Antitumor Activity and Cellular Accumulation of a New Platinum Complex, (−)-(R)-2-Aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) Monohydrate, in Cisplatin-sensitive and -resistant Murine P388 Leukemia Cells

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We have examined the cytotoxicity and accumulation of (−)-(R)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate (DWA2114R) in parent and cisplatin-resistant mouse P388 leukemia cells (P388 and P388/DDP), in comparison with those of cisplatin (CDDP) and carboplatin (CBDCA). The degrees of resistance to CDDP and CBDCA, expressed as the ratio of IC_{50} for P388/DDP cells to IC_{50} for P388 cells, were 75-33 and 100-27, respectively, under the conditions of 2–24 h exposure to each drug at a density of 10^6 cells/ml. The corresponding values (25-7) for DWA2114R were relatively low. Accumulations of CDDP and CBDCA were reduced in P388/DDP cells; however, no reduction in accumulation of DWA2114R was observed at various exposure periods and concentrations of the drugs. The accumulations of CDDP in P388 and P388/DDP cells at drug concentrations corresponding to the IC_{50} values for drug exposure periods of 2–24 h were 0.41–0.97 and 13.1–33.7 ng $Pt/10^7$ cells, respectively, suggesting that an intracellular mechanism of resistance against CDDP could be activated in P388/DDP cells. P388/DDP cells also showed relatively low resistance to DWA2114R via this mechanism in comparison with CDDP and CBDCA. From the relationship between structure and activity of several Pt-complexes, these different properties of DWA2114R compared with CDDP and CBDCA could be due not only to the differences in carrier ligand structure but also to the properties of the whole molecule associated with the carrier ligand and leaving group.

Key words: Antitumor platinum complex — Cisplatin-resistant P388 leukemia cell line — Platinum complex accumulation in cell

Cisplatin (CDDP)[5] has been one of the most potent anticancer chemotherapeutic agents against experimental and clinical tumors.[1, 2] However, CDDP has severe side effects such as nephrotoxicity, neurotoxicity, nausea and vomiting.[3, 4] Because of these toxicities, many attempts have been made to develop CDDP analogs with equivalent or higher antitumor activity and lower toxicity than CDDP.[5–10] In addition, the appearance of CDDP-resistant tumors has often been reported.[11, 12] Therefore, the activities of newly developed CDDP analogs against CDDP-resistant tumors are of interest.[10, 13–15]

A new platinum complex, (−)-(R)-2-aminomethyl-pyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate (DWA2114R), currently undergoing

Phase III studies in Japan, has been demonstrated to have equivalent antitumor activities to CDDP against various rodent tumors and much lower nephrotoxicity than CDDP.[16, 17] It has also been demonstrated that CDDP-resistant mouse L1210 leukemia cells had lower cross-resistance to DWA2114R than to CDDP and carboplatin (CBDCA).[18]

In this study, we have examined the cytotoxicity and accumulation of DWA2114R in CDDP-resistant mouse P388 leukemia cells, in comparison with those of CDDP and CBDCA. We have also studied the relationship between the structure and activity of DWA2114R. The results obtained from these experiments indicate that DWA2114R has activity against a CDDP-resistant murine tumor.

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[5] Abbreviations: CDDP, cisplatin; DWA2114R, (−)-(R)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate; CBDCA, carboplatin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; FCS, fetal calf serum; PBS, phosphate-buffered saline.
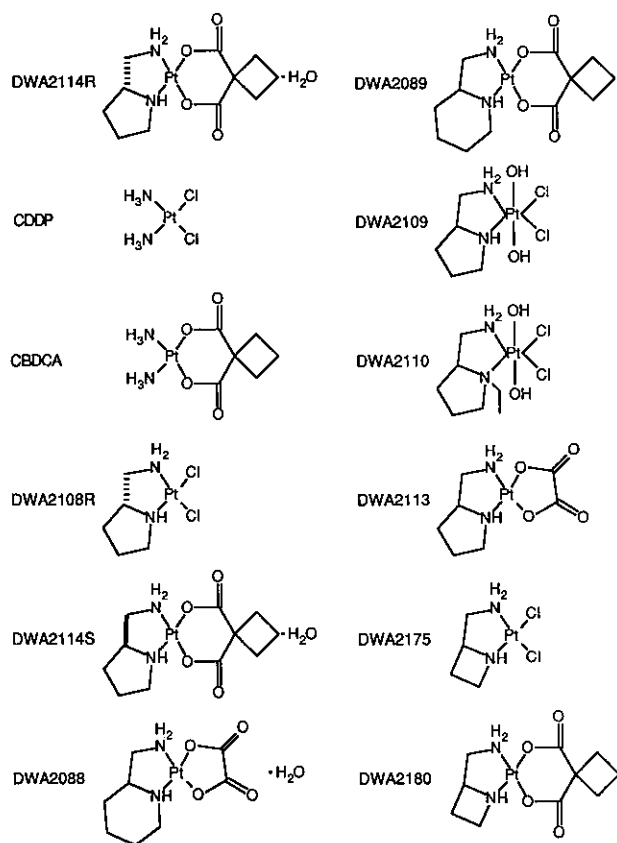
MATERIALS AND METHODS

