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Pharmacokinetic Evaluation of (Glycolato-*O,O'*)diammine Platinum(II) in Lung Lymph in Sheep

Tomonobu Koizumi,[1,4] Keishi Kubo,[1] Shirou Shinozaki,[1] Shigeru Koyama,[1] Toshiya Amari,[1] Toshihide Hayano,[1] Keisaku Fujimoto,[1] Toshio Kobayashi,[1] Morie Sekiguchi,[1] Ryosuke Sakai,[2] Taeyuki Ohshima[3] and Ken-ichi Miyamoto[3]

[1]*The First Department of Internal Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390,* [2]*Hokuriku Gakuin Junior College, I-11 Mitsukoji-machi, Kanazawa 920-13 and* [3]*Hokuriku University, School of Pharmacy, Ho-3 Kanagawa-machi, Kanazawa 920-11*

The pharmacokinetics of (glycolato-*O,O'*)diammine platinum(II) (254-S), especially the distribution and behavior in the lung lymph in sheep, was investigated and compared with that of *cis*-diamminedichloroplatinum(II) (CDDP). The blood and lung lymph fluid were collected from the carotid artery and a lung lymph fistula, respectively, in conscious sheep following intravenous infusion of 100 mg/body of 254-S and CDDP for 30 min. The concentrations of these platinum complexes were measured by using atomic absorption spectrometry. We analyzed the data using an anatomically based model including part of the lymphatic circulation. The ultrafilterable platinum of 254-S showed much larger area under the curve (AUC) and transfer rate constants than that of CDDP, even though the mean residence times were the same. The total platinum showed the opposite pharmacokinetic behavior. In anesthetized sheep, when lung tissue samples were obtained by biopsy at the same times as those of blood and lung lymph sampling after infusion of these drugs, 254-S distributed in lung tissue appeared to move more easily into lung lymph than CDDP, which tended to be retained in lung tissue. These differences in pharmacokinetic behavior between 254-S and CDDP seemed to be caused by differences in their strength of protein binding; the association constants of 254-S for plasma and lymph protein were much less than those of CDDP. From these results, 254-S may have favorable therapeutic effects on intrathoracic malignacies such as lung cancer and lymph metastasis.

Key words: Pharmacokinetics — Cisplatin — 254-S — Lung lymph — Sheep

cis-Diamminedichloroplatinum(II) (CDDP[5]) is an important antineoplastic drug for the treatment of lung cancer,[1–3] but its use is limited due to its various toxicities, including gastrointestinal and renal toxicities. (Glycolato-*O,O'*)diammine platinum(II) (254-S) was developed as a second-generation platinum coordination compound, showing less nephrotoxicity and having better activity against a variety of experimental cancer cell lines than CDDP.[4,5] It has been shown that 254-S has the same antitumor activity as CDDP in patients with non-small-cell lung cancer in a phase II study.[6,7] However, while many pharmacokinetic studies of platinum complexes have been done in patients[8–11] and several animals,[12–14] little work has been reported on the behavior in the major target organ, lung and lymph.

In this study, we investigated the pharmacokinetics of 254-S in plasma, lung lymph, and lung tissue of sheep, compared with those of CDDP, and we suggest that 254-S is likely to be useful for the treatment of lung cancer and lymph metastasis.

[4] To whom correspondence and reprint requests should be addressed.

[5] The abbreviations used are: 254-S, (glycolato-*O,O'*)diammine platinum(II); CDDP, *cis*-diamminedichloroplatinum(II).

MATERIALS AND METHODS

Materials CDDP and 254-S were kindly supplied by Nippon Kayaku Co., Tokyo and Shionogi & Co., Osaka, respectively. These compounds were dissolved in normal saline immediately before injection. Other chemicals used were of reagent grade.

Animal preparations Adult sheep weighing 28 to 35 kg used in the following experiments had normal renal function (serum creatinine values of < 1.5 mg/dl), and lung lymph-to-plasma protein concentration ratio (< 0.65). No animal had previously received platinum compounds, and each was studied a single time. Animals were prepared to form chronic lung lymph fistulas for the collection of lung lymph fluid, using the method of Staub *et al.*[15] Briefly, three sheep in a group were anesthetized with intravenous pentobarbital sodium (12.5 mg/kg), and then ventilated with 0.5–1.0% halothane using positive pressure ventilation. Through a right thoracotomy in the sixth intercostal space, the efferent lymphatic channel from the caudal mediastinal node (CMN) was cannulated with a thin silicon tube. This tube was secured and brought to the outside. Through a second thoracotomy in the ninth intercostal space, the tail of the CMN was ligated at the free margin of the inferior pul-

monary ligament to eliminate contamination with non-pulmonary lymph. We inserted catheters into the right carotid artery and extrajugular vein for the collection of blood samples and for drug infusion, respectively. The animals were allowed to recover for at least 7 days after the surgical procedures.

