Distribution of Radiolabeled 1-(4-Amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea Hydrochloride in Rat Brain Tumor: Intraarterial versus Intravenous Administration

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ABSTRACT

To assess the rationale of intraarterial (i.a.) 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea chemotherapy, distribution of 14C-labeled 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea in rat glioma was studied after i.a. or i.v. infusion.

Immediately after infusion, the tumor located in the hemisphere of intracarotid infusion received 4.6-fold higher radioactivity than the tumor located contralaterally to intracarotid infusion and 2.8-fold higher radioactivity than i.v. infusion. The difference was kept up to 30 min after i.a. infusion. Autoradiographic observation indicated rather uniform distribution of the tracer in the central portion of i.a. infusion. However, in the periphery of i.a. infusion, distribution of the tracer was nonhomogeneous.

The results indicate that i.a. 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea chemotherapy is useful when the tumor has high blood flow and is located in the center of an infused area.

INTRODUCTION

Nitrosourea derivatives have been widely used for the treatment of malignant brain tumors because of their lipophilic nature and being able to cross the blood-brain barrier. Pharmacokinetics of nitrosoureas in the brain tumor has been reported previously by several authors (1-6). Theoretically, lipidsoluble nitrosoureas should distribute to the tumor and brain tissue depending on their molecular weight, their lipophilicity, and local tissue blood flow (7). However, no previous report has shown tissue distribution of nitrosoureas in detail with correlation to histological appearance. In this report, we have undertaken a quantitative autoradiographic technique to study pharmacokinetic distribution of [ethylene-14C]ACNU3 in the autochthonous brain tumor model. With this system we tried to simulate uptake and distribution of the nitrosourea in the human brain tumors.

Furthermore, after the appearance of a report by Levin et al. (8) indicating advantage of i.a. BCNU, many clinical trials have shown that i.a. nitrosourea (BCNU and ACNU) was effective for primary (9-16) and metastatic (14, 17, 18) brain tumors. However, this mode of treatment was not always effective. Although part of this reason can be attributed to ineffectiveness of the nitrosourea against the tumor, the other important factor is uptake of the nitrosourea by the tumor. Factors affecting uptake of i.a. administered ACNU were also analyzed in this study.

MATERIALS AND METHODS

Experimental Glioma. A method to develop ethynitrosourea-induced glioma was the same as that in previous studies (19, 20). Briefly, a single dose of ethynitrosourea (50 mg/kg) was given s.c. to newborn Sprague-Dawley rats on the third day after birth. These rats were used for experimentation at 5 to 10 mo of age.

Tracer. [Ethylene-14C]ACNU (specific activity, 9.5 μCi/mg) was synthesized by the Central Research Laboratory, Sankyo Co., Ltd., Tokyo, Japan, and supplied to us as a powder. The same lot of the radiolabeled ACNU was used for the entire study to avoid the different specific activity and purity of the tracer. The radiochemical purity was ascertained to be 97% by thin-layer chromatography using two solvent systems, i.e.; chloroform:methanol (3:1) and normal butyl alcohol: methanol:water (9:1:1).

Procedures for Isotope Injection and Sampling. Twenty ethynitrosourea-treated rats were used for experimentation. Animals received 0.5 ml of 2% Evans blue 3 h prior to isotope injection for delineation of the area of blood-brain barrier disruption. Under anesthesia with ketamine hydrochloride, animals were placed on the wood plate with their extremities fixed by rubber bands. The femoral artery and vein were cannulated with polyethylene tubes. Arterial blood pressure and blood gases were monitored just prior to isotope injection. For i.c. administration study, a polyethylene catheter was placed into the common carotid artery through the external carotid artery, and the origin of the external carotid artery was ligated, so that the tracer injected into the common carotid artery reached to the internal carotid artery.

For i.v. study, 100 μCi/kg of [14C]ACNU dissolved in 1.0 ml of normal saline were injected at a constant rate for 1 min through the catheter placed in the femoral vein. For i.c. injection study, the same amount of 14C-labeled ACNU was injected at a constant rate for 1 min with an infusion. Periodical arterial blood sampling was made during the experimental period. Ten-μl blood samples were placed in counting vials, and radioactivity was counted. The animals were sacrificed at 1, 5, and 30 min after starting injection by rapid i.v. injection of saturated potassium chloride. The brain was removed, frozen with freon gas, and stored at −25°C until sectioned. Each group consisted of 3 to 4 animals.