Chemicals Compounds of the DWA series (Fig. 1) were synthesized in our laboratory.[16, 19] CDDP was purchased from Aldrich Chemical Co. Inc. CBDCA was also synthesized according to US patent 4140707[20] in our laboratory. These drugs were dissolved in 0.9% NaCl or RPMI-

Fig. 1. Chemical structures of Pt complexes used in this paper.

μM 2-mercaptoethanol at a density of 2×10^6 cells/ml. The cell suspension was added to 6-well tissue culture plates and exposed to various concentrations of drugs at 37°C under a 5% CO_2 atmosphere. At various intervals (1–24 h) after drug addition, the cells were collected and washed with phosphate-buffered saline (PBS) three times by centrifugation ($200g$, 4°C, 5 min). The cell pellets were transferred to glass tubes with small portions of PBS. The Pt content of the cells was determined as described below. The experiments were carried out in duplicate.

Pt determination Pt content of cells was determined by a method similar to that of Pera and Harder.[22] Briefly, cell suspension in PBS was lyophilized, digested in 60% HNO_3 and evaporated until dry. The residue was dissolved in 0.1 N HNO_3 and Pt content was measured by flameless atomic absorption spectrophotometry using a Model AA-8500 MK II (Nippon Jarrell-Ash Co., Ltd., Kyoto) equipped with a heated graphite furnace. Values for each sample were determined in triplicate. Statistical analyses were carried out by using Student's t test.

Determination of drug sensitivity Sensitivities of P388 and P388/DDP cells to DWA2114R, CDDP and CBDCA were determined by measuring the inhibition of cell growth in response to continuous or pulse exposure.

For continuous exposure, ascitic cells prepared from tumor-bearing mice in the same manner as described for drug uptake experiments were exposed to various concentrations of drug at a density of 2.5×10^4 cells/ml at 37°C under a 5% CO_2 atmosphere in 96-well tissue culture plates for 72 h in duplicate. The resultant cell number was measured in terms of MTT formazan formation.[23] *In vitro* drug sensitivity was expressed as the concentration of drug producting 50% inhibition of growth (IC_{50}).

In order to investigate the relationship between drug sensitivity and uptake, IC_{50} values were determined as follows. Ascitic cells prepared from tumor-bearing mice were exposed to various concentrations of drug at a density of 10^6 cells/ml at 37°C under a 5% CO_2 atmosphere for various time intervals. The cells were then washed with RPMI1640 medium containing 10% FCS and 50 μM 2-mercaptoethanol by centrifugation ($200g$, 4°C, 5 min), diluted 400-fold (2.5×10^4 cells/ml) with the same medium and incubated at 37°C under a 5% CO_2 atmosphere in quadruplicate for 72 h in total. The resultant cell number was measured as described above.

Drug efflux Ascitic cells prepared from tumor-bearing mice were exposed to 50 μM drug at a density of 10^6 cells/ml as described above. After 2 h the cells were washed, resuspended in fresh medium, and Pt accumulated in the cells was determined using an aliquot of the cell suspension. The remainder was further incubated for 2 h under the same conditions in fresh medium. The

1640 medium immediately before use. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co., RPMI1640 from Gibco Ltd., fetal calf serum (FCS) from Boehringer Mannheim, kanamycin from Meiji Seika Co., Ltd. and 2-mercaptoethanol from Tokyo Kasei Co., Ltd. **Cells** A CDDP-resistant subline (P388/DDP) of P388 was established from the parent cell line, P388.[10] P388 and P388/DDP cells were maintained in DBA/2 mice by weekly transplantaion of 10^6 ascites cells.

Drug uptake Drug uptake studies were carried out basically as described by Waud.[21] Ascitic cells were aseptically harvested from mice bearing P388 or P388/DDP 7 days after implantation of 10^6 cells and washed with RPMI1640 medium by centrifugation ($200g$, 4°C, 5 min). The cells were resuspended in 0.017 M Tris-HCl (pH 7.2) containing 0.75% NH_4Cl and incubated at 4°C for 1 min to lyse contaminating erythrocytes. The cells were collected by centrifugation ($200g$, 4°C, 5 min), washed twice with RPMI1640 medium and resuspended with RPMI1640 medium containing 10% FCS and 50

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cells were collected and washed with PBS 3 times by centrifugation (200*g*, 4°C, 5 min). The Pt content of the cells was then determined. The difference in Pt content between the two cell preparations reflects the extent of drug efflux.

