10^5 cells were diluted in a 10 μl volume. This volume was injected with a cone-pointed Hamilton syringe through a cranial burr hole made by a dental drill at the right coronal suture, 4 mm lateral to the sagittal suture. The syringe had a stopper device at 4 mm depth. The tumour cell injection, therefore, initiated tumour growth just below and in the cortex of the right frontal lobe.

Treatment groups

The animals were randomised to each of five treatment groups: controls (10 rats) vehicle only, i.v. ACNU alone (10 rats); i.a. ACNU alone (10 rats); i.v. ACNU and hyperthermia (11 rats); and i.a. ACNU and hyperthermia (11 rats).

In previous experiments [23], hyperthermia produced with the same procedure as in the present study (treatment temperature 42.4°C and duration 45 min), had no effect on survival after treatment/sham procedure (average survival: 14.0 days for controls, 14.8 days after hyperthermia). Therefore, no group was treated with hyperthermia alone in the present experiment.

Anaesthesia

Between 15 and 20 min before the start of the tumour implantation or treatment, 1.0 ml/kg of a solution of midazolam 1.25 mg, fentanyl 0.05 mg and fluanisone 2.5 mg/ml was given, with equal parts intramuscularly (i.m.) and subcutaneously (s.c.). When necessary, anaesthesia was maintained with half of the initial dose.

Before treatment, 1 ml/kg of atropine 0.06 mg/ml was given s.c. to prevent bronchial and laryngeal spasm. Half of the initial dose was given after treatment, before removal of the catheters.

After treatment, flumazenil 0.01 mg was given i.m. to prevent respiratory depression, and the corticosteroid drug betamethasone (0.90 mg, Celeston Chronodose, Schering-Plough, Kenilworth, New Jersey, U.S.A.) was given s.c. to prevent brain oedema to all animals including controls. In the betamethasone solution, half the dose is rapidly acting, and the rest is salts with prolonged action. Previous experiments showed no influence of the drug on symptom-free survival [22].

Bronchial and laryngeal spasm, respiratory depression and brain oedema were all frequent problems during the development of this treatment model, but vanished after introduction of atropine, flumazenil and betamethasone in the anaesthesia procedure [23].

Catheterisation procedure

Polyethylene catheters (Portex PP 25, outer diameter 0.62 mm) were used. To reduce the diameter of the part of the catheter introduced i.a. or i.v., this part was heated in warm water, stretched as far as possible and the distal end cut off. The catheter was then filled with heparin 100 I.E./ml.

The position and fixation of the i.a. catheters inserted in all animals, are shown in Figure 1. The skin incision was made in the midline from 5 mm caudal of the lower jaw to the jugulum. The right part of the sternohyoid muscle was divided just caudal to the hyoid bone. The right common carotid artery was identified lateral to the trachea, and then carefully mobilised. The blood flow was stopped by a clip 8–9 mm caudal to the carotid bifurcation. Access was then established to the carotid bifurcation and the proximal parts of the internal and external carotid artery. The external carotid artery was ligated 5 mm cranial to the bifurcation, and the internal carotid artery blocked by a metallic clip 3 mm cranial to the bifurcation. Care was taken to avoid damage to the vagal nerve. The catheter was inserted through an opening in the external carotid artery down to the clip on the common carotid artery. The catheter was fixed in this position by a suture. Removal of the clips on the common and internal carotid arteries allowed blood flow along the catheter to the internal carotid artery (Figure 1). In all animals, including controls, a catheter was introduced 22 mm into the left femoral vein to the inferior cava. The skin wounds were then closed by tape during treatment. After treatment, the catheters were removed, and the external carotid artery and the left femoral vein were ligated.

