Local Distribution into Brain Tumor and Pharmacokinetics of 4-Pyridoxate Diammine Hydroxy Platinum, a Novel Cisplatin Derivative, after Intracarotid Administration in Rats with 9L Malignant Glioma: Simultaneous Brain Microdialysis Study

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Local distribution into brain tumor and the pharmacokinetics of 4-pyridoxate diammine hydroxy platinum (PyPt), a novel cisplatin derivative, were examined using rats implanted with 9L glioma and compared with cisplatin. PyPt (5.0 mg/kg) and cisplatin (3.5 mg/kg) were administered as selective intracarotid infusions for 30 min to the rats. Dialysates from extracellular fluid (ECF) in tumor and non-tumor brain tissues were collected by simultaneous microdialysis. The amount of platinum was determined by atomic absorption spectrophotometry, as representative of the drug administered. Plasma concentration of total and protein unbound platinum, and urinary excretion amount and tissue distribution of total platinum were also determined. Unbound platinum was accumulated preferentially in the brain tumor tissue ECF after drug administration, while there was little distribution into normal tissue ECF of the brain. In the brain tumor, the values of the unbound platinum AUC and MRT, where AUC is the area under the concentration–time curve and MRT is the mean residence time, for PyPt were 1.7 and 1.3 times larger than with cisplatin, respectively. The brain tumor distribution coefficient (the ratio of brain tumor ECF platinum AUC to plasma protein unbound platinum AUC) for PyPt (0.85) was higher than that for cisplatin (0.69), indicating that the local amount of platinum distributed into the glioma is enhanced by PyPt rather than by cisplatin. The binding to plasma proteins of PyPt (23%) was lower than that of cisplatin (65%). The total platinum concentration in tissues after administration of PyPt was significantly lower than that of cisplatin in the kidney, liver and spleen. In addition, the urinary excretion amount of total platinum after the administration of PyPt was significantly larger than that of cisplatin. These results suggested that PyPt is easily eliminated by rapid urinary excretion because of its reduced interaction with plasma proteins and poor distribution to the kidney or reticuloendothelial tissues such as the liver and spleen.

It is concluded that PyPt is an effective cisplatin derivative for the treatment of gliomas with the added advantage of enhancing local distribution of drug into the brain tumor and reducing its accumulation in the kidney, which has previously caused severe nephrotoxicity.

Key words microdialysis; cisplatin derivative; brain tumor chemotherapy; drug delivery system; 9L glioma rat; pharmacokinetics

Cisplatin (Fig. 1) is the most active anticancer agent found to date and is widely used in the treatment of many solid cancers.²⁾ Intravenous administration of cisplatin has been shown to be beneficial in treating malignant brain tumors.³⁾ Moreover, intracarotid administration of cisplatin has been considered for the treatment of malignant gliomas with the advantage of increasing the local concentration of drug exposed to tumors.^{4–6)} However, major dose-limiting toxic effects, mainly on the kidney, blood and neurons, have limited the clinical application of cisplatin.⁷⁾ Thus, many platinum complex analogues have been synthesized to increase antitumor activity and minimize the toxicity associated with cisplatin. Carboplatin is a representative second-generation platinum complex analogue with similar antitumor activity, but reduced toxicity, compared with cisplatin. It is now clinically used in the treatment of a variety of tumors, including malignant gliomas.⁸⁾ In a previous study, we synthesized 4-pyridoxate diammine hydroxy platinum (PyPt, Fig. 1), a novel cisplatin derivative, and indicated that it has high antitumor activities against brain tumors *in vitro* and *in vivo*.⁹⁾

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 direct and continuous monitoring of an unbound drug in

blood,^{10–12)} in the anterior chamber of the eye,¹³⁾ and in extracellular fluid (ECF) of other tissues such as liver,¹⁴⁾ dermis^{10,15)} and muscle.^{16,17)} This technique offers several advantages in that no blood need be drawn, and a large number of samples can be collected from a single experimental animal without loss of fluid volume. Several researchers have applied brain microdialysis to neuropharmacokinetic studies.^{18,19)} Determination of the unbound drug concentration on both sides of the blood–brain barrier is important for

Fig. 1. Structures of 4-Pyridoxate Diammine Hydroxy Platinum (PyPt) and Cisplatin

A, PyPt; B, cisplatin.