RESULTS

Drug sensitivity of cells The sensitivities of P388 and P388/DDP cells to DWA2114R, CDDP and CBDCA are shown in Table I. P388/DDP cells were found to be resistant to CDDP and CBDCA in comparison with P388 cells, while P388/DDP cells were relatively more sensitive to DWA2114R than to CDDP or CBDCA for any period of drug exposure. The data also indicated that DWA2114R has relatively higher *in vitro* time-dependence of cytotoxicity against both cell lines, in particular P388/DDP cells, than CDDP or CBDCA, with CDDP showing the least time-dependence.

Relationship between resistance and cellular accumulation of drugs To determine the relationship between resistance and cellular accumulation of the drugs in sensitive and resistant cell lines, the accumulations for various exposure periods or at various concentrations of the drugs in RPMI1640 containing 10% FCS were measured in each cell line.

Cellular accumulations of these drugs at 50 μM increased nearly linearly for approximately 8 h in both parent and CDDP-resistant cell lines (Fig. 2). Furthermore, cellular accumulations for a 2 h exposure period were increased almost linearly with drug concentration over the range from 25 to 200 μM in both cell lines (Fig. 3).

Accumulations of CDDP and CBDCA in P388/DDP cells were much lower than those in P388 cells for various concentrations and priods of exposure time of the drugs. However, accumulation of DWA2114R in P388/DDP cells was almost equal to or greater than that in

Table I. Time-dependent Cytotoxicity of Pt Complexes to P388 and P388/DDP Cells[a]

Drug exposure (h)	Cytotoxicity (IC$_{50}$; μM)								
	DWA2114R			CDDP			CBDCA		
	P388	P388/DDP	Ratio[b]	P388	P388/DDP	Ratio[b]	P388	P388/DDP	Ratio[b]
2	6.11	153.6	25	0.34	25.5	75	10.54	1055.6	100
4	3.55	44.2	12	0.25	11.3	45	5.73	415.0	72
8	2.45	33.7	14	0.20	8.9	45	4.49	260.7	58
24	1.33	9.1	7	0.15	4.9	33	2.63	71.0	27

a) P388 and P388/DDP cells were exposed with each drug at a density of 10^6 cells/ml at 37°C.
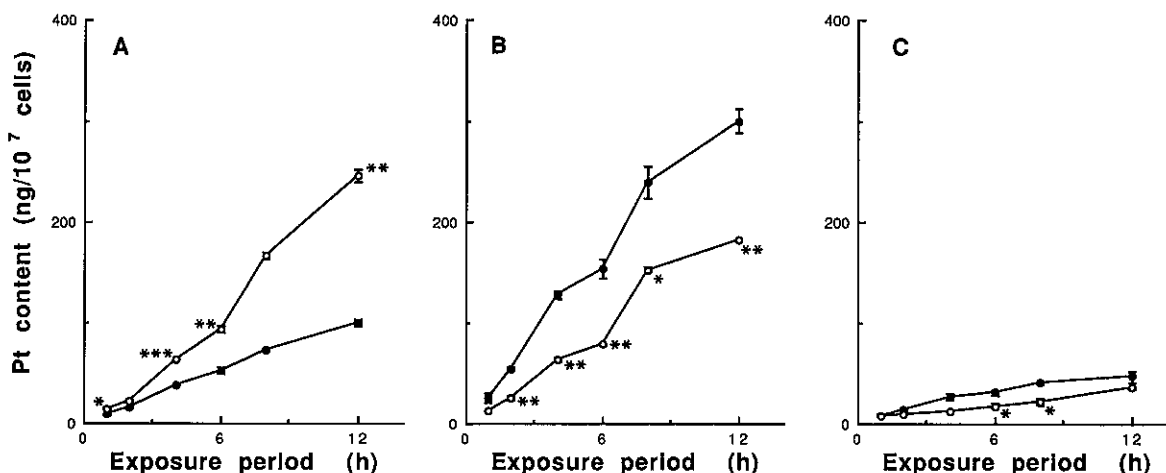*b*) Ratio of (IC$_{50}$ vs. P388/DDP cells)/(IC$_{50}$ vs. P388 cells).