ACNU

1-(4-amino-2-methylpyrimidine-5-yl) methyl-3-(2-chloro-ethyl)-3-nitrosourea was dissolved in sterile water to a 5 mg/ml solution, prepared daily and stored at 5°C in darkness. During develop-

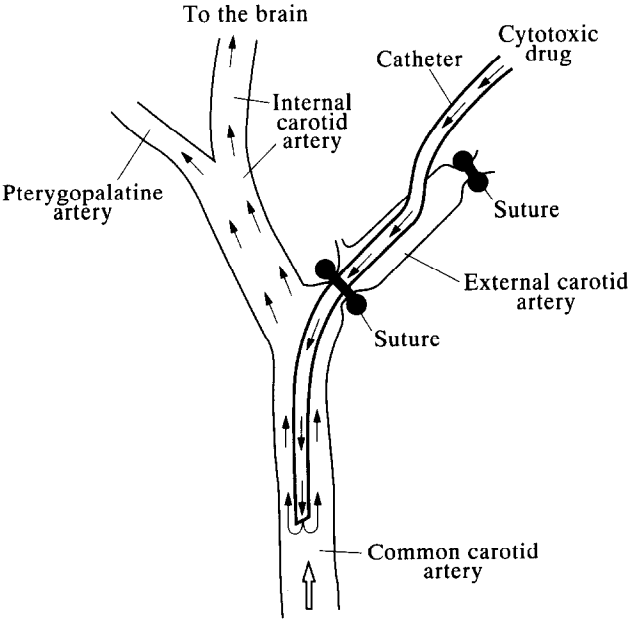

Figure 1. Position of catheter in the carotid artery during infusion of ACNU.

ment of the i.a. chemotherapy method, toxicity was evaluated when increased doses of ACNU were administered. Based on these data, and toxicity data from the first experiment with i.a. ACNU where 15 mg/kg was used [23], the ACNU dose was increased to 18 mg/kg in the present study. During infusion of ACNU, the animal was lying free in a prone position, to avoid any pressure on the catheter and the carotid arteries.

A precision-controlled syringe pump (Terumo STC-521) with 1 ml syringes was used. The infusion period was 18 min. This was divided into three periods of 6 min, the infusion rates in these periods were 0.875, 1.0 and 1.125 mg/kg min, respectively. The position of the head was altered slightly between each 6 min period. The change in infusion rate, and head position was used to avoid the well recognised phenomenon of marginalisation, that is drug streaming along the wall of the vessel due to laminar blood flow in a high pressure flow system. Marginalisation may result in uneven drug distribution at the branching of the artery.

Hyperthermia

The animals were fixed in a restraining frame, and hyperthermia was induced by microwaves from the BSD-1000 clinical hyperthermia system. A Bowman temperature probe was inserted through the cranial burr hole at a 45° angle with the horizontal plane, in a lateral to medial direction. The probe had a stopper device 4 mm from the end, bringing the temperature-sensitive tip of the probe to approximately 2.5 mm into brain tissue, in the medial periphery of, or just medial to, the tumour [22]. A locally designed applicator with a physical aperture of 2 × 3 cm closed with silicone rubber was operated at 700 MHz. The applicator was placed with its centre above the burr hole. Through the applicator, deionised water (32.1°C, range 31.5–32.7°C) was continuously circulated to achieve surface cooling by direct contact with the scalp. Hyperthermia was continuously controlled by the temperature measured at the tip of the Bowman probe. The desired temperature during treatment was 42.4°C, according to reports on threshold values of normal brain tolerance to hyperthermia [26], and previous experience in the present brain tumour model [22, 23]. In all animals, the desired temperature was reached within 8 min after the start of microwave heating. The average temperature during the 45 min treatment period was 42.3°C in both groups given hyperthermia.

All animals not treated with thermochemotherapy underwent sham procedures, where temperature probes were inserted into the brain tumour area, and the hyperthermia applicator without circulating cooling water was placed in position for 2 min. Earlier studies have shown that a 45 min sham procedure had no effect on survival, and this shorter procedure was used for practical reasons.