Experimental protocols We conducted the following two experiments.

Experiment 1: Experiments were done with the animals awake and standing. The dose of CDDP and 254-S was fixed at 100 mg/body, based on the data of the phase II study. CDDP or 254-S was given to the animal by an intravenous drip infusion for 30 min via the extrajugular vein. No additional hydration or diuretic agent was given to any animal. Lung lymph and blood samples were collected every 30 min after the start of the drug infusion. The observations were made over 24 h after the start of infusion.

Experiment 2: To measure the drug distribution in lung tissue, experiments were done in sheep anesthetized with pentobarbital sodium and halothane. Open lung biopsies were done by a modification of the method of Meyrick and Brigham[16] before and 30 min, 1 h, 1.5 h, and 2 h after the start of the drug infusion. Briefly, the lung was inflated to a constant pressure of 40 cm H_2O under anesthesia. We clamped the peripheral portion of the lung, and cut it along the clamp. After that, the cut surface of the lung was sutured around the clamp, and then the sutures were tightened immediately after the clamp was removed. Lung lymph and blood samples were obtained at the same time as the lung biopsy.

Sample analysis Blood and lung lymph samples were drawn in heparin-containing syringes, and immediately centrifuged at 600g for 15 min. To remove protein, as soon as the plasma and supernatant of lung lymph were prepared, portions of them were filtered through an Amicon MPS-3 filter (Amicon Corporation, Danvers, MA) by centrifugation at 1500g for 20 min at 4°C. Tissue samples were digested in nitric acid, and after evaporation to dryness, the residue was solubilized in dilute hydrochloric acid. All plasma, lung lymph, ultrafiltrates, and tissue samples were stored at −70°C until assayed for platinum. Platinum in these samples was measured at 265.9 nm by using a Hitachi Z 8000 polarized Zeeman atomic absorption spectrophotometer (Hitachi Ltd., Tokyo) as described previously.[17, 18]

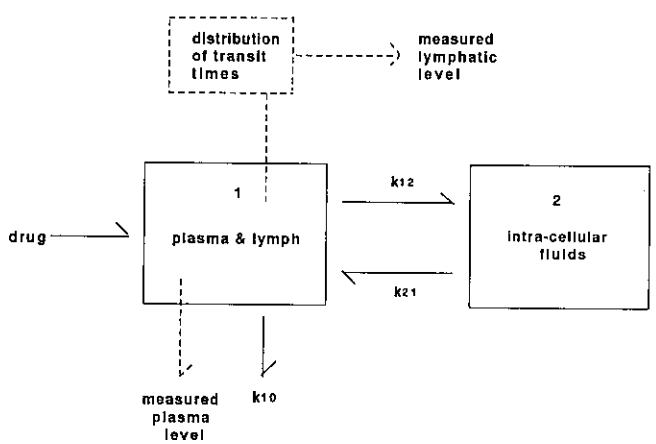

Fig. 1. Mathematical model for pharmacokinetic analysis of drug concentrations in plasma and thoracic duct lymph.[19]

Pharmacokinetic analysis The analysis of concentration-time curves was done on the basis of a model including part of the lymphatic circulation proposed by Blanc *et al.*,[19] using the nonlinear least-squares regression program MULTI, written by Yamaoka *et al.*[20] The mathematical model with plasma levels and thoracic duct lymphatic concentrations is shown in Fig. 1. That is,

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

$$C_L = 0 \qquad \text{if } 0 \leq t < \tau$$

$$C_L = G[A\nu(e^{-\nu(t-\tau)} - e^{-\alpha(t-\tau)})/(\alpha-\nu) + B\nu(e^{-\nu(t-\tau)} - e^{-\alpha(t-\tau)})/(\beta-\nu)] \qquad \text{if } t \geq \tau$$

where C_p, C_L, G and τ are plasma concentration, lymphatic concentration, a constant expressing the drug's partition between plasma and lymph and a time lag in the lymphatic time course, respectively. A and B are the intercepts at zero time. α, β and ν are the rate constants.

Protein binding data were calculated by Scatchard analysis using the nonlinear least-squares program. Statistical analysis was done by using Student's t test.