Fig. 2. Pt incorporations of P388 and P388/DDP cells using Pt complexes at 50 μM for various exposure times. A, DWA2114R; B, CDDP; C, CBDCA. ●, P388; ○, P388/DDP. Bars indicate mean±SD. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.
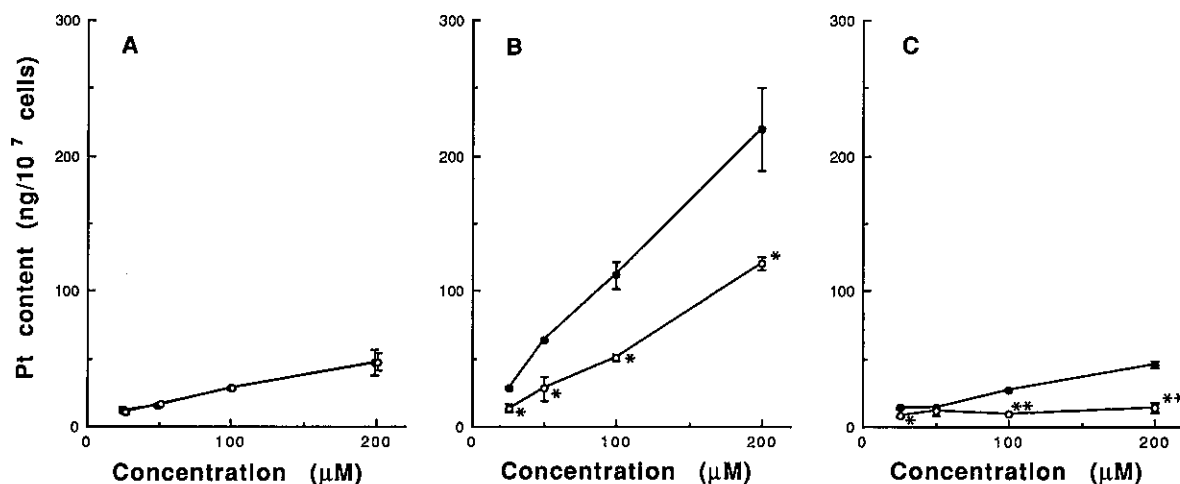
Fig. 3. Pt incorporations of P388 and P388/DDP cells at various concentrations of Pt complexes for 2 h. A, DWA2114R; B, CDDP; C, CBDCA. ●, P388; ○, P388/DDP. Bars indicate mean±SD. *, $P<0.05$; **, $P<0.01$.

Table II. Efflux of Pt Complexes from P388 and P388/DDP Cells[a]

Drug	Pt amount (ng/10^7 cells)[b]			
	P388 cells		P388/DDP cells	
	Before efflux	After 2 h efflux	Before efflux	After 2 h efflux
DWA2114R	19.3±0.4	16.6±0.7 (86%)[c]	24.7±1.9	25.0±2.6 (101%)
CDDP	88.3±8.5	85.7±4.7 (97)	35.8±3.5	35.7±2.7 (100)
CBDCA	15.5±2.0	12.8±0.4 (83)	8.9±0.8	6.4±0.7 (72)

a) Each cell preparation was treated with 50 μM of each Pt complex 37°C for 2 h. After the treatment, the cells were washed and the Pt content of the cells was determined using an aliquot of the treated cells. Residual cell fractions were incubated for a further 2 h at 37°C in fresh medium. The cells were washed, and Pt remaining in them was also determined.
b) Mean±SD.
c) Values in parentheses are % of Pt in the cells after 2 h efflux to that before efflux.

P388 cells for at any concentration and exposure period of the drugs. These data suggest that reduced accumulation of CDDP and CBDCA could be one component of resistance in P388/DDP cells, while lower cross-resistance to DWA2114R in P388/DDP cells could be due to equal or greater accumulation in P388/DDP cells in comparison with P388 cells.

Since the Pt content of P388/DDP cells after 2 h efflux was not greatly reduced in comparison with P388 cells, the mechanism of drug efflux is probably not significantly activated in P388/DDP (Table II).

The cellular accumulations of the drugs under conditions which produced comparable effects were determined at concentrations corresponding to the IC$_{50}$ values of the drugs.

As shown in Table III, P388/DDP cells accumulated 30- to 40-fold higher amounts of CDDP and about 20-

fold higher amounts of DWA2114R than P388 cells; CBDCA gave results intermediate between CDDP and DWA2114R. Since the accumulation of CDDP in P388/DDP cells was greatly increased in comparison with P388 cells when treated at concentrations corresponding to the IC$_{50}$ values, some intracellular resistance mechanism(s) against CDDP could be activated in P388/DDP cells. A similar phenomenon was observed using CBDCA and DWA2114R; however, the ratio of accumulation of DWA2114R in P388/DDP cells to that in P388 cells was lower than seen with CDDP for all periods of drug exposure. Therefore, P388/DDP cells appeared to be less resistant to DWA2114R than to CDDP or CBDCA with respect to this intracellular resistance mechanism.

