Intra-arterial ACNU therapy for malignant brain tumors
Experimental studies and preliminary clinical results

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The authors examined the growth rate of mouse 203 glioma cells in vitro and found it to be markedly
inhibited after exposure to ACNU for 5 minutes at a drug concentration of 100 μg/ml. Rats that had undergone
intracranial implantation of T1 neurogenic tumor were treated by 5 mg/kg of ACNU administered either
intravenously or intra-arterially. The median survival times for the control animals and the animals undergoing
intravenous or intracarotid administration of ACNU were 23, 29, and 46 days, respectively. The difference in
survival time between the intravenous and intracarotid administration groups was statistically significant (p <
0.01) when examined by the Cox-Mantel test.

In a clinical trial, 17 patients with glioblastoma were treated by ACNU, eight intravenously and nine by the
intra-arterial route. The drug was given in doses of 2 to 3 mg/kg at least twice before and twice after a course
of postoperative radiotherapy. Intra-arterial administration was performed over a period of 5 minutes under
local anesthesia. The median postoperative survival time for the patients in the intra-arterial group was 12.5
months, compared with 9.0 months for those in the intravenous group. The survival rate for the intra-arterial
group was slightly higher, although statistically not significant, probably because the number of cases was
small. The degree of thrombocytopenia due to ACNU tended to be less marked in the intra-arterially treated
patients. The theoretical advantages of the intra-arterial administration of ACNU are discussed.

KEY WORDS • ACNU • chemotherapy • brain tumor • drug administration •
inha-arterial administration

NITROSOUREA compounds are supposed to express their anti-tumor effects mainly by means of
the alkylation of DNA (deoxyribonucleic acid) by the diazonium ions that are the labile biotransformed
products of these drugs.6,8 The drug ACNU (1-(4-amino-2-methyl pyrimidine-5-yl)-methyl-3-(2-chlo-
roethyl)-3-nitrosourea hydrochloride) is one of the most powerful nitrosourea compounds and is known to be
water-soluble as well as lipid-soluble.5,9,10 Recently, ACNU has been widely used in Japan for the treatment
of malignant brain tumors, and its clinical effectiveness after systemic intravenous administration has been
reported by various investigators.12

The idea of intra-arterial chemotherapy for malignant brain tumors is not new; this route of administration
has been attempted since the 1950's in order to deliver larger amounts of drugs and to lessen systemic side effects.3,11,13 Primary malignant brain tumors are particularly suitable for this kind of treatment, because they do not usually metastasize to other organs and are
fed by one or two main arteries, such as the internal carotid artery (ICA) or the vertebral artery, which are
accessible by percutaneous puncture or selective catheterization. Although previous studies with metho-
trexate, vincristine, and other drugs have been generally unsatisfactory, the recent development of the nitroso-
urea compounds has provoked a renewed interest in this method of treatment, supported by the findings of
modern pharmacokinetic studies.1,2,7,8,14

When the usual systemic dose of 100 mg of ACNU is administered intravenously as a bolus injection in a
human adult, it is possible to achieve a drug concentration of only about 4 μg/ml in the peripheral blood, even
in the 5-minute period immediately following administration.2,12 Supposing that the cerebral blood flow is
50 ml/100 gm brain tissue/min and that about 400 gm of brain tissue is supplied by one ICA, then a theoretical
drug concentration of more than 100 μg/ml will be achieved in the arterial blood distal to the injection site
if 100 mg of ACNU is gradually injected into the ICA over a period of 5 minutes (Fig. 1).

The purpose of this paper is to present the results of
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Materials and Methods

In Vitro Experiments

Mouse 203 glioma cells were used for the in vitro experiments. These cells were derived from ependymoblastoma that had originally been induced in a C57BL mouse by methylcholanthrene and serially transplanted and maintained in our laboratory. The effects of drug concentration and duration of contact with ACNU were examined in a preparation of 203 glioma cells at a concentration of $5 \times 10^5$ cells, explanted in different wells. After 4 hours, when the cells had become attached to the floor of the wells, they were exposed to various concentrations of ACNU, ranging from 10 µg/ml to 1000 µg/ml, over various lengths of time from 1 minute to 30 minutes. The cells were then gently washed three times with Eagle’s minimum essential medium (MEM) and cultured in Eagle’s MEM containing 5% fetal calf serum in an incubator with 5% CO₂ at 37°C. Viable cells were counted sequentially by using the trypan blue dye exclusion method.