During hyperthermia or sham procedure, the rectal temperature was measured, and maintained between 35 and 37°C by covering the animal in a cotton sheet if necessary.

Treatment procedure

Treatment was carried out 10–12 days after tumour implantation. The rats were anaesthesised and the scalp skin incision from the tumour implantation re-opened to give access to the burr hole. Both the i.v. and i.a. catheters were inserted in all animals and ACNU was infused i.v. or i.a. The rats were fixed in a restraining frame immediately after infusion. A Bowman probe was then inserted, and microwave hyperthermia was given or sham procedure for hyperthermia carried out. The catheters were removed and the incisions closed. To prevent post-treatment hypothermia [23], the rats were kept in an isolette at 24°C until waking.

Treatment evaluation

Weight loss, symptoms from the right eye, and signs of neurological disturbance of the right hemisphere were registered to evaluate treatment toxicity. Eye toxicity was graded 1 when signs of irritation were present but not permanent, with no macroscopic disturbances of the cornea. Toxicity was graded 2 if opaqueness of the cornea was present. Neurological toxicity was evaluated 24 h after treatment. It was graded 1 when walking was normal, but spontaneous adduction of the left forefoot was present when the animal was elevated from the ground. Presence of atactic gait was evaluated as grade 2 neurological toxicity.

Animals developing signs of tumour recurrence, such as passivity, weight loss or reduced co-ordination of movement, were killed by CO_2 inhalation. Time from treatment to the first indication of symptom development, and to killing of the animals, were noted.

After CO_2 inhalation, a small mark with India ink was made on the brain surface of the right frontal lobe through the cranial burr hole. The brain was removed and cut in a coronal plane through the coloured mark in the brain surface. The greatest coronal diameter and the depth of the tumour were measured. If the tumour reached the brain surface, the greatest diameter and the perpendicular diameter on the surface were measured.

Statistics

Survival and toxicity data were analysed statistically by the Mann–Whitney test.

RESULTS

Survival of the rats after treatment is shown in Figure 2 and Table 1. ACNU i.v. had no notable effect on survival. ACNU i.a. alone improved survival compared to controls and animals treated with ACNU i.v. ($P < 0.05$ for both).

Combining hyperthermia with chemotherapy improved treatment outcome, both for i.v. and i.a. drug administration ($P < 0.01$). However, survival was significantly longer if hyperthermia was combined with i.a. rather than i.v. ACNU ($P < 0.01$), with more than a doubling of post-treatment survival in animals given thermochemotherapy with i.a. ACNU compared to controls. Treatment statistics from the thermochemotherapy groups showed no notable difference concerning average temperature during treatment (42.3°C), or energy input during hyperthermia treatment between the groups given ACNU i.a. or i.v.

Differences in time from onset of slight symptoms to death, and volume, position and macroscopic appearance of tumour after death, were not significant (Table 1).

There was no mortality due to treatment toxicity. The animals were in good general health the day after treatment. In the thermochemotherapy groups, weight loss came later ($P < 0.05$) and was more pronounced ($P < 0.01$) in animals receiving i.a. chemotherapy than in those given ACNU i.v., while the addition of hyperthermia to i.a. or i.v. chemotherapy had no significant influence on weight loss (Table 1).

No eye toxicity occurred. Five animals had grade 1 neurological toxicity, one treated with i.a. ACNU alone, two with i.v. ACNU and hyperthermia, and two with i.a. ACNU and hyperthermia.