Autoradiographic Preparation. Frozen brains were cut into 60-μm-thick sections in a cryostat, thaw-mounted onto a cover glass, and dried on a hot plate at 60°C. The cover glasses were attached to the X-ray film (Kodak SB-5) in the dark room. Standard plates containing a known amount of [14C]methylenemacrylate (supplied by Radiochemical Centre, Amersham, England) were also attached to the X-ray film for quantitative measurement of the tissue radioactivity from the absorbance of the X-ray film. After exposure for 2 wk, the X-ray film was developed. Densitometric measurements of the local absorbance in the autoradiogram were made by a densitometer (Sakura PDA-15; manufactured by Konishirouku, Tokyo, Japan). The calibration curve for each film was obtained from the absorbance of the standards. Calculation was made by the programmed computer. The representative brain sections were stained with hematoxylineosin for histological evaluation.

RESULTS

Peripheral Arterial [14C]ACNU Levels. Blood radioactivity levels sampled from the femoral artery after i.v. and i.c. injection are depicted in Fig. 1. The peak blood level in the femoral arter
artery was achieved concurrently at the end of 1-min injection. At this time the peak blood level of i.v. injection was significantly (P < 0.01) lower than that of i.e. injection. However, at 2, 5, 15, and 30 min after injection, no statistical difference in the blood radioactivity level was noted between i.e. and i.v. injection. Points; mean; bars, SE. Statistical significance by t test: **, P < 0.01; *, P < 0.05; NS, not significant.

Fig. 2. Levels of [14C]ACNU and its equivalents in the cortex and white matter after i.v. or i.c. injection. Data were expressed as the percentage of injected dose per g of tissue. Columns, mean; bars, SE. Statistical significance by t test: **, P < 0.01; *, P < 0.05; NS, not significant.

[14C]ACNU and Its Equivalents in Brain Tissue. Levels of [14C]ACNU equivalents in the cortex and white matter are shown in Fig. 2. At the end of injection (1 min), the [14C]ACNU level of i.a. infused cortex was 4.2-fold higher than that in the noninfused side and 2.5-fold higher than the level attained by i.v. injection. The difference was kept for 30 min of the experimental period. At 5 and 30 min after injection, i.a. infused cortex contained radioactivity levels 5.2- and 3.7-fold higher than that in the noninfused side, respectively. Even in the white matter, the difference between i.c. and i.v. infusion was evident. At 1 min after injection, [14C]ACNU levels of i.a. infused white matter were 2.8-fold higher than that of the noninfused side. At 5 and 30 min after injection, i.a. infused white matter contained radioactivity levels 3.6- and 3.5-fold higher than that of the noninfused side, respectively. The tissue radioactivity level after i.v. infusion tended to be higher than that in the noninfused hemisphere of the i.a. injection, although the data did not reach statistical significance.

Tumor Radioactivity Level. Distribution of radioactivity in the tumor tissue varied depending on the part of the tumor. However, for comparison, the highest absorbance of the tumor was obtained for measurement of radioactivity. The data are shown in Fig. 3. Immediately after injection (1 min), the tumor in the infused hemisphere contained 1.65% of injected radioactivity per g of tissue, which was 4.6-fold higher than the radioactivity level of the tumor located contralaterally to the i.c. infusion and 2.8-fold higher than that attained by i.v. injection.

The difference was less significant at 5 and 30 min after injection. Yet, at 5 min after injection, i.a. infused tumor contained 4.7-fold higher radioactivity than the tumor located in the noninfused hemisphere. At 30 min after injection, the difference of tumor radioactivity levels in the infused hemisphere and noninfused hemisphere was 5.8-fold, and it remains statistically significant (P < 0.01). The radioactivity levels in the tumor of i.v. [14C]ACNU injection tended to be higher than that in the noninfused side of i.c. injection. However, no statistical significance was noted among those groups.