In Vivo Experiments

In vivo experiments were conducted on 4- to 6-week old male Wistar rats. A concentration of $5 \times 10^5$ T1 tumor cells was transplanted into the right frontal lobe. The T1 tumor used was a rat neurogenic tumor originally induced in a Wistar rat by transplacentalethyl nitrosourea. Two weeks later, the animals were anesthetized with Nembutal (sodium pentobarbital), and both the right ICA and the internal jugular vein in the neck were exposed under an operative microscope. A 5-mg/kg dose of ACNU was injected into either the ICA or the internal jugular vein over a period of 2 minutes. The survival rate of the animals treated by intracarotid administration was compared to that of both the untreated control animals and the animals that had received the same amount of the drug intravenously into the internal jugular vein.

The mortality rate from toxicity and the rate of weight gain of normal rats were examined after the intracarotid and the intravenous administration of various doses of ACNU. After sacrifice, the brain tissue of the animals was examined in histological preparations stained with hematoxylin and eosin.

Clinical Studies

In the period between 1978 and 1981, we treated 36 glioma patients with ACNU, administered intravenously in 20 and intra-arterially in 16. All of the patients were also treated by surgery and irradiation. The ACNU was administered in doses of 2 to 3 mg/kg at least twice before and twice after the course of postoperative radiotherapy, and in some cases every 8 weeks thereafter. The intra-arterial administration was performed over a period of 5 minutes under local anesthesia by the methods used for percutaneous carotid angiography or Sel-dinger catheter angiography. Since all patients received radiotherapy, which frequently suppresses the number of white blood cells, the degree of thrombocytopenia as a relatively specific measure of ACNU toxicity was compared between the intra-arterially and the intravenously treated groups.

Results

In Vitro Experiments

In preparations with a drug concentration of 10 µg/ml, substantial growth inhibition was noted only after exposure to the drug for 30 minutes (Fig. 2 left). Drug concentrations of 100 µg/ml resulted in a marked growth inhibition, even after exposure for 5 minutes (Fig. 2 center). In wells with a drug concentration as high as 1000 µg/ml, a marked growth inhibition was observed, even after an exposure time as short as 1 minute (Fig. 2 right). It should be emphasized that a concentration of 100 µg/ml for 5 minutes in the arterial blood is clinically achievable by the intra-arterial administration of ACNU.

In Vivo Experiments

All of the untreated rats died of tumor growth within 29 days of transplantation, and all animals that were treated by intravenous ACNU died within 41 days. However, 50% of the animals that were treated by intracarotid ACNU survived free of tumor for the observation period of 12 weeks. The median survival times for the control animals and the animals undergoing either intravenous or intracarotid administration of ACNU were 23, 29, and 46 days, respectively (Fig. 3). The difference in survival rates between the intravenous and intracarotid administration groups was statistically significant ($p < 0.01$) when examined by the Cox-Mantel test.

There were no deaths from drug toxicity after the
intracarotid administration of 10 mg/kg of ACNU in normal rats, but half of the animals that had received 50 mg/kg of ACNU intravenously died within 4 weeks (Table 1). There was slightly less increase in body weight in the group of animals that had received intracarotid administration. This was ascribed to the fact that the animals in the intracarotid administration group were slightly younger than those used for the intravenous administration. Accordingly, the difference in weight gain was considered to be insignificant.

Histological examination of the rat brains 4 weeks after the intracarotid administration of 10 mg/kg of ACNU revealed no remarkable changes in the hematoxylin and eosin-stained specimens.

