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In-vivo Microdialysis Study of the Distribution of Cisplatin into Brain Tumour Tissue after Intracarotid Infusion in Rats With 9L Malignant Glioma

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Abstract

Simultaneous brain microdialysis in tumour and non-tumour tissues has been used for kinetic determination of the local distribution of an anticancer agent, cisplatin, in rats.

Rat brain was implanted with 9L malignant glioma and cisplatin (3·5 mg kg^{-1}) was administered as a selective intracarotid infusion for 30 min to rats prepared for brain microdialysis. The amount of platinum in the dialysate collected from tumour and non-tumour brain tissues was determined by atomic absorption spectrophotometry, as representative of cisplatin. Total and free platinum concentrations in plasma were also measured. Free platinum is accumulated preferentially in the tumour tissue and the brain tumour distribution coefficient (the ratio of brain tumour platinum AUC to plasma free platinum AUC, where AUC is the area under the platinum concentration–time curve) was 0·69, although there was little distribution into normal brain tissue. Drug binding to plasma proteins was 65%.

It is concluded that simultaneous microdialysis is an easy and available method for assessing in-vivo local pharmacokinetics and distribution of cisplatin in tumour and non-tumour tissues of the brain.

There is considerable current interest in improving drug delivery to increase therapeutic efficacy and reduce systemic side-effects. Brain tumour chemotherapy is generally associated with severe side-effects that limit the amount of therapy possible. One of the major approaches to reducing systemic side-effects of anticancer agents is local drug administration to target organs or tissues. However, the local quantitative pharmacokinetic behaviour of drugs in brain tumour or non-tumour tissues has not been well described, although such information is important for assessing the efficacy of brain tumour chemotherapy.

Cisplatin is the most active anticancer agent and is widely used in the treatment of many solid cancers (Loehrer & Einhorn 1984). Intravenous administration of cisplatin has been shown to be beneficial in treating malignant brain tumours (Stewart et al 1983), but major dose-limiting toxic effects, mainly on the kidney, blood and neuron, have limited its clinical application (Von Hoff et al 1979); accordingly, intracarotid administration of cisplatin has been proposed for the treatment of malignant glioma (Feun et al 1984; Mahaley et al 1989). However, the distribution of cisplatin among brain tumour and normal tissues after intracarotid administration still remains obscure.

Microdialysis is an in-vivo sampling technique enabling the determination of test substances in the extracellular space of most body tissues with minimum tissue damage. It allows continuous monitoring of an unbound drug in blood (Nakashima et al 1994) and other tissues (Matsuyama et al 1994a, b; Nakashima et al 1996b, 1997) without loss of fluid volume. We have recently applied microdialysis to a neuropharmacokinetic study in rats (Nakashima et al 1996a). Determination of the free drug concentration on both sides of the blood–brain barrier is important for characterizing the transport processes across that barrier.

The aim of this study was to determine local drug concentrations and distribution parameters in brain tumour tissue and normal brain tissue using simultaneous brain microdialysis. The transport of cisplatin to the brain tumour across the blood–brain barrier was studied after selective intracarotid administration to rats implanted with 9L malignant glioma.

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Materials and Methods

Materials

Cisplatin powder was purchased from Sigma (St Louis, MO). Phosphate buffered saline (PBS) was freshly prepared and filtered (0·2 μm) before use. A cisplatin solution for intracarotid infusion (0·88 mg mL^{-1}) was prepared in PBS and filtered (0·2 μm) before administration. This solution was diluted with PBS to a final concentration of 8·8 μg mL^{-1}, as cisplatin, for in-vivo retrodialysis loss calibration experiments. Platinum standard solution (1000 ppm) was obtained from Wako Pure Chemical (Osaka, Japan). Other chemicals were of special reagent grade.