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characterizing the transport processes across that barrier. We have recently applied simultaneous microdialysis of tumor and non-tumor brain tissues for assessing the *in vivo* local pharmacokinetics and distribution of cisplatin after selective intracarotid administration to rats implanted with 9L malignant glioma.²⁰⁾

In this paper, to reveal the benefit of PyPt for brain tumor chemotherapy, we investigated local distribution into brain tumor and the pharmacokinetic properties of PyPt using rats with 9L glioma, and compared the results with cisplatin. After selective intracarotid administration of the drug, the blood concentration–time profile was measured and the concentration–time profile in the ECF of tumor and non-tumor brain tissues was determined by a simultaneous microdialysis method. The unbound drug distribution parameter was calculated both in tumor and in non-tumor brain tissues. The urinary excretion amount and tissue distribution of the drug were also examined.

MATERIALS AND METHODS

Materials PyPt was synthesized in our laboratories as described previously.⁹⁾ Cisplatin powder was purchased from Sigma Chemical Co., (St. Louis, U.S.A.). Phosphate buffered saline (PBS) was freshly prepared and filtered (0.2 μ m) before use. A PyPt solution (1.25 mg/ml) and a cisplatin solution (0.88 mg/ml) were prepared in PBS and filtered (0.2 μ m) before administration. Platinum standard solution (1000 ppm) was obtained from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All 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 in length and 0.5 mm in 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 μ l/min during the experimental period.

Tumor Cell Line Rat 9L malignant glioma cell line was obtained from The American Type Culture Collection (Rockville, U.S.A.) and cultured in Dulbecco's modified Eagles medium (Gibco BRL, Grand Island, U.S.A.) supplemented with 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μ g/ml) in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C.

Animals and Brain Tumor Model Male Fischer-344 rats, 240–260 g, were housed in cages in an air-conditioned room, and had free access to a standard laboratory diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and water. All animal experiments in the present study conformed strictly to the Nagasaki University Guideline for Animal Experimentation. Rats were anesthetized by halothane inhalation, then mounted on a stereotaxic instrument. The skull was exposed and a small hole was drilled through the cranium. A volume of 10 μ l tumor cell suspension, 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.²¹⁾ The injection was administered with a 25-gauge needle attached to a 10 μ l chromatography syringe (701RN, Hamilton, Reno, U.S.A.).

The hole was immediately sealed with bone wax, the scalp incision was sutured, and povidone iodine was applied to prevent infection.

Experimental Procedures Rats implanted with 9L glioma cells were used for brain microdialysis, blood and urine samplings, and tissue collection studies. Drug treatment, as a selective intracarotid infusion, was carried out 21 d after tumor inoculation. For drug administration into the internal carotid artery, rats were anesthetized as above, the carotid bifurcation at the neck portion was exposed, and the proximal branches of the external carotid artery and pterygopalatine artery were burnt off using a microscope. A cannula was inserted into the common carotid artery through the external carotid artery.

For brain microdialysis and blood sampling studies, subsequently, the femoral vein of the rat was also cannulated, then the microdialysis probe perfused with PBS was stereotactically inserted into the tumor area (the right caudate nucleus inoculated with the tumor cell suspension), and another probe perfused with PBS was also inserted into the caudate nucleus of the left hemisphere (the non-tumor area). The localization of the membrane of the microdialysis probe in the brain was verified by visual inspection of the 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 a 4 ml/kg volume of PyPt solution into the right internal carotid artery at a constant rate for 30 min. The total dose of PyPt administered was 5.0 mg/kg. The dialysate samples were collected into small sample tubes every 10 min for 120 min. Whole blood samples (400 μ l) were also collected at various times (10–120 min) after the start of drug infusion, and were immediately centrifuged to separate plasma. The plasma sample (150 μ l) was divided in half and analyzed for total and unbound platinum concentrations, unbound platinum being determined after ultrafiltration of the plasma sample (Ultrafree C3-LGC, Nippon Millipore Co., Ltd., 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 PyPt solution (12.5 μ g/ml) at a flow rate of 3 μ l/min was inserted into the tumor and the normal regions of another rat brain in the same manner as for microdialysis. Dialysate was then collected into a small sample tube every 20 min for 120 min. The *in vivo* recovery by retrodialysis loss was calculated as follows¹⁹⁾:

$$\text{recovery} = (C_{\text{in}} - C_{\text{out}}) / C_{\text{in}} \quad (1)$$

where C_{in} and C_{out} are, respectively, the concentrations in the perfusate and in the dialysate.

Two other groups of four rats, implanted with 9L glioma cells, were used for urine and tissue collection studies. The internal carotid artery was cannulated as above for drug administration, and the bladder of the rat was also cannulated for urine sampling. A volume of 4 ml/kg of the PyPt solution or the cisplatin solution was administered into the right internal carotid artery at a constant rate for 30 min. The total doses of PyPt and cisplatin were 5.0 and 3.5 mg/kg, respectively. These doses correspond to 2.3 mg/kg platinum. Following the start of drug infusion, urine samples were col-

Fig. 2. Concentration–Time Profiles for Total (A) and Unbound (B) Platinum (as Total and Protein Unbound Drugs) in Plasma after Selective Intracarotid Infusion of PyPt (5.0 mg/kg) and Cisplatin (3.5 mg/kg) in Rats Implanted with 9L Malignant Glioma

Symbols: (●) PyPt; (○) cisplatin. Each point represents the mean $\pm$ S.D. of results from 4 experiments. The profiles of cisplatin were previously published data.²⁰⁾

lected into small sample tubes at intervals of 30 to 60 min for 360 min. Thereafter, the rat was deeply anesthetized with ether, and all blood from the circulation was removed by perfusing the heart with saline (above 100 ml). After sacrifice of the rat, tissue samples were obtained from the kidney, liver and spleen.

Platinum Analysis The concentration of platinum in the dialysate, plasma, plasma ultrafiltrate, urine and tissues was determined by flameless atomic absorption spectrophotometry (Z-8000, Hitachi, Ltd., Tokyo, Japan) according to the procedure described previously.⁹⁾ Standards (0.2–2.0 $\mu\text{g/ml}$) were prepared from platinum standard solution by dilution with 0.1 M HCl. The standard curve was linear for the entire range of standard samples. The limit of assay detection was 0.02 $\mu\text{g/ml}$.

Data Analysis Pharmacokinetic parameters were calculated using the MULTI program.²²⁾ 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:

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

$$C_{(t-30)} = A e^{-a(t-30)} + B e^{-b(t-30)} \quad (3)$$

where t is the time after the start of drug infusion, $C_{(t-30)}$ is the drug concentration at time $(t-30)$ min, and $C_{\max}$ 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 the 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:

$$\text{binding} = (AUC_{\text{total}} - AUC_{\text{unbound}}) / AUC_{\text{total}} \quad (4)$$

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

The brain tumor distribution coefficient for the drug was calculated by the equation:

$$\text{brain tumor distribution coefficient} = AUC_{\text{tumor}} / AUC_{\text{unbound}} \quad (5)$$

where AUC_{tumor} is the AUC value for the unbound platinum in the brain tumor ECF. The normal brain distribution coefficient for the drug was calculated by the equation:

$$\text{normal brain distribution coefficient} = AUC_{\text{non-tumor}} / AUC_{\text{unbound}} \quad (6)$$

where $AUC_{\text{non-tumor}}$ is the AUC value for the unbound platinum in the non-tumor brain ECF.

Statistical analysis was carried out using Student's t -test.

RESULTS

There was no statistical difference between the *in vivo* recovery by retrodialysis losses of platinum in the tumor and non-tumor regions of the brain during the perfusion microdialysis probe with PyPt solution. The average value was $12.6 \pm 1.5\%$ (mean $\pm$ S.D.; $n=6$).