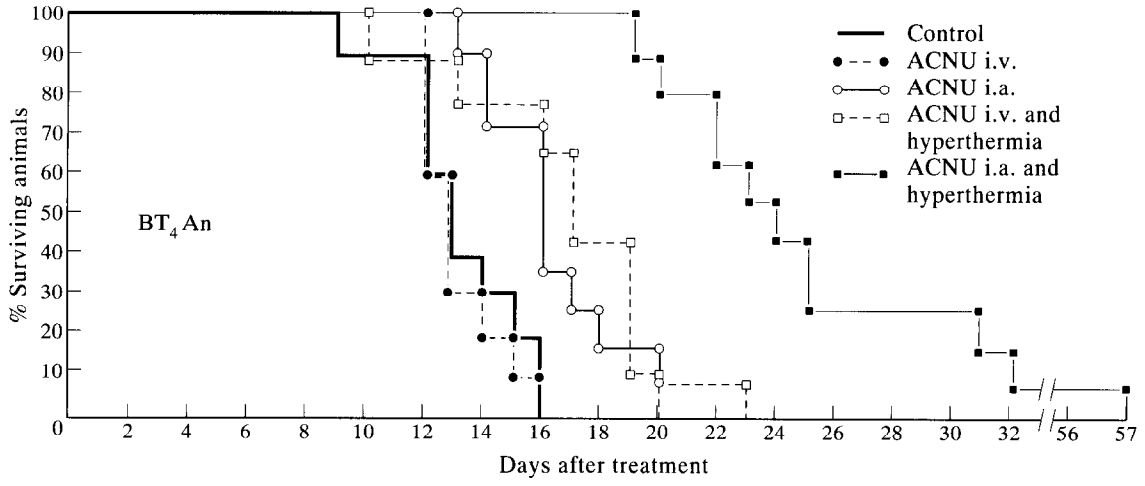

Figure 2. Survival of rats in the different treatment groups after treatment/sham procedures.

Table 1. *Survival and tumour data*

	Days from treatment to symptoms	Days from treatment to death	Relative nadir weight	Days from treatment to nadir weight	Tumour coronal diameter (mm)	Tumour depth (mm)	Greatest tumour diameter (surface) (mm)	Tumour perpendicular diameter (surface) (mm)
Control	12.1 ± 0.7	13.3 ± 0.7	0.90 ± 0.018	7.7 ± 1.1	7.4 ± 0.34	6.7 ± 0.26	9.0 ± 0.37	7.7 ± 0.21
ACNU i.v.	12.1 ± 0.5	13.1 ± 0.5	0.87 ± 0.016	8.1 ± 0.54	6.8 ± 0.25	6.3 ± 0.30	7.9 ± 0.43	6.9 ± 0.23
ACNU i.a.	15.0 ± 1.2	16.2 ± 1.1	0.83 ± 0.020	10.3 ± 0.97	7.2 ± 0.43	5.9 ± 0.31	8.4 ± 0.50	7.7 ± 0.37
ACNU i.v. + HT	15.5 ± 0.9	16.5 ± 0.9	0.88 ± 0.011	7.3 ± 0.58	7.5 ± 0.41	6.6 ± 0.34	8.0 ± 0.47	7.4 ± 0.43
ACNU i.a. + HT	25.9 ± 3.2	26.9 ± 3.2	0.82 ± 0.012	9.6 ± 0.82	6.9 ± 0.66	5.8 ± 0.44	8.0 ± 0.99	6.8 ± 0.64

The data are mean values ± standard error of the mean. i.v., intravenous; i.a., intra-arterial; HT, hyperthermia.

DISCUSSION

Both for BCNU [27] and ACNU [10], human data show drug uptake in the brain tumour area to be markedly enhanced following i.a. drug administration, compared to conventional i.v. infusion. Similar animal data have been presented with ACNU [28]. The effect of altered drug administration was reflected in experiments by Bullard and associates [29], who compared i.v. and intracarotid (i.a.) infusion of BCNU in the treatment of the 9L gliosarcoma in rats, showing that the greatest prolongation of survival was reached at a lower dose, when BCNU was administered i.a. compared to i.v. A correlation between improved effect on the tumour and increased local drug exposure with i.a. drug administration is indicated in the present tumour model: in a previous experiment [23], i.a. ACNU alone with a lower dose than in this study (15 instead of 18 mg/kg) was without significant effect on the BT_4An brain tumour. In the present experiment, survival increased when the same ACNU dose was given i.a. instead of i.v., and this difference was even more evident when chemotherapy was combined with hyperthermia. The value of increased drug uptake from i.a. drug delivery, therefore, may indicate potential for increased effect on primary brain tumours.