Resistance and chemical structure of drugs The above data strongly suggest that DWA2114R could have different properties from CDDP and CBDCA. The major

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Table III. Pt Incorporations of P388 and P388/DDP Cells at IC_{50} Values of Pt Complexes at Various Exposure Periods

| Drug exposure period at IC_{50}[a] (h) | Pt amount (ng/10^7 cells)[b] | | | | | | | | |
| | DWA2114R | | | CDDP | | | CBDCA | | |
	P388	P388/DDP	Ratio[c]	P388	P388/DDP	Ratio[c]	P388	P388/DDP	Ratio[c]
2	2.57±0.19	57.2±4.2	22	0.41±0.06	13.1±2.9	32	3.97±0.11	96.7±7.3	24
4	2.44±0.16	43.6±2.3	18	0.49±0.04	15.0±0.5	31	3.55±0.07	84.0±3.9	24
8	4.13±0.23	83.1±7.2	20	0.60±0.06	23.7±2.0	40	5.07±0.41	133.0±4.7	26
24	10.50±0.53	170.0±5.9	16	0.97±0.04	33.7±1.4	35	8.16±0.23	163.0±3.8	20

a) IC_{50} values for each period of time for each drug in Table I were used.
b) Mean±SD.
c) Ratio of (Pt amount of P388/DDP cells)/(Pt amount of P388 cells).

Table IV. Cytotoxicities of Pt Complexes against P388 and P388/DDP *in vitro*[a]

| Drug | Cytotoxicity (IC_{50}; μM) | | |
	P388/DDP	P388	Ratio[b]
DWA2114R	38.8	3.36	12
CDDP	19.2	0.32	60
DBDCA	260.1	4.21	62
DWA2108R	125.0	1.93	65
DWA2114S	22.8	3.55	6
DWA2088	115.4	3.86	30
DWA2089	98.1	10.93	9
DWA2109	>249.8[c]	9.76	>26
DWA2110	123.4	7.42	17
DWA2113	40.2	1.80	22
DWA2175	77.3	1.06	73
DWA2180	22.8	2.01	11

a) P388 and P388/DDP cells were continuously exposed to each drug at a density of 2.5×10^4 cells/ml at 37°C for 72 h.
b) Ratio of (IC_{50} vs. P388/DDP cells)/IC_{50} vs. P388 cells).
c) Greater than.

difference in chemical structure between DWA2114R and CDDP or CBDCA is the structure of the carrier ligand (Fig. 1). To determine whether the difference in resistance of P388/DDP cells to DWA2114R in comparison to CDDP and CBDCA could be due simply to the different carrier ligand structure, we examined the relationships between structures of Pt complexes and sensitivities of P388/DDP cells. The structures of various Pt complexes used and the sensitivities of P388 and P388/DDP cells to these Pt complexes are shown in Fig. 1 and Table IV, respectively. Table IV shows the following relationship between structure and activity: (1) P388/DDP cells have high cross-resistance to Pt complexes which share the structure of either the carrier ligand or the leaving group of CDDP; (2) P388/DDP cells have
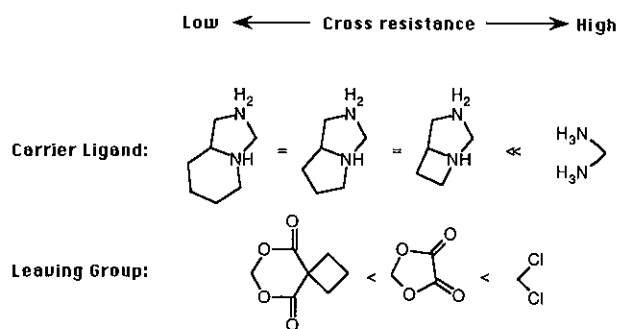

Fig. 4. Resistance of P388/DDP cells in relation to structures of carrier ligands and leaving groups of Pt complexes.
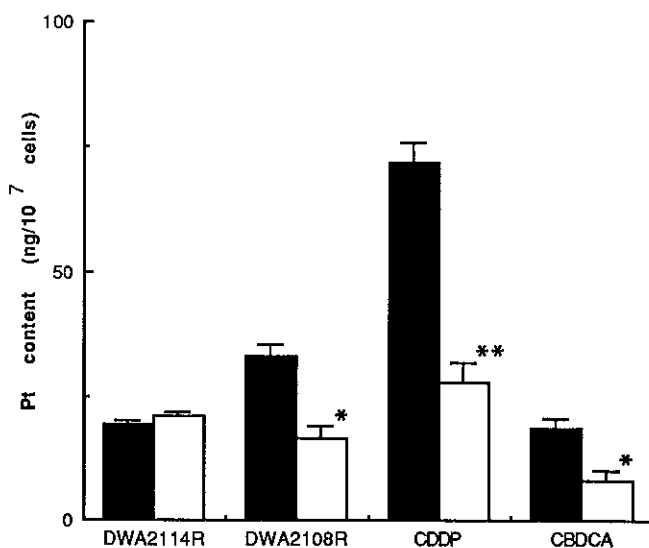

Fig. 5. Pt incorporation of P388 and P388/DDP cells using various Pt complexes at 50 μM for 2 h. ■, P388; □, P388/DPP. Bars indicate mean±SD. *, P<0.05; **, P<0.01.

relatively low cross-resistance to Pt complexes which have both the carrier ligand and leaving group structures of DWA2114R or similar types. This relationship is shown diagrammatically in Fig. 4.