Autoradiograms of i.v. Injection. As indicated above, distribution of radioactivity in the tumor was nonhomogenous. Therefore, several autoradiograms are presented, and distribution of radioactivity was discussed in relation to histological sections. At 1 min the tumor received higher radioactivity than the brain tissue. However, distribution of [14C]ACNU in the tumor was not uniform (Fig. 4, left). The histologically viable part contained high levels of radioactivity, whereas the necrotic part had no uptake. Furthermore, in some parts where no
histological necrosis was found, uptake of $[^{14}C]ACNU$ was low. At 5 min after injection, the tumor again contained higher levels of radioactivity than brain tissue (Fig. 5). Furthermore, the radioactivity level in the tumor was proportional to the degree of Evans blue staining, which is indicative of the degree of blood-brain barrier disruption. Small tumors which were not stained by Evans blue had the same radioactivity levels as brain tissue, and they were indistinguishable from brain tissue in the autoradiograms (Fig. 5). Radioactivity levels in the cortex and white matter were also identical and indistinguishable in the autoradiograms.

**Autoradiograms of i.e. Injection.** $[^{14}C]ACNU$ administered i.a. had a different pattern of distribution from i.v. injection. At 1 min after i.a. infusion, a certain heterogeneity of distribution was present in the autoradiograms. The maximum tissue concentration was attained at the center of the MCA territory, where radioactivity was highly accumulated in the tumor, cortex, and white matter (Fig. 6). In contrast, the level of $[^{14}C]ACNU$ in the periphery of the MCA territory was much lower than in the center of MCA territory. Furthermore, the pattern of distribution was nonhomogenous. The cortex and some part of the tumor had high levels of radioactivity, whereas the white matter and less viable part of the tumor had low levels. Non-homogeneity of distribution was also noted in the territory of the anterior cerebral artery and posterior cerebral artery, which may be explained by the pattern of local blood flow. In the noninfused hemisphere, the distribution pattern of $[^{14}C]ACNU$ to the tumor and brain tissue was the same as i.v. infusion (Fig. 6), although the actual values were somewhat lower than i.v. infusion (Figs. 2 and 3).

At 5 min after i.a. infusion, a similar pattern of distribution was attained. Tumor infused i.a. contained high levels of radioactivity, but the necrotic part and periphery of the tumor where blood flow was presumably low had low radioactivity (Fig. 7, left). Even at 30 min after i.a. infusion, tumor and brain tissue of the infused side retained higher levels of radioactivity than those of the noninfused side (Fig. 7, right).

**DISCUSSION**

ACNU is a nitrosourea derivative and effective for malignant neoplasms (21). Although ACNU can be dissolved in water, its log $P$ (log of octanol/water partition coefficient) is 0.92 (22), indicating that a lipid-soluble nature is also present. Because of this liposolubility and therefore potential to pass across the blood-brain barrier or -tumor barrier readily, several experimental and clinical trials have been made for the treatment of malignant gliomas (22-25). Furthermore, ACNU can be administered safely through the i.a. route because there is no necessity for using alcohol as a vehicle solution. Clinical trials of i.a. ACNU administration have been initiated with certain success (12). However, distribution of ACNU in the tumor and brain tissue after i.a. administration as well as i.v. administration remains uncertain. Therefore, we have undertaken autoradiographic presentation of $[^{14}C]ACNU$ distribution in the chemically induced rat glioma.

The blood radioactivity levels of i.e. injection tended to be lower than i.v. injection, although statistical significance was achieved only at 1 min after injection (Fig. 1). One cause of this difference was uptake of radioactivity in the infused tissue. As pointed out by Bullard et al. (26), the internal carotid artery of the rat branches off a major extracranial branch (pterygopalatine artery) before it enters intracranially. About two-thirds of the blood flow goes to the pterygopalatine artery, and only a third of the blood flow enters the intracranial cavity. The tissue supplied by the pterygopalatine artery (mainly muscle) may have a potential to trap the infused radioactivity and may be a cause of difference in blood radioactivity levels between i.e. and i.v. injection. The difference in blood radioactivity levels might affect the tissue radioactivity levels and may result in higher radioactivity levels in the tissue of i.v. injection than in the tissue of the noninfused side of i.e. injection, although no statistical significance was obtained (Figs. 2 and 3).

Immediately after i.v. injection, ACNU was distributed to the brain tissue apparently by local blood flow. Cortex and caudo-putamen which had adequate blood flow showed high levels of...
[14C]ACNU uptake, whereas corpus callosum which is in a low blood flow state had small uptake of ACNU. In the viable part of the tumor, high uptake of [14C]ACNU was apparent by the autoradiographs (Fig. 4). As we reported previously (19), the viable part of this tumor has high blood flow. Therefore, it is reasonable to conclude that [14C]ACNU was taken up by the tumor and brain tissue mainly depending on their local blood flow. Even 5 min after injection, the distribution profile was similar to that of 1 min.