The concentration–time profiles of total and protein unbound platinum in plasma after the start of intracarotid infusion of PyPt are shown in Fig. 2, along with our previously published data concerning cisplatin.²⁰⁾ While PyPt and cisplatin were administered with total doses corresponding to 2.3 mg/kg platinum, the highest concentration of total platinum (*i.e.* as total drug) in plasma was 4.6 ± 0.8 and 6.7 ± 0.3 $\mu\text{g/ml}$, respectively. PyPt was significantly lower ($p < 0.05$) than cisplatin. In contrast, the highest concentration of unbound platinum (*i.e.* as protein unbound drug) in plasma after the administration of PyPt was 3.7 ± 0.8 $\mu\text{g/ml}$, which was almost equal to that of cisplatin (3.9 ± 0.4 $\mu\text{g/ml}$). Thereafter, all plasma-decay curves showed typical biexponential kinetics. The profiles of the drug concentrations in plasma were analyzed pharmacokinetically, and the results are shown in Table 1. The total platinum AUC and MRT values for PyPt were significantly lower than those for cisplatin. The total

Table 1. Pharmacokinetic Parameters for Total and Unbound Platinum (as Total and Protein Unbound Drugs) in Plasma after Selective Intracarotid Infusion of PyPt (5.0 mg/kg) and Cisplatin (3.5 mg/kg) in Rats Implanted with 9L Malignant Glioma

Parameter	PyPt		Cisplatin	
	Total platinum	Unbound platinum	Total platinum	Unbound platinum
<i>AUC</i> (min $\mu\text{g/ml}$)	459 $\pm$ 81*	355 $\pm$ 91	779 $\pm$ 51	270 $\pm$ 36
<i>MRT</i> (min)	136 $\pm$ 25*	145 $\pm$ 35*	210 $\pm$ 23	89 $\pm$ 15
<i>CL</i> (ml/min/kg)	6.5 $\pm$ 2.2*	—	3.2 $\pm$ 1.1	—
<i>AUC</i> _{unbound} / <i>AUC</i> _{total} ratio	0.77	—	0.35	—

Each value represents the mean $\pm$ S.D. of results from 4 experiments. * $p < 0.05$, compared with cisplatin. *AUC* is the area under the platinum concentration–time curve; *MRT* is the mean residence time; *CL* is the total body clearance. The parameters of cisplatin were previously published data.²⁰⁾

Fig. 3. Concentration–Time Profiles for Unbound Platinum (as Protein Unbound Drug) in Tumor and Non-tumor Regions of the Brain after Selective Intracarotid Infusion of PyPt (5.0 mg/kg) and Cisplatin (3.5 mg/kg) in Rats Implanted with 9L Malignant Glioma

Symbols: (●) tumor for PyPt; (○) tumor for cisplatin; (▲) non-tumor for PyPt; (△) non-tumor for cisplatin. *In vivo* relative recovery of platinum was 12.6% and 10.7% for PyPt and cisplatin, respectively. Each point represents the mean $\pm$ S.D. of results from 5 experiments. The profiles and the *in vivo* recovery of cisplatin were previously published data.²⁰⁾

platinum *CL* value for PyPt was significantly larger than that for cisplatin. On the other hand, in the unbound platinum parameters no statistical difference was observed in the *AUC* value between PyPt and cisplatin, while the *MRT* value for PyPt was 1.6 times larger than that for cisplatin. The ratio of the *AUC* for unbound platinum to that for total platinum in PyPt and cisplatin was 0.77 and 0.35, so 23% and 65% of the drug administered was bound to plasma proteins, respectively.