In addition, we determined the accumulation of DWA-2108R, which has a carrier ligand of the DWA2114R type and a leaving group of the CDDP type, in comparison with DWA2114R, CDDP and CBDCA (Fig. 5). Accumulation of DWA2108R in P388/DDP cells was much lower than in P388 cells, similar to the results obtained in the cases of CDDP and CBDCA, while accumulation of DWA2114R in P388/DDP cells was almost equal to that in P388 cells, as shown in Fig. 2.

DISCUSSION

Various mechanisms have been proposed to explain resistance to CDDP, such as differences in drug transport, differences in glutathione content and differences in DNA crosslinking between sensitive and resistant cells.[13, 15, 21, 24-29] In P388/DDP cells, the accumulations of CDDP and CBDCA were reduced in experiments using the same concentrations of the drugs in comparison with the parent P388 cells (Figs. 2 and 3), as reported elsewhere.[30] Furthermore, in the accumulation experiments at the IC_{50} values, which have almost the same effect on the cell growth, P388/DDP cells accumulated much greater amounts of CDDP and CBDCA in comparison with the parent P388 cells (Table III). Therefore, it appears that the resistance of P388/DDP cells to CDDP and CBDCA is due to (a) reduction of drug accumulation, which could involve membrane transport of the drug and (b) activation of an intracellular resistance mechanism.

Since accumulation of DWA2114R was not reduced but rather increased (Figs. 2 and 3), P388/DDP cells appeared less able to resist DWA2114R through mechanism (a). With respect to mechanism (b), P388/DDP cells appeared to show relatively low resistance to DWA-2114R in comparison with CDDP and CBDCA (Table III). From these results, it appears that DWA2114R could be effective against CDDP-resistant tumor cells which have mechanisms of resistance that are similar to those of P388/DDP cells.

Since accmulations of CDDP and CBDCA were reduced and that of DWA2114R was not altered (or was increased) in the same P388/DDP cells, there could be multiple mechanisms of uptake of Pt-complexes, and the mechanisms of uptake in P388/DDP cells could be altered from those of the parent P388 cell line. Mauldin et al.[31] have reported that one CDDP analog, (d,l-trans-1,2-diaminocyclohexane)malonatoplatinum(II), could be changed to different forms with different cell accumu-

lation rates and have proposed that multiple mechanisms could contribute to accumulation of the drug.

From a comparison of activities and chemical structures, it appears that the differences in activities between DWA2114R and CDDP or CBDCA could be due to not only the structure of the carrier ligand but also the properties of the whole molecule associated with both carrier ligand and leaving group (Figs. 4 and 5, Table IV). It has been suggested that the structure of the carrier ligand could be directly relate to the properties of Pt complexes with respect to resistance and accumulation in CDDP-resistant L1210 leukemia cells; however, in the case of CDDP-resistant P388 leukemia cells, the properties of Pt complexes could be due to not only differences in the structures of the carrier ligands but also those of the whole molecular properties related to both carrier ligand and leaving group.[13] The P388/DDP cells used in this paper might have similar properties to the CDDP-resistant P388 leukemia cells previously reported.

Recently, Misawa et al. reported that a CDDP-resistant human ovarian tumor cell line, NOS2CR, showed low resistance to DWA2114R.[32] Reduction of accumulation of CDDP in NOS2CR cells in comparison to the parent cells, NOS2, might be one resistance mechanism in this CDDP-resistant cell line. The accumulation of DWA2114R in NOS2CR cells was similar to that in NOS2 cells, while that of CDDP and CBDCA in the former cells was markedly decreased. Therefore, it was concluded that the low resistance of NOS2CR cells to DWA2114R reflected unchanged drug accumulation. These results suggested that the transport mechanism of NOS2CR cells for DWA2114R might be different from that of CDDP.

Furthermore, Kikuchi et al. have reported that another CDDP-resistant human ovarian tumor cell line, KFr, also showed low resistance to DWA2114R in comparison with CDDP and several CDDP derivatives including CBDCA, 254S and NK121.[33] One of the possible resistance mechanisms of KFr cells for CDDP is reduction of accumulation of this drug, although not all the resistance can be explained in terms of changes in drug transport. In fact, the decrease of DWA2114R accumulation in KFr cells compared with KF cells was lower than that of CDDP. Therefore, it is likely that the low resistance of KFr cells to DWA2114R derives from differences in drug transport mechanisms.