Microdialysis system

The microdialysis system consisted of a CMA/100 microinjection pump and CMA/10 microdialysis probes with a

dialysis membrane 3 mm length and 0·5 mm o.d. (Carnegie Medicin, Stockholm, Sweden). The probe was connected to the microinjection pump and was perfused continuously with PBS at a rate of 3 μL min^{-1} during the experimental period.

Animals and the brain tumour model

Rat 9L malignant glioma cells were cultured at 37°C in Dulbecco's modified Eagle medium (Gibco BRL, Grand Island, NY) supplemented with 10% foetal calf serum, penicillin (100 units mL^{-1}), and streptomycin (100 μg mL^{-1}) according to the procedure described by Barker et al (1973). Experiments were performed on male Fischer-344 rats, 240–260 g. Rats were anaesthetized by fluothane inhalation and mounted on stereotaxic instruments. The skull was exposed and a small hole was drilled through the cranium. Tumour cell suspension (10 μL) containing 5×10^6 viable cells was injected into the caudate nucleus of the right hemisphere (co-ordinates: rostral +1·5 mm, lateral +2·5 mm, ventral −6·5 mm, relative to bregma and the dura surface) according to the Paxinos & Watson atlas (1986). Injection was administered with a 25-gauge needle attached to a 10 μL chromatography syringe (701RN, Hamilton, Reno, NV).

Experimental procedure

Brain microdialysis was performed under the same inhalation anaesthesia 21 days after tumour inoculation. For drug administration into the internal carotid artery, the carotid bifurcation in the neck region was exposed, and the proximal branches of external carotid artery and pterygo-palatine artery were burnt off, using a microscope, followed by insertion of a cannula into the common carotid artery through the external carotid artery. The femoral vein of the rat was also cannulated for blood sampling. Subsequently, the microdialysis probe perfused with PBS was stereotaxically inserted into the tumour area (the right caudate nucleus inoculated with the tumour cell suspension). Another probe perfused with PBS was inserted into the caudate nucleus of the left hemisphere (the non-tumour area). The location of the membrane of the microdialysis probe in the brain was verified by visual inspection of brain slices at the end of the experiment.

Sixty minutes after complete insertion of both microdialysis probes, an infusion pump (Type 235, ATOM, Tokyo, Japan) was used to infuse cisplatin solution (4 mL kg^{-1}) into the right internal carotid artery at a constant rate for 30 min. The total dose of cisplatin administered was 3·5 mg kg^{-1}.

The dialysate samples were collected into small sample tubes every 10 min for 120 min. Whole blood samples (400 μL) were also collected at various times (10–120 min) after the start of drug infusion and immediately centrifuged for 10 min at 3000 rev min^{-1} to separate the plasma. The plasma sample was divided into two and analysed for total and free platinum concentrations, free platinum being determined after ultrafiltration of the plasma sample (150 μL) at 3500 rev min^{-1} for 30 min (Ultrafree C3-LGC, Nippon Millipore, Tokyo, Japan).

The in-vivo relative recovery of platinum through the microdialysis probe was estimated by determining the retrodialysis loss in-vivo. The microdialysis probe perfused with cisplatin solution (8·8 μg mL^{-1}) at a flow rate of 3 μL min^{-1} was inserted into the tumour and normal regions of another rat brain in the same manner as for microdialysis. Dialysate was collected into a small sample tube every 20 min for 120 min. The in-vivo retrodialysis loss was calculated as:

1 − (concentration in dialysate/concentration in perfusate) (1)

(Wang & Welty 1996).

Platinum analysis

The concentration of platinum in dialysate, plasma and plasma ultrafiltrate was determined by flameless atomic absorption spectrophotometry (Z-8000, Hitachi, Tokyo, Japan) according to the procedure described previously (Nakashima et al 1989).