The concentration–time profiles of unbound platinum (as protein unbound drug) in tumor and non-tumor regions of the brain after the start of intracarotid infusion of PyPt are shown in Fig. 3, along with our previously published data concerning cisplatin.²⁰⁾ The administration of PyPt resulted in high levels of unbound platinum in the brain tumor ECF, but there was little distribution of the platinum in the normal brain ECF. This result was similar to the finding with cisplatin. In the brain tumor, the highest concentration of unbound platinum was reached immediately after the end of the drug infusion, followed by an exponential decrease in all tested cases. The maximum concentration of PyPt was $4.8 \pm 0.8 \mu\text{g/ml}$, which was close to that of cisplatin ($4.7 \pm 1.7 \mu\text{g/ml}$). Table 2

Table 2. Pharmacokinetic Parameters for Unbound Platinum (as Protein Unbound Drug) in Tumor and Non-tumor Regions of the Brain after Selective Intracarotid Infusion of PyPt (5.0 mg/kg) and Cisplatin (3.5 mg/kg) in Rats Implanted with 9L Malignant Glioma

Parameter	PyPt		Cisplatin	
	Tumor	Non-tumor	Tumor	Non-tumor
<i>AUC</i> (min $\mu\text{g/ml}$)	311 $\pm$ 62*	11 $\pm$ 3	186 $\pm$ 58	10 $\pm$ 1
<i>MRT</i> (min)	55 $\pm$ 7*	—	42 $\pm$ 5	—
K_e (min^{-1})	0.027 $\pm$ 0.005*	—	0.059 $\pm$ 0.021	—
<i>AUC</i> _{tumor} / <i>AUC</i> _{unbound} ratio	0.85	—	0.69	—
<i>AUC</i> _{non-tumor} / <i>AUC</i> _{unbound} ratio	—	0.03	—	0.04

Each value represents the mean $\pm$ S.D. of results from 5 experiments. * $p < 0.05$, compared with cisplatin. *AUC* is the area under the platinum concentration–time curve; *MRT* is the mean residence time; K_e is the elimination rate constants. The parameters of cisplatin were previously published data.²⁰⁾

Fig. 4. Cumulative Excretion Amount of Total Platinum (as Total Drug) in Urine after Selective Intracarotid Infusion of PyPt (5.0 mg/kg) and Cisplatin (3.5 mg/kg) in Rats Implanted with 9L Malignant Glioma

Symbols: (●) PyPt; (○) cisplatin. Each point represents the mean $\pm$ S.D. of results from 4 experiments.

summarizes the pharmacokinetic parameters for unbound platinum (as protein unbound drug) in the brain. In the brain tumor, the values of unbound platinum *AUC* and *MRT* for PyPt were 1.7 and 1.3 times larger than with cisplatin, respectively. On the other hand, the K_e value of unbound platinum for PyPt was 2.2 times less than that for cisplatin. The brain tumor distribution coefficient for PyPt and cisplatin was 0.85 and 0.69, respectively; PyPt was higher than cisplatin. On the other hand, the normal brain distribution coefficient for PyPt and cisplatin was 0.03 and 0.04, respectively; there was no statistical difference between them.

Figure 4 shows the urinary excretion amount of total platinum (as total drug) after the start of the drug infusions. Platinum was excreted rapidly after the administration of PyPt compared with cisplatin. Total urinary excretion amount for 360 min was 33 ± 4 and $15 \pm 3\%$ for PyPt and cisplatin, respectively. Thus, PyPt excretion was 2.2 times greater than cisplatin.

Table 3 summarizes the concentration of total platinum (as total drug) in the kidney, liver and spleen 360 min after the start of the drug infusions. Total organ platinum concentration after the administration of PyPt was significantly lower than that of cisplatin in every tissue.

Table 3. Tissue Concentration of Total Platinum (as Total Drug) 360 min after Selective Intracarotid Infusion of PyPt (5.0 mg/kg) and Cisplatin (3.5 mg/kg) in Rats Implanted with 9L Malignant Glioma

Tissue	Concentration ($\mu\text{g/g}$)	
	PyPt	Cisplatin
Kidney	$26.8 \pm 6.7^*$	51.1 ± 9.1
Liver	$2.3 \pm 0.8^*$	7.4 ± 2.8
Spleen	$0.6 \pm 0.5^*$	2.3 ± 0.8

Each value represents the mean $\pm$ S.D. of results from 4 experiments. * $p < 0.05$, compared with cisplatin.