At 30 min after injection, [14C]ACNU equivalents in the brain tissue attained low levels, and the difference between the cortex and white matter was less significant. This is probably because backflux of lipid-soluble ACNU from brain parenchyma to capillary vessels is also fast, and ACNU is extracted from brain tissue rapidly. However, in the large tumors which were stained by Evans blue, tissue radioactivity tended to stay in high levels up to 30 min of the experimental period. As the large tumor of this model has increased capillary permeability (20), the water-soluble moiety of [14C]ACNU metabolites (27) may tend to stay longer in those areas. This is in contrast with the data by Levin et al. (1), in which i.v. administered 14C-labeled 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea distributed equally into murine ependymoblastoma, brain adjacent to the tumor, and normal distant brain at 7 to 24 min after injection. The difference was probably as a result of water solubility of radiolabeled degradation products.

The distribution profile of i.a. administered [14C]ACNU was quite different from that of i.v. injection. At the central part of infusion (MCA territory), the radioactivity distributed rather uniformly in the tumor, cortex, and white matter, whereas at the peripheral part, distribution was not uniform. This nonhomogeneity of distribution was probably due in part to a blood-streaming phenomenon reported recently by Blacklock et al. (28). If we assume that the mean cerebral blood flow of the rat is 1.0 ml/g/min and that the tissue weight of the infused MCA territory is about 2 g, 2 ml of blood per min are supplied to the MCA territory. When we infuse 1 ml of radioactive solution for 1 min from the carotid artery, 0.33 ml of the solution enters the MCA territory, and 0.67 ml of the solution goes to the pterygopalatine artery (26). Therefore, 17% of intravascular blood was substituted by the radioactive solution at the center of the infused area, and the drug was completely mixed with blood and distributed rather uniformly. On this occasion, the main factor of tissue distribution was local blood flow and tissue vascular volume, although local blood flow might be partially modified during i.a. infusion.

In contrast, the amount of radioactive solution at the periphery of the infused area might be much smaller than at the center of the infusion, because the solution is diluted by the blood flow coming from the other circulation, and a blood-streaming phenomenon might occur. The phenomenon is pointed out in Fig. 6. The MCA territory is solely supplied by the internal carotid artery, and mixing of blood flow from the other circulation may not occur. However, in the territory of the posterior cerebral artery (hippocampus, thalamus, temporoooccipital cortex), blood flow from internal carotid artery might be mixed with blood flow from the basilar artery, and nonhomogeneity of distribution may result in those areas.

The other factor which affects distribution of [14C]ACNU is local blood flow in the tumor. As we reported previously (19), intratumoral blood flow is nonhomogenous in this tumor model. However, as a general principle, a small tumor has low blood flow, and when the tumor reaches a certain size (2 mm), neovascularization of the tumor occurs, and the center of the tumor has an increase in blood flow. This principle is preserved well in this experiment. Yet, in some tumors which were large but histologically benign and probably had low blood flow (Fig. 6), the [14C]ACNU level was quite low. Although we do not have direct evidence that this tumor had low blood flow, the histologically benign tumor had poor vascularity and might have had low blood flow (19). This kind of low blood flow state might be an obstacle for i.a. nitrosourea therapy. Therefore, measurement of tumor blood flow is beneficial for anticipation of the effect of i.a. nitrosourea therapy.

During i.a. BCNU therapy, leukoencephalopathy has been occurring in the white matter of the infused territory (13), and it is thought to be a dose-dependent event (29). Although leukoencephalopathy has not been reported during i.a. ACNU therapy, a high concentration of [14C]ACNU in the white matter of the infused territory is suggestive of an occurrence of leukoencephalopathy by BCNU. To avoid the toxic effects of nitrosourea to the infused brain tissue, a selective increase in tumor blood flow might be considered. To date, only a few trials have been made to increase tumor blood flow (30), but it
DISTRIBUTION OF i.a. AND i.v. ACNU

Fig. 6. [14C]ACNU autoradiographs (top) and corresponding histological sections (bottom) at 1 min after right carotid artery injection of the tracer.

Fig. 7. [14C]ACNU autoradiographs (top) and histological sections (bottom) at 5 min (left) or 30 min (right) after i.e. injection of the tracer.

may have a future to increase effectiveness of i.a. nitrosourea therapy without leukoencephalopathy.

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