Data analysis

Pharmacokinetic parameters were calculated using the MULTI program (Yamaoka et al 1981). Platinum concentration data after the end of drug infusion in brain microdialysis and blood sampling experiments were fitted to the following exponential and biexponential equations:

$$C_{(t-30)} = C_{max} e^{-Ke(t-30)} \quad (2)$$

$$C_{(t-30)} = Ae^{-a(t-30)} + Be^{-b(t-30)} \quad (3)$$

where t is time after the start of drug infusion, $C_{(t-30)}$ is the drug concentration at time (t-30) min, C_{max} is the maximum drug concentration. K_e is the elimination rate constant, and A, a, B and b are the biexponential equation constants.

The area under the platinum concentration–time curve (AUC) from time 0 (the start of drug infusion) to time infinity for blood sampling was calculated by the linear trapezoidal rule. For the microdialysis data, platinum concentration measured in dialysate is a time-averaged concentration. Accordingly, the AUC was obtained as the sum of the products of the measured concentration and the collection time interval with the addition of C_n/K_e, where C_n is the concentration of the last sample measured.

Non-compartmental analysis was performed to obtain the mean residence time (MRT) for platinum in both brain and plasma. The extent of drug binding to plasma proteins was calculated by the equation:

$$\text{Binding} = (AUC_{total} - AUC_{free})/AUC_{total} \quad (4)$$

where AUC_{total} and AUC_{free} are, respectively, the AUC values for total and free platinum (present as cisplatin) in plasma.

Statistical analysis was performed by use of Student's *t*-test.

Results

There was no statistical difference between the in-vivo retrodialysis losses of platinum in the tumour and the non-tumour regions of brain. The average value was 10·7 ± 1·6% (mean ± s.d.; n = 6).

The concentration–time profiles of free platinum (i.e. as unbound cisplatin) in tumour and non-tumour regions of brain after the start of intracarotid infusion of cisplatin (3·5 mg kg^{-1}) are shown in Fig. 1.

Administration of cisplatin resulted in high levels of platinum in the brain tumour but there was little accumulation of free platinum in the non-tumour tissues of the brain. In the brain tumour, the highest concentration of free platinum (4·7 ± 1·7 μg mL^{-1}) was reached immediately after the end of the drug infusion; the concentration then decreased exponentially in all tested cases. The profiles of the drug concentrations

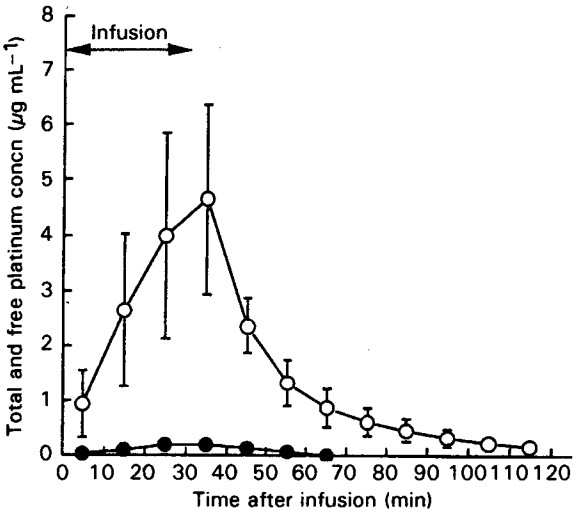

FIG. 1. Concentration–time profiles for free platinum (as unbound cisplatin) in tumour and non-tumour regions of the brain after selective intracarotid infusion of cisplatin (3·5 mg kg^{-1}) in rats implanted with 9L malignant glioma: ○, tumour; ●, non-tumour. In-vivo relative recovery of platinum was 10·7%. Each point represents the mean ± s.d. of results from five experiments.