Clinical Studies
The difference in survival rates was assessed only in patients with glioblastoma, eight of whom were treated by intravenous administration and nine by intra-arterial administration. Kaplan-Meier analysis showed that the postoperative survival rate for the intra-arterial group was slightly higher than for the intravenous group, although statistically it was not significant (Fig. 4 left). The survival rate after the first administration of ACNU

![Figure 2](image_url)

**FIG. 2.** The growth rates of mouse 203 glioma cells in vitro after exposure to 10 μg/ml (left), 100 μg/ml (center), and 1000 μg/ml (right) of ACNU for various periods of time.

![Figure 3](image_url)

**FIG. 3.** The survival rates of rats with intracranial T1 tumors after either the intracarotid or the intravenous administration of 5 mg/kg of ACNU.
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![Graphs showing survival rates and platelet counts](image)

**Fig. 4.** Survival rates in patients with glioblastoma treated with ACNU. Eight patients underwent intravenous injection and nine intra-arterial injection. Triangles denote the number of survivors. Postoperative rates (left) and after the first administration of ACNU (right).

For the intra-arterial group was also slightly higher than for the intravenous group, although again not statistically significant (Fig. 4 right). We hope that the superiority of intra-arterial administration in prolonging survival may be proven if a sufficient number of cases are accumulated.

Platelet counts were decreased maximally in the 4th week after drug administration. In the patients treated by intravenous administration, platelet counts diminished below 30% of the initial level in about 25% of the cases (Fig. 5 left). On the other hand, the patients treated by intra-arterial administration showed a less marked decrease of platelets; only one case had a decrease below 30% of the initial level (Fig. 5 right).

**TABLE 1**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>No. of Animals</th>
<th>Mortality Rate (%)</th>
<th>Increase in Body Weight (%)</th>
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<tr>
<td>intravenous</td>
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<tr>
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<td>6</td>
<td>0</td>
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<tr>
<td>5</td>
<td>6</td>
<td>0</td>
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* Determinations were made 4 weeks after intravenous or intracarotid administration.

**Fig. 5.** Percentage changes in platelet (PLT) counts of the patients with glioblastoma treated with ACNU administered intravenously (left) or intra-arterially (right).
**Illustrative Cases**

**Case 1.** This 42-year-old man was treated for recurrent malignant astrocytoma by the intracarotid administration of 100 mg of ACNU. He had received a course of radiotherapy 4 years previously. Computerized tomography (CT) scans showed complete disappearance of the recurrent tumor after the intracarotid ACNU therapy (Fig. 6).

**Case 2.** This 60-year-old woman received intracarotid ACNU for a recurrent glioblastoma 7 months after the initial treatment by surgery and radiotherapy. The dose of ACNU was reduced to 50 mg in this case because of the patient’s general condition. The CT scans showed nearly complete resolution of the recurrent tumor after the ACNU therapy (Fig. 7).

**Discussion**

Local intra-arterial drug administration to a tumor has been considered to have an advantage over the systemic intravenous route because it facilitates delivery of a larger amount of the drug directly to the tumor and minimizes systemic toxicity. Since the 1950’s, a number of clinical studies on the treatment of malignant brain tumors have been conducted using intra-arterial infusion of various drugs, including nitrogen mustard, vincristine, and methotrexate. This method was abandoned because of unsatisfactory clinical results and the occasional serious embolic complications related to the long period of infusion.

Recently, however, a renewed interest in intra-arterial chemotherapy has arisen on the basis of the findings of modern pharmacokinetic and pharmacodynamic studies. Blasberg reviewed the parameters associated with the intra-arterial administration of drugs. Intra-arterial infusion offers an advantage over the intravenous route only during the initial passage of the drug through the brain and/or tumor capillaries. The concentration of the drug in the blood during the second and subsequent passages through the brain and/or tumor capillaries will be similar to that achieved by intravenous administration. For the same degree of systemic toxicity, a considerably larger amount of drug can be delivered to the brain and/or tumor by the intra-arterial route in comparison to the intravenous route, if the rates of biotransformation, metabolism, and excretion during the first passage are high.