RESULTS

Pharmacokinetics in conscious sheep The changes in the plasma and lung lymph concentrations of total platinum after the intravenous infusion of 100 mg/body of CDDP and 254-S in conscious sheep are shown in Fig. 2. The plasma concentrations of total platinum for the two platinum compounds declined in a biexponential fashion. The peak plasma concentration of total platinum was slightly higher for 254-S than that for CDDP (254-S: 9.26±0.15 μg/ml, CDDP: 7.80±0.47 μg/ml). The peak lung lymph concentrations of total platinum were reached 1 h after the start of infusion (7.20±0.16 μg/ml for 254-S and 5.14±0.63 μg/ml for CDDP) and decayed triexponentially. We also measured platinum concentrations in the ultrafiltrate of plasma and lymph. Then, the data were analyzed using an anatomically based model including part of the lymphatic circulation.[19] Overall, the model gives satisfactory global non-linear least-squares fitting of plasma and lymphatic data. The pharmacokinetic parameters of total and ultrafilterable

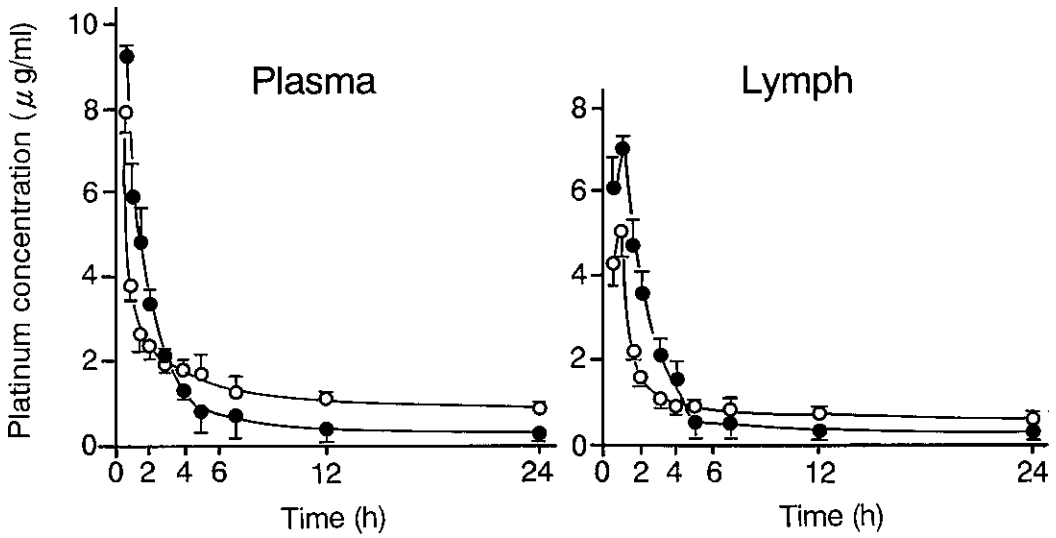

Fig. 2. Plasma and lung lymph levels of total platinum in conscious sheep treated with a 30-min drip infusion of 100 mg/body of CDDP (○) or 254-S (●). The points with bars represent means±SD of three sheep.

Table I. Pharmacokinetic Parameters of Platinum in Conscious Sheep Treated with 100 mg/kg of CDDP or 254-S

Parameter	CDDP		254-S	
	Total	Ultrafilterable	Total	Ultrafilterable
$t_{1/2\alpha}$ (h)	0.34±0.07[a)]	0.39±0.04	1.00±0.02**	0.14±0.04**
$t_{1/2\beta}$ (h)	23.21±5.51	2.33±0.66	103.74±63.59	1.15±0.31*
k_{12} (h^{-1})	1.61±0.36	0.32±0.09	0.33±0.22**	1.85±0.83*
k_{21} (h^{-1})	0.24±0.04	0.40±0.10	0.04±0.05**	2.47±1.49
k_{10} (h^{-1})	2.27±0.06	1.64±0.39	0.34±0.25	1.55±0.57
MRT (h)	29.85±7.65	1.21±0.45	68.06±67.65	1.32±0.22
V_d (liter)	42.39±4.69	17.58±6.42	152.14±129.57	7.98±2.44
Cl (liter/h)	1.48±0.37	14.57±1.12	2.81±2.03	6.07±1.60**
AUC (mg×h/liter)	71.02±20.11	6.89±0.55	48.89±29.23	17.26±4.49*
Lymph-partition	0.63±0.04	0.62±0.11	0.83±0.04**	0.75±0.15
Lag time (min)	28.38±1.25	28.19±0.44	21.05±12.54	23.48±3.74

a) Means±SD (n=3).
* Significantly different from the CDDP group at $P<0.05$.
** Significantly different from the CDDP group at $P<0.01$.