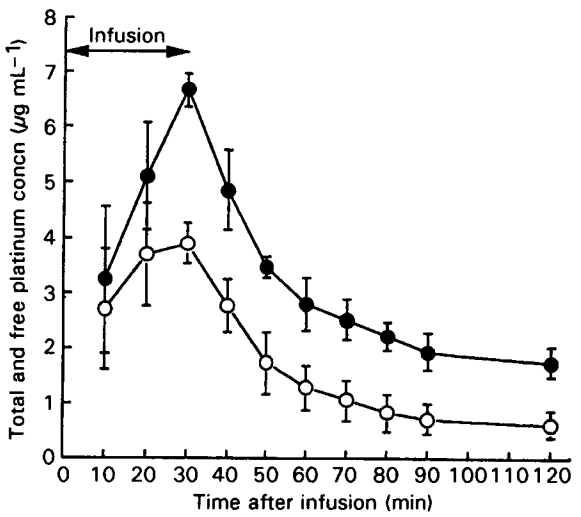

FIG. 2. Concentration–time profiles for total and free platinum (as total and unbound cisplatin) in plasma after selective intracarotid infusion of cisplatin (3·5 mg kg^{-1}) in rats implanted with 9L malignant glioma: ●, total platinum; ○, free platinum. Each point represents the mean ± s.d. of results from four experiments.

in the brain were analysed pharmacokinetically and the results are shown in Table 1.

The AUC of free platinum in the brain tumour was 18·6 times greater than for the non-tumour region of the brain; although these values were statistically different, there was no statistical difference between their MRT values. The elimination rate constant of free platinum in the tumour was 0·059 ± 0·021 min^{-1}.

The concentration–time profiles of total and free platinum in plasma after the start of the drug infusion are shown in Fig. 2.

The highest concentrations of total and free platinum in plasma were 6·7 ± 0·3 μg mL^{-1} and 3·9 ± 0·4 μg mL^{-1}, respectively. Thereafter both plasma-decay curves showed typical biexponential kinetics. Table 2 summarizes the pharmacokinetic parameters for total and free platinum (as total and unbound cisplatin) in the plasma.

Although there were no statistical differences between the elimination rate constants for total and free platinum, the AUC and MRT values were significantly different. The ratio of the AUC for free platinum (270 ± 36 min μg mL^{-1}) to that for total platinum (779 ± 51 min μg mL^{-1}) was 0·35, so 65% of the cisplatin was bound to plasma proteins. The brain tumour distribution coefficient (ratio of the AUC for free platinum in brain tumour to that for free platinum in plasma) was 0·69, although the normal brain distribution coefficient (ratio of the AUC for free platinum in brain non-tumour tissue to that for free platinum in plasma) was 0·04.

Table 1. Pharmacokinetic parameters of free platinum (as unbound cisplatin) in tumour and non-tumour regions of the brain after selective intracarotid infusion of cisplatin (3·5 mg kg^{-1}) in rats implanted with 9L malignant glioma.

Parameter	Tumour	Non-tumour
AUC (min μg mL^{-1})	186 ± 58*	10 ± 1
MRT (min)	42 ± 5	47 ± 15
K_e (min^{-1})	0·059 ± 0·021*	0·031 ± 0·009
$AUC_{tumour}/AUC_{non\text{-}tumour}$ ratio	18·6	

Each value represents the mean ± s.d. of results from five experiments. *$P < 0.01$ compared with non-tumour tissue. AUC is the area under the platimum concentration–time curve; MRT is the mean residence time; K_e is the elimination rate constant.

Discussion

Cisplatin is the most effective anticancer agent in the treatment of patients with malignant brain tumours. However, the intravenous administration of cisplatin is limited by toxic effects, mainly on kidney, blood and neuron. Accordingly, there has been considerable interest in the efficacy of intracarotid administration of cisplatin for malignant gliomas (Feun et al 1984; Mahaley et al 1989). This has the advantage of increasing

Table 2. Pharmacokinetic parameters for total and free platinum (as total and unbound cisplatin) in plasma after selective intracarotid infusion of cisplatin (3·5 mg kg^{-1}) in rats implanted with 9L malignant glioma.