DISCUSSION

There is considerable current interest in improving drug delivery to increase therapeutic efficacy and to reduce systemic side-effects. Brain tumor chemotherapy is generally associated with severe side-effects which 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. Accordingly, intracarotid administration of cisplatin has been evaluated for the treatment of malignant gliomas.⁴⁻⁶ However, the accumulation of cisplatin in the kidney causes severe nephrotoxicity. Moreover, resistance to cisplatin frequently appears in many tumor cell lines.² Therefore, many approaches have been used to synthesize novel platinum complex analogues which have a broader spectrum of antitumor activity, reduced toxicity, and a lack of cross-resistance. Recently there has been great interest in the synthesis of cisplatin derivatives with improved pharmacokinetic properties.

We previously reported that PyPt, synthesized newly in our laboratories, has shown antitumor activities against brain tumors *in vitro* and *in vivo*.⁹ Briefly, in *in vitro* drug sensitivity studies using human glioma cell lines T98G and A172, the antitumor activity of PyPt was lower than cisplatin in T98G, whereas the antitumor activity of PyPt was significantly higher than cisplatin in A172. These findings indicated that PyPt was more effective than cisplatin against this particular human glioma cell line. Furthermore, in animal studies using rats implanted with 9L malignant glioma, the prolonged survival time compared with the control animals after treatment using PyPt was equal to that of cisplatin, although it showed a lower *in vitro* antitumor activity against the rat 9L glioma cell line than cisplatin. In addition, when we determined total platinum concentrations in brain tumor 60 min after start of intracarotid infusion for 30 min of PyPt and cisplatin at doses corresponding to 0.92 mg/kg platinum, the concentration in the case of PyPt was approximately double that of cisplatin. To reveal the usefulness of PyPt for brain tumor chemotherapy, in this study we carried out a further examination using rats with 9L glioma. This 9L rat glioma model is the generally adopted experimental model for brain tumor chemotherapy because the implanted tumor is of glial origin and tumor take is 100%.²³

Recently, using a simultaneous brain microdialysis method, we have successfully demonstrated the *in vivo* local pharmacokinetics and distribution of cisplatin administered *via* the carotid artery in tumor and neighboring non-tumor brain tissues of rats with 9L glioma.²⁰ The advantage of this

method is that simultaneous sampling of an unbound drug both in tumor and non-tumor regions of the target organ can be achieved in a single experimental animal. Previous to this literature, De Lange *et al.*²⁴ and Devineni *et al.*²⁵ also used brain microdialysis as a tool to investigate drug distribution in brain tumors. These authors monitored the drug concentration profiles in plasma and in brain dialysate after the administration of methotrexate, an anticancer drug, to normal and glioma bearing rats, and described individually the drug transport parameters of an intact brain and a tumor bearing brain. However, it is important to examine the local quantitative pharmacokinetic behavior of drugs between tumor and neighboring non-tumor regions of the same brain for assessing the efficacy of brain tumor chemotherapy, because the disadvantage of this chemotherapy is a toxic effect on the neighboring normal tissues of the brain. In the present study, therefore, we determined the local concentrations and distribution parameters of PyPt administered *via* the carotid artery using the simultaneous microdialysis of tumor and normal brain tissues in rats with 9L glioma.

To estimate the unbound drug concentration in brain tissue ECF from measured microdialysis data, microdialysis probe recovery for test drugs must be determined *in vivo*. In our studies, 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 zidovudine¹⁸ and gabapentin¹⁹ under *in vivo* and *in vitro* conditions. When we examined the *in vivo* recovery for cisplatin there was no statistical difference between platinum recovery in the tumor and non-tumor regions of the brain.²⁰ In this study, PyPt also showed no statistical difference between the two. Therefore, the concentrations of unbound platinum in the brain tissue ECF were calculated using the average value of recovery measured in both regions.