platinum of sheep are summarized in Table I. Although data for 254-S showed some scatter, the total platinum had a much longer half-life ($t_{1/2\beta}$) and a longer mean residence time (MRT) than CDDP. The area under the curve (AUC) of total platinum from 254-S was smaller than that of CDDP. On the other hand, the ultrafilterable platinum of 254-S showed significantly larger AUC than that of CDDP ($P<0.01$), even though the MRTs were the same. The transfer rate constants (k_{12} and k_{21}) were significantly larger in total CDDP and less in ultrafilterable CDDP than for total and ultrafilterable 254-S, respectively, while the V_d and Cl values showed the opposite tendencies in these compounds.

Plasma-lung lymph partition This study also indicated that 254-S had significantly higher partition into lymph than CDDP (Table I). Figure 3 shows the changes in the ratio of total platinum concentration in lung lymph to plasma after the infusion of these drugs. The ratios were similar for these platinum complexes during the initial decay phase, but the ratio was significantly and consistently higher for 254-S than for CDDP during the second decay phase.

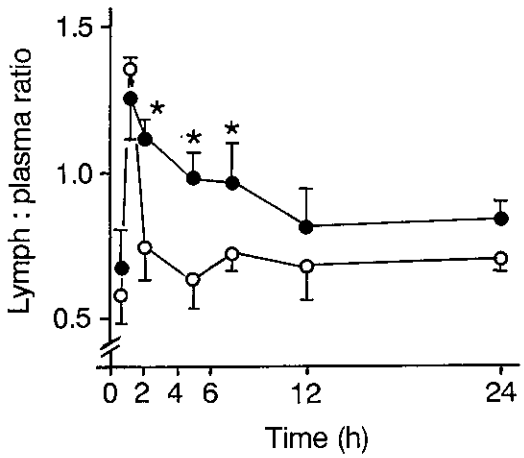

Fig. 3. Ratio of total platinum concentrations in lung lymph to plasma after a 30-min infusion of 100 mg/kg of CDDP (○) or 254-S (●). The points with bars represent means±SD of three sheep. * Significantly different from CDDP ($P<0.05$).

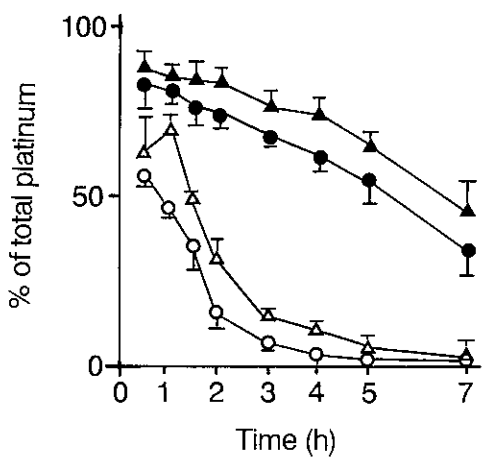

Fig. 4. Percentage of ultrafilterable platinum in plasma (○, ●) and lung lymph (△, ▲) after a 30-min infusion of 100 mg/body of CDDP (open symbols) or 254-S (closed symbols). The points with bars represent means±SD of the three sheep.

Protein binding Figure 4 shows the course of the ultrafilterable fraction of the two platinum complexes in plasma and lung lymph *in vivo*. After the infusion of CDDP, ultrafilterable platinum in plasma rapidly fell to a negligible amount by 4 h with a similar result in lung lymph. However, the levels of ultrafilterable platinum of 254-S were about 32% and 44% in plasma and lymph, respectively, even 7 h after the infusion. The association constant (K) and the binding capacity (nP) for the first class of binding sites of protein in plasma and lymph were analyzed and are shown in Table II. The protein-binding potency of CDDP was over 100-fold stronger than that of 254-S.