However, a major problem in i.a. treatment of brain tumours with the nitrosoureas, is ophthalmic toxicity and the damage to neighbouring normal brain tissue [4, 11–13]. Ophthalmic infusion may both cause pain [30] and harbour a risk of long term retinal damage [31]. With more advanced procedures, infusion can be made beyond the ophthalmic artery [8, 14]. Such procedures do not diminish brain tissue toxicity, and with infusion in smaller vessels closer to the tumour area requiring a slower infusion rate, drug marginalisation and uneven distribution could be increased [32–35].

A method to substantially enhance the local cytotoxic effect solely in the tumour area is, therefore, valuable since it increases the effect on the tumour, or enables the same local tumour toxicity with lower drug doses. Although hyperthermia in the present study significantly improved the effect of both i.a. and i.v. ACNU, showing the potential of this treatment modality, it was the combination of i.a. drug infusion and hyperthermia that yielded major extension of survival. During development of this brain tumour model, average symptom-free survival after implantation of 10^5 cells was 20.4 days, and was increased to approximately 30 days when reducing the number of implanted cells from 10^5 to 10^3 [21]. A doubling of symptom-free survival after thermochemotherapy with i.a. ACNU, therefore, indicates a substantial reduction of clonogenic cells remaining in the tumour area after treatment. In this small animal model, repeated i.a. treatment is not possible. In larger species with brain tumours, where such treatment is anatomically possible, the impact of repeated treatment on survival could be substantial.

The tumours recurred with similar size, shape, position and marcroscopic appearance in all groups. This fact, and the similar time from onset of slight symptoms to killing of the animals in all groups, indicates that the increased survival after treatment with i.a. ACNU and hyperthermia were due to delayed tumour

recurrence, and not caused by better tolerance for a tumour growing in a different position or intracranial mechanisms gradually compensating for an increased tumour volume.

Weight loss was mainly due to drug administration i.a. instead of i.v., and did not increase significantly by addition of hyperthermia. The neurological side-effects were moderate, but the limited number of animals with side-effects did not allow any statistical evaluation.

The value of regional drug administration is not obvious if the cytotoxic drug has a moderate first pass effect, and a high peak concentration is of minor importance for the total drug exposure to the tumour. However, in thermochemotherapy, i.a. drug administration is able to elevate drug concentration in the tumour during the heating period, when interaction of the two modalities is most pronounced for several cytotoxic drugs [36, 37]. Investigation of the combination of i.a. drug delivery and hyperthermia, therefore, should not necessarily be limited only to drugs with a high first pass effect.

In the VX-2 carcinoma implanted in rabbits, brain hyperthermia combined with BCNU i.v. resulted in extended survival compared to untreated controls [38]. The study documented brain hyperthermia to be a tolerable treatment for the animals, but the design of the study makes it difficult to assess whether it is BCNU or hyperthermia, or an interaction of drug and heat, which is responsible for the improved survival. An enhancement of intraperitoneal BCNU or i.a. ACNU via combination with local brain hyperthermia has been shown in the BT_4An cerebral glioma model [22, 23]. As far as we know, these results and the present study are the first examples of increased survival, by combining hyperthermia tolerable to the normal brain with chemotherapy in an animal *in situ* glioma model. The present experiments indicate that thermochemotherapy not only has potential in the treatment of malignant brain tumours, but that thermochemotherapy with i.a. drug administration may yield an even greater effect on these currently incurable neoplasms. However, normal brain tolerance to hyperthermia [39] and thermochemotherapy has to be investigated more extensively before brain thermochemotherapy should be pursued in patients with reasonable survival expectancy.

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