Parameter	Total platinum	Free platinum
AUC (min μg mL^{-1})	779 ± 51*	270 ± 36
MRT (min)	210 ± 23*	89 ± 15
K_{12} (min^{-1})	0·032 ± 0·006**	0·041 ± 0·004
K_{21} (min^{-1})	0·026 ± 0·010*	0·010 ± 0·001
K_e (min^{-1})	0·009 ± 0·003	0·009 ± 0·005
Vd_c (mL kg^{-1})	344 ± 16	–
Vd_t (mL kg^{-1})	484 ± 236	–
Vd_{ss} (mL kg^{-1})	829 ± 229	–
CL (mL kg^{-1} min^{-1})	3·2 ± 1·1	–
AUC_{free}/ACU_{total} ratio	0·35	
$AUC_{non\text{-}tumour}/AUC_{free}$ ratio	0·04	
AUC_{tumour}/AUC_{free} ratio	0·69	

Each value represents the mean ± s.d. of results from four experiments. *$P < 0.01$ and **$P < 0.05$, compared with free platinum. AUC is the area under the platinum concentration–time curve; MRT is the mean residence time; K_{12} and K_{21} are the distribution rate constants; Vd_c and Vd_t are the distribution volumes of central and tissue comparments, respectively; Vd_{ss} is the steady state distribution volume; CL is the total body clearance.

its local concentration in the region of the tumour (Shani & Wolf 1989), but there is the disadvantage of toxicity in neighbouring normal brain tissues (Newton et al 1989). The local distribution parameters of cisplatin between the brain tumour and normal brain tissues after intracarotid administration have not yet been thoroughly studied. Therefore, we used simultaneous microdialysis of tumour tissue and normal brain tissue, in the 9L rat glioma model, to determine local concentrations and distribution parameters of cisplatin administered via the carotid artery.

The in-vivo microdialysis probe recovery for platinum was determined by the retrodialysis method in which the relative loss of drug from the perfusate is assumed to be equal to its relative gain by the perfusate in the microdialysis probe. This assumption was found to be valid for zidovudine (Wang et al 1993) and gabapentin (Wang & Welty 1996) under in-vivo and in-vitro conditions. In this study there was no statistical difference between the in-vivo recovery of platinum in tumour and non-tumour tissues of brain. Therefore, the concentrations of free platinum in brain extracellular fluid were calculated using the average value of recoveries measured in both regions.

This study shows that when cisplatin is administered by selective intracarotid infusion the uptake and duration of free platinum in the 9L glioma are significantly higher than in the normal brain. Yamashima et al (1993) reported that the tumour vasculature of the 9L glioma enabled increased permeability compared with normal brain tissues, because of the different ultrastructure of the endothelial cells of the tumour vessels, such as increased fenestrations, swelling, and disrupted junctions. We therefore suggest that cisplatin is easily transferred into the tumour interstitial space through the abnormal vessels. There was little uptake of free platinum (i.e. unbound cisplatin) in the normal brain. Cisplatin is sparingly soluble not only in water but also in lipid; accordingly it did not permeate the blood–brain barrier via normal vessels. Although the normal brain distribution coefficient was 0·04, the brain tumour distribution coefficient was 0·69, which suggests that unbound cisplatin in plasma crosses the tumour vessels freely. These results from our studies support the usefulness of the selective intraarterial chemotherapy of cisplatin for malignant gliomas in clinical application.

Recently Palsmeier & Lunte (1994) applied microdialysis to the pharmacokinetic study of 3-amino-1,2,4-benzotriazine-1,4-di-*N*-oxide (SR 4233), a new class of bioreductive antineoplastic agents, in rats carrying 3924A hepatoma, however a drug transport parameter from systemic circulation to a target tumour was not shown pharmacokinetically. In this study, we have demonstrated a kinetic parameter for drug delivery to a tumour and to neighbouring normal tissue. In conclusion, simultaneous microdialysis is an easy and available method for assessing in-vivo local pharmacokinetics and distribution of the anticancer drug cisplatin in the tumour and non-tumour tissues of the brain.

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