The biological half-life of nitrosourea compounds is extremely short. Levin, et al. reported that the biological half-life of BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) in the blood is 15 to 16 minutes. The half-life of ACNU in a neutral solution in vitro was reported to be 25 minutes. The passive transport of nonpolar lipid-soluble drugs, such as nitrosourea compounds, across the endothelial cells of the brain capillaries is usually considered to be unrestricted. Since the capillary permeability constant is relatively high for these drugs, capillary exchange is not usually considered to be a rate-limiting factor. Harada, et al. performed a pharmacokinetic analysis of a two-compartment open
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FIG. 8. Superselective administration of ACNU by means of a balloon-catheter technique. The drug was injected from the tip of the balloon (left) during temporary occlusion of the middle cerebral artery (right). Arrows indicate the balloon.

model of distribution of ACNU in patients with glioma, and reported that the half-life of distribution after intravenous administration was 6.3 to 6.7 minutes and that the equilibrium was reached at 44.7 to 53.7 minutes, indicating the rapid distribution of the drug to the glioma tissue from the blood.

Other parameters, including binding to serum protein, tumor cell uptake, and diffusion in the extracellular fluid, have an important bearing on the maximum delivery of drug to the brain and/or tumor. However, they are intrinsic to the specific drugs and less important when comparison is made between intra-arterial and the systemic intravenous administration.

According to Blasberg, an ideal drug for intra-arterial administration has the following characteristics: 1) the time course of the drug action should be short; 2) a significant fraction of the drug should be taken up by the brain and/or tumor, or be biotransformed or metabolized during the first passage through the brain and/or tumor capillaries, or be excreted by the lungs; 3) the physical characteristics of the drug should be such that it rapidly equilibrates across the brain and/or tumor capillaries and across the cell membranes.

Theoretically, therefore, highly labile drugs have a selective advantage for intra-arterial administration. The rapid-acting and degraded alkylating agents, such as the nitrosoureas, are probably closest to being the ideal intra-arterial drug available at the present time.

Levin, et al., reported that the intracarotid administration of BCNU in the monkey resulted in a 190% to 280% higher drug level than could be achieved by the intravenous route in the infused hemisphere and a 130% to 280% higher level than in the non-infused hemisphere. Yamada, et al. (unpublished data, 1982), using autoradiography, reported that the intracarotid administration of carbon-14-labeled ACNU to rats with intracranial tumors produced a drug level five times higher after 1 minute and 1.5 to 2 times higher after 30 minutes than when the same drug was administered by the intravenous route.

The results of our animal experiments support the superiority of intracarotid over intravenous administration. In our clinical trial, we obtained encouraging results with intra-arterial ACNU therapy; although we did not obtain a statistically significant difference due to the small number of cases. Thrombocytopenia as a major sign of systemic drug toxicity also tended to be less marked with intra-arterial administration. We experienced no complications attributable to intra-arterial administration when the infusion time was limited to 5 minutes. The technique is the same as for percutaneous carotid angiography. In experienced hands, the procedure is easily performed at the bedside or in an outpatient clinic.

It has been pointed out that when the rate of blood flow is lowered, the artery and brain tissue exposures to drugs following intra-arterial administration are markedly elevated. In order to improve the intra-arterial delivery of ACNU, we are developing a superselective balloon-catheter technique. It is technically feasible to introduce the catheter tip as far as the main trunk of the anterior, middle, or posterior cerebral arteries. In a feasibility study, we have treated three patients by this method (Fig. 8), and there were no complications. We believe that this technique will be extremely useful in selected cases because it will be possible not only to increase local drug concentration but also to decrease the local capillary blood flow during drug administration by inflating the proximal balloon.

Conclusions

Our experimental studies revealed the superiority of the intra-arterial method of administering ACNU compared to intravenous administration. Our clinical data are still too premature to form a conclusion, but it appears that intra-arterial administration of ACNU is
more effective and less toxic than intravenous administration. We believe that our results with intra-arterial ACNU therapy for primary malignant brain tumors are encouraging and that further investigations should be conducted.

References


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