REFERENCES

1) Rosenberg, B., VanCamp, K., Trosko, J. E. and Mansour, V. H. Platinum compounds: a new class of potent antitumor agents. *Nature*, **222**, 385–386 (1969).
2) Rozencweig, M., Von Hoff, D. D., Slavik, M. and Muggia, F. M. *Cis*-diamminedichloroplatinum(II). A new anticancer drug. *Ann. Intern. Med.*, **86**, 803–812 (1977).
3) Von Hoff, D. D., Schilsky, R., Reichert, C. M., Reddick, R. L., Rozencweig, M., Young, R. C. and Muggia, F. M. Toxic effects of *cis*-dichlorodiammineplatinum(II) in man. *Cancer Treat. Rep.*, **63**, 1527–1531 (1979).
4) Krakoff, I. H. Nephrotoxicity of *cis*-dichlorodiammineplatinum(II). *Cancer Treat. Rep.*, **63**, 1523–1525 (1979).
5) Burchenal, J. H., Kalaher, K., Dew, K. and Lokys, L. Rationale for development of platinum analogs. *Cancer Treat. Rep.*, **63**, 1493–1498 (1979).
6) Connors, T. A., Cleare, M. J. and Harrap, K. R. Structure-activity relationships of the antitumor platinum coordination complexes. *Cancer Treat. Rep.*, **63**, 1499–1502 (1979).
7) Brown, D. B., Khokhar, A. R., Hacker, M. P., Lokys, L., Burchenal, J. H., Newman, R. A., McCormack, J. J. and Frost, D. Synthesis and antitumor activity of new platinum complexes. *J. Med. Chem.*, **25**, 952–956 (1982).
8) Lelieveld, P., Van der Vijgh, W. J. F., Veldhuizen, R. W., Van Velzen, D., Van Putten, L. M., Atassi, G. and Danguy, A. Preclinical studies on toxicity, antitumour activity and pharmacokinetics of cisplatin and three recently developed derivatives. *Eur. J. Cancer Clin. Oncol.*, **20**, 1087–1104 (1984).
9) Carter, S. K., Canetta, R. and Rozencweig, M. Carboplatin: future directions. *Cancer Treat. Rev.*, **12**, (Suppl. A), 145–152 (1985).
10) Tashiro, T., Kawada, Y., Sakurai, Y. and Kidani, Y. Antitumor activity of a new platinum complex, oxalato (*trans-l*-1,2-diaminocyclohexane)platinum (II): new experimental data. *Biomed. Pharmacother.*, **43**, 251–260 (1989).
11) Ozols, R. F. and Young, R. C. Chemotherapy of ovarian cancer. *Semin. Oncol.*, **11**, 251–263 (1984).
12) Inoue, K., Mukaiyama, T., Mitsui, I. and Ogawa, M. *In vitro* evaluation of anticancer drugs in relation to development of drug resistance in the human tumor clonogenic assay. *Cancer Chemother. Pharmacol.*, **15**, 208–213 (1985).
13) Kraker, A. J. and Moore, C. W. Accumulation of *cis*-diamminedichloroplatinum(II) and platinum analogues by platinum-resistant murine leukemia cells *in vitro*. *Cancer Res.*, **48**, 9–13 (1988).
14) Wilkoff, L. J., Dulmadge, E. A., Trader, M. W., Harrison, S. D., Jr. and Griswold, D. P., Jr. Evaluation of *trans*-tetrachloro-1,2-diaminocyclohexane platinum(IV) in murine leukemia L1210 resistant and sensitive to *cis*-diamminedichloroplatinum(II). *Cancer Chemother. Pharmacol.*, **20**, 96–100 (1987).
15) Behrens, B. C., Hamilton, T. C., Masuda, H., Grotzinger,