When PyPt was administered by selective intracarotid infusion, there was little uptake of unbound platinum (*i.e.* unbound PyPt) in the normal brain tissue, and the value of the normal brain distribution coefficient for PyPt was almost equal to that of cisplatin. Cisplatin is sparingly soluble not only in water but also in lipid; accordingly, it does not permeate the blood-brain barrier *via* normal vessels. The present microdialysis study shows that PyPt is also barely transferred into the normal interstitial space through normal vessels. On the other hand, the uptake of unbound platinum in the 9L glioma after the administration of PyPt was significantly higher than that in the normal brain, similarly to cisplatin administration. Yamashima *et al.*²⁶ reported that the tumor vasculature of the 9L glioma enabled increased permeability compared with normal brain tissues, because of the different ultrastructure of the endothelial cells of the tumor vessels such as increased fenestrations, swelling, and disrupted junctions. We therefore suggested that PyPt and cisplatin were easily transferred into the tumor interstitial space through the abnormal vessels. Thus, PyPt would show brain tumor and plasma unbound platinum concentration-time profiles similar to cisplatin. Unbound PyPt and cisplatin in plasma have crossed brain tumor vessels freely. However, interestingly, the duration of unbound platinum in the 9L glioma was different between them. The slow elimination rate of unbound PyPt

from the glioma resulted in a significantly larger value of the brain tumor unbound platinum *AUC* for PyPt compared with that for cisplatin. The finding that the retention of unbound platinum in blood circulation for PyPt was longer than that for cisplatin probably reflected in part this elimination characteristics of PyPt in the brain tumor. However, the brain tumor distribution coefficient for PyPt was 0.85, which was 1.2 times higher than that for cisplatin, suggesting that there was a difference in the brain tumor distribution characteristics of unbound platinum between them. Platinum complex produced from cisplatin is predominantly conjugated with glutathione intracellularly. Using a mouse brain capillary endothelial cell line monolayer, Homma *et al.*²⁷⁾ reported that the efflux transport system of this glutathione conjugate was expressed on both luminal and abluminal sides of the blood-brain barrier. Ueda *et al.*²⁸⁾ reported that an active transport system for the glutathione-platinum complex conjugate was markedly expressed on a cisplatin-resistant tumor cell line. Therefore, enhanced platinum distribution into the 9L glioma in the case of PyPt administration is probably related to the difference in conjugating activity with intracellularly existing glutathione between the platinum complex produced from PyPt and that from cisplatin. The affinity between glutathione and the platinum complex produced from PyPt must be poorer compared with that in the case of cisplatin; accordingly, unbound platinum was more slowly eliminated from the 9L glioma by PyPt than by cisplatin. These results suggest that PyPt may have the potential to enhance local drug delivery into the brain tumor. Furthermore, the present study reveals that PyPt has some pharmacokinetic properties which may overcome the problem of systemic side-effects of cisplatin. Cisplatin showed poor urinary excretion because of high plasma protein binding and extensive tissue distribution to the kidney and reticuloendothelial tissues such as the liver and spleen. In contrast, PyPt showed reduced interaction with plasma proteins and poor distribution to these tissues, and thus it was easily eliminated from the circulating blood via rapid urinary excretion. The *in vivo* binding affinity of PyPt with plasma proteins was less than that of cisplatin; accordingly, the amount of drug distributed into the tissues would be diminished by PyPt compared with cisplatin. PyPt may have great potential for reducing systemic side-effects in brain tumor chemotherapy. Pyridoxic acid is a safe and clinically well established agent, easily transported to the brain, and with high renal excretion. The significantly high local distribution into brain tumor and rapid urinary excretion of PyPt are probably related to the pharmacokinetic properties of pyridoxic acid.

It is concluded that PyPt is an effective cisplatin derivative for the treatment of gliomas, with several advantages: it enhances local distribution of a drug into the brain tumor and reduces its accumulation in the kidney which has caused severe nephrotoxicity, and its simultaneous microdialysis is an easy and available method for assessing *in vivo* local pharmacokinetics and distribution of the anticancer drug in the target tumor and non-target normal tissue of the brain.

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