Table II. Protein Binding Parameters of CDDP and 254-S in Plasma and Lung Lymph from Sheep

Drug	K (μM)	nP (μM)
Plasma		
CDDP	20.83	5.27
254-S	0.14	6.61
Lung lymph		
CDDP	21.28	3.16
254-S	0.25	3.72

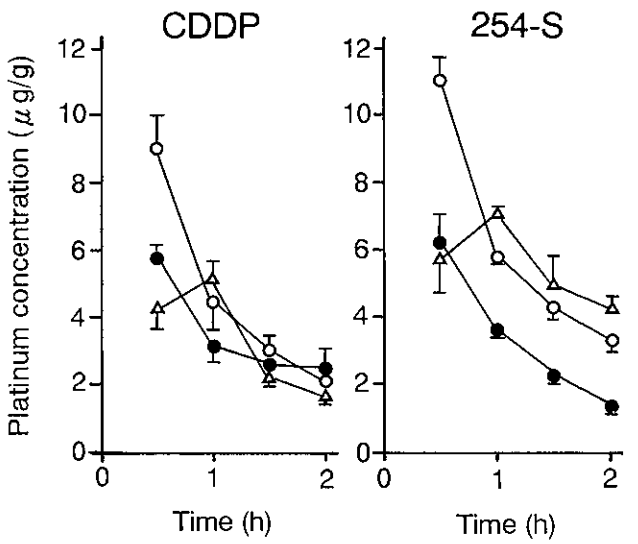

Fig. 5. Course of total platinum levels in plasma (○), lung lymph (△), and lung tissue (●) in anesthetized sheep treated with a 30-min drip infusion of 100 mg/body of CDDP or 254-S. The points with bars represent means±SD of three sheep.

Distribution to lung tissue in anesthetized sheep The changes of platinum concentration in lung tissue after the infusion of 254-S and CDDP were measured in anesthetized sheep. The pharmacokinetic behaviors of total platinum in plasma and lung lymph in the early decay phase after the infusion of these platinum complexes were almost the same as those in conscious sheep. The peak concentrations in lung tissue were observed at 30 min (just after the infusion), and then the concentration of 254-S exponentially decreased, but the decay rate of CDDP slowed down from 90 min (Fig. 5).

DISCUSSION

Staub *et al.*[15] have developed a steady-state, unanesthetized sheep preparation with a chronically instrumented lung lymph fistula that permits continuous measurement of net fluid and protein flow from the lung. Since the lymph obtained from the sheep is confirmed to be of pulmonary origin,[15,21] this model has been widely adopted for the study of lung fluid and protein exchange.[22-24] We measured total and free (ultrafilterable) platinum concentrations in plasma and lung lymph fluid in conscious sheep following intravenous infusion of 100 mg/body of two platinum complexes, CDDP and 254-S, for 30 min. The pharmacokinetic behaviors of platinum in the plasma from the sheep treated with 254-S and CDDP were almost the same as those from humans, reported by Sasaki *et al.*[10] Therefore, we thought that the sheep model was useful for evaluating the pharmacokinetics of the platinum complexes. In this study, we analyzed the pharmacokinetic data using an anatomically based model including part of the lymphatic circulation proposed by Blanc *et al.*[19] The pharmacokinetic data indicated that the free platinum of 254-S showed significantly larger AUC and transfer rate constants than those of CDDP, despite having the same MRT, while the total platinum showed the opposite pharmacokinetic behaviors. This seems to be attributable to the protein binding potency of 254-S being about 100-fold less than that of CDDP. Indeed, the free platinum fraction of 254-S was maintained at levels as high as 30–50% of total platinum even 7 h after the intravenous infusion, while the free fraction of CDDP quickly declined to a negligible level. As free platinum in the blood is regarded as the therapeutically active component,[18,25] these pharmacokinetic characteristics of the free form suggest that greater efficacy of 254-S than CDDP can be expected in cancer chemotherapy.

A further noteworthy result in this study is the difference of the pharmacokinetic behaviors in the lung lymph between 254-S and CDDP. 254-S had a consistently higher lung lymph-to-plasma concentration ratio than CDDP. In addition, the total platinum in lung lymph was higher than that in plasma as well as in lung tissue from anesthetized sheep treated with 254-S from 1–2 h, whereas after the infusion of CDDP the level in lung lymph was lower than those in plasma and lung tissue for most of the period. These data suggest that 254-S distributed in lung tissue is easily transferred to lung lymph, whereas CDDP may rather be accumulated in lung tissue. This is consistent with their protein binding characteristics; CDDP showed more potent binding to macromolecules than 254-S, thereby reducing the amount of CDDP available for transport from lung intestinum to lung lymph flow. The better transfer of 254-S into lung lymph from the lung tissue should have a favorable effect on therapeutic activity against metastatic lymph nodes.

In conclusion, our results presented here may provide some insights into chemotherapeutic strategy for intrathoracic malignancies, especially lung cancer. Pharmacokinetic analysis of lung lymph is an important factor in the evaluation of agents with cytotoxic activity against intrathoracic malignancies.

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