K. R., Whang-Peng, J., Louie, K. G., Knutsen, T., McKoy, W. M., Young, R. C. and Ozols, R. F. Characterization of a *cis*-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res.*, **47**, 414–418 (1987).
16) Morikawa, K., Honda, M., Endoh, K., Matsumoto, T., Akamatsu, K., Mitsui, H. and Koizumi, M. Synthesis, antitumor activity, and nephrotoxicity of the optical isomers of 2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II). *J. Pharm. Sci.*, **80**, 837–842 (1991).
17) Matsumoto, T., Endo, K., Akamatsu, K., Kamisango, K., Mitsui, H., Koizumi, K., Morikawa, K., Koizumi, M. and Matsuno, T. Comparison of the antitumor effects and nephrotoxicity-inducing activities of two new platinum complexes, (−)-(R)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate, and its enantiomeric isomer. *Br. J. Cancer*, **64**, 41–46 (1991).
18) Akamatsu, K., Endo, K., Matsumoto, T., Kamisango, K., Morikawa, K., Koizumi, M., Mitsui, H., Koizumi, K. and Matsuno, T. Antitumor activity of a new platinum complex, (R)-(−)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II), against *cis*-diamminedichloroplatinum(II)-resistant murine leukemia cell line. *Jpn. J. Cancer Chemother.*, **17**, 73–78 (1990).
19) Morikawa, K., Honda, M., Endoh, K., Matsumoto, T., Akamatsu, K., Mitsui, H. and Koizumi, M. Synthesis and antitumor activities of platinum complexes of unsymmetrical alicyclic diamines as carrier ligands. *J. Pharm. Sci.*, **79**, 750–753 (1990).
20) Cleare, M. J. and Hoeschele, J. D. US Patent 4140707 (1989) [*Chem. Abstr.*, **81**, 21172 (1974)].
21) Waud, W. R. Differential uptake of *cis*-diamminedichloroplatinum(II) by sensitive and resistant murine L1210 leukemia cells. *Cancer Res.*, **47**, 6549–6555 (1987).
22) Pera, M. F., Jr. and Harder, H. C. Analysis for platinum in biological material by flameless atomic absorption spectrometry. *Clin. Chem.*, **23**, 1245–1249 (1977).
23) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**, 55–63 (1983).
24) Andrews, P. A., Velury, S., Mann, S. C. and Howell, S. B. *Cis*-diamminedichloroplatinum(II) accumulation in sensitive and resistant human ovarian carcinoma cells. *Cancer Res.*, **48**, 68–73 (1988).
25) Shionoya, S., Lu, Y. and Scanlon, K. J. Properties of amino acid transport systems in K562 cells sensitive and resistant to *cis*- diamminedichloroplatinum(II). *Cancer Res.*, **46**, 3445–3448 (1986).
26) Strandberg, M. C., Bresnick, E. and Eastman, A. The significance of DNA cross-linking to *cis*-diamminedichloroplatinum(II)-induced cytotoxicity in sensitive and resistant lines of murine leukemia L1210 cells. *Chem.-Biol. Interact.*, **39**, 169–180 (1982).

27) Masuda, H., Ozols, R. F., Kai, G.-M., Fojo, A., Rothenberg, M. and Hamilton, T. C. Increased DNA repair as a mechanism of acquired resistance to *cis*-diamminedichloroplatinum(II) in human ovarian cancer cell lines. *Cancer Res.*, **48**, 5713–5716 (1988).

28) Hamilton, T. C., Winker, M. A., Louie, K. G., Batist, G., Behrens, B. C., Tsuruo, T., Grotzinger, K. R., McKoy, W. M., Young, R. C. and Ozols, R. F. Augmentation of adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and -sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. *Biochem. Pharmacol.*, **34**, 2583–2586 (1985).

29) Wang, Y., Teicher, B. A., Shea, T. C., Holden, S. A., Rosbe, K. W., Al-Achi, A. and Henner, W. D. Cross-resistance and glutathione-S-transferase-π levels among four human melanoma cell lines selected for alkylating agent resistance. *Cancer Res.*, **49**, 6185–6192 (1989).

30) Tashiro, T. and Sato, Y. Characterization of acquired resistance to *cis*-diamminedichloroplatinum(II) in mouse leukemia cell lines. *Jpn. J. Cancer Res.*, **83**, 219–225 (1992).

31) Mauldin, S. K., Plescia, M., Richard, F. A., Wyrick, S. D., Voyksner, R. D. and Chaney, S. G. Displacement of the bidentate malonate ligand from (*d,l-trans*-1,2-diamino-cyclohexane)malonatoplatinum(II) by physiologically important compounds *in vitro. Biochem. Pharmacol.*, **37**, 3321–3333 (1988).

32) Misawa, T., Kikkawa, F., Oguchi, H., Morikawa, Y., Kawai, M., Maeda, O., Iwata, M., Kano, T., Furuhashi, Y. and Tomoda, Y. Accumulation of *cis*-diamminedichloroplatinum(II) and its analogues in sensitive and resistant human ovarian carcinoma cells. *Oncology*, (1992), in press.

33) Kikuchi, Y., Iwano, I., Miyauchi, M., Kita, T., Sugita, M., Tenjin, Y. and Nagata, I. Possible mechanisms of resistance to *cis*-diamminedichloroplatinum (II) of human ovarian cancer cells. *Jpn. J. Cancer Res.*, **81**, 701–706 (1990).