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Cytotoxicity of *cis*-[((1*R*,2*R*)-1,2-Cyclohexanediamine-*N*,*N*′)bis(myristato)]-platinum (II) Suspended in Lipiodol in a Newly Established Cisplatin-resistant Rat Hepatoma Cell Line

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The cytotoxic activity of *cis*-[((1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′)bis(myristato)]platinum (II) (SM-11355) was evaluated in a cisplatin (CDDP)-resistant tumor cell line, and compared with that of CDDP. H4-II-E/CDDP with acquired resistance to CDDP was established by continuous exposure of a rat hepatic tumor line, H4-II-E, to increasing concentrations of CDDP over 12 months. Compared with the parental cell line, this cell line exhibited an 8.8-fold increase in resistance to CDDP and was not cross-resistant to 1,2-diaminocyclohexane platinum (II) dichloride (DPC). There were no differences in sensitivity to six non-platinum antitumor drugs between H4-II-E and H4-II-E/CDDP, which suggests that H4-II-E/CDDP is not multidrug-resistant. Intracellular platinum accumulation and the formation of a platinum-DNA adduct following CDDP exposure were significantly reduced in H4-II-E/CDDP compared to the parental cell line. The acquired CDDP resistance in H4-II-E/CDDP appeared to be predominantly due to reduced CDDP uptake. H4-II-E/CDDP was also resistant to CDDP suspended in Lipiodol (CDDP/Lipiodol), but was not cross-resistant to SM-11355 suspended in Lipiodol (SM-11355/Lipiodol). Also, there were no differences in intracellular platinum accumulation or the formation of platinum-DNA adducts after SM-11355/Lipiodol exposure between H4-II-E and H4-II-E/CDDP. These results suggest that acquired CDDP resistance in H4-II-E/CDDP does not influence the cytotoxic activity of SM-11355/Lipiodol.

Key words: Lipiodol — Cisplatin — Hepatocellular carcinoma — Sustained release — Resistance

Cisplatin (CDDP) is one of the most effective antitumor agents for the treatment of a variety of human malignancies.[1)] However, many tumors are intrinsically resistant to CDDP. In addition, initially sensitive tumors commonly develop acquired resistance to CDDP during the course of treatment. The development of resistance is a major limiting factor in curative treatment. Studies involving sensitive and acquired CDDP-resistant murine and human tumor cell lines have shown that CDDP resistance is multifocal, and may be due to reduced intracellular accumulation of CDDP, elevated intracellular thiol levels, increased DNA repair, increased tolerance to DNA damage due to CDDP, or a combination of these factors.[2–6)] Attempts have been made to overcome CDDP resistance, both *in vitro* and *in vivo*, using novel platinum compounds and various agents that affect these resistance mechanisms.[7, 8)]

Intra-hepatic arterial administration of antitumor drugs using Lipiodol, an oily lymphographic agent, as a carrier, has been widely used to treat hepatocellular carcinoma.[9)] Styrene maleic acid neocarzinostatin (SMANCS) and various water-soluble drugs have been administered in this manner.[10–13)] CDDP suspended in Lipiodol (CDDP/Lipiodol) has been reported to be extremely effective against primary hepatocellular carcinoma.[14, 15)] However, CDDP-resistant tumors with no sensitivity to CDDP/Lipiodol are occasionally encountered. Also, because CDDP has a low affinity for Lipiodol, it is poorly retained within the suspension with Lipiodol upon administration. The antitumor drug SM-11355 is a lipophilic platinum complex that has been developed as a suitable agent for therapy using Lipiodol due to its lipophilicity.[16)] SM-11355 has a high affinity for Lipiodol, and SM-11355 suspended in Lipiodol is a stable suspension. Previous studies have demonstrated that SM-11355 is superior to CDDP for co-administration with Lipiodol in comparison with CDDP.[17–21)] The carrier ligands of platinum in SM-11355 differ from those in CDDP. It has been reported that CDDP-resistant cell lines exhibit no cross-resistance with platinum complexes containing different carrier ligands.[22–25)] Thus, SM-11355/Lipiodol can be expected to show sufficient antitumor activity in CDDP-resistant hepatic tumors.

In the present study, we investigated the sensitivity of a novel CDDP-resistant cell line, H4-II-E/CDDP, to various antitumor drugs. We also examined the mechanism of CDDP resistance within this cell line, and the cytotoxic activity of SM-11355/Lipiodol on this cell line.

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MATERIALS AND METHODS

Drugs *Cis*-[((1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′)bis(myristato)]platinum (II) (SM-11355) and 1,2-diaminocyclohexane platinum (II) dichloride (DPC) were prepared by Sumitomo Pharmaceuticals Co. (Osaka). The structures of these compounds are illustrated in Fig. 1. Cisplatin (CDDP) was produced by Sigma Aldrich Japan K. K. (Tokyo). Lipiodol Ultra-Fluid (Lipiodol), Laboratorie Guerbert (Paris, France), was purchased from Mitsui Pharmaceuticals Co., Ltd. (Tokyo). Carboplatin was purchased from Bristol-Myers Squibb K. K. (Tokyo), doxorubicin, mitomycin C and 5-fluorouracil from Kyowa Hakko Co. (Tokyo), etoposide from Nihon Kayaku Co. (Tokyo), methotrexate from Wyeth Lederly Japan Co. (Tokyo), and vincristine from Eli Lilly Japan Co. (Hyogo).

Preparation of drug suspensions SM-11355 suspended in Lipiodol was prepared by adding Lipiodol to SM-11355 and shaking. CDDP suspended in Lipiodol was prepared by adding Lipiodol to CDDP powder and mixing in a mortar.

Cell culture Rat hepatoma H4-II-E cells were kindly provided by Sumitomo Pharmaceuticals Co. H4-II-E cells were maintained in minimum essential medium containing 10% fetal bovine serum (FBS) at 37°C in humidified 5% CO_2 air. CDDP-resistant H4-II-E/CDDP cells were established by stepwise exposure of H4-II-E cells to increasing concentrations of CDDP over a period of 12 months; the cells were initially treated with 0.3–0.5 μg/ml of CDDP for 3 months, and the CDDP concentration was increased to a final value of 1.0 μg/ml.

Population-doubling time determinations Cells (1×10^5) were seeded into 25-cm^2 tissue culture flasks; cells in duplicate flasks were then detached by trypsinization and counted at 24-h intervals for up to 120 h.

Cytotoxicity assays of drugs in solution and in Lipiodol Cytotoxic activities of drug solutions were assessed using WST-1 assays.[26] H4-II-E and H4-II-E/CDDP cells were seeded into separate 96-well plates at 5×10^2 cells/0.1 ml/well and incubated overnight. Serial drug dilutions were then added to sets of five wells. Drug exposure was maintained for 7 days. To study the effects of 2-h exposure to a given concentration of drug, the drug was removed from the cells after 2 h, and the cells were then incubated for a further 7 days in 0.2 ml of drug-free growth medium. To determine drug cytotoxicity, 20 μl of a solution containing 2 m*M* WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfate) and 0.2 m*M* 1-methoxy PMS (1-methoxy-5-methylphenazinium methosulfate) was added to each well followed by a 24-h incubation period. The absorbance of each well was measured at 450 nm with a reference wavelength at 650 nm using an Immuno Reader NJ-2001 (Inter Med Japan, Tokyo). IC_{50} was defined as the concentration of drug at which a 50% reduction in the cell survival curve of a given cell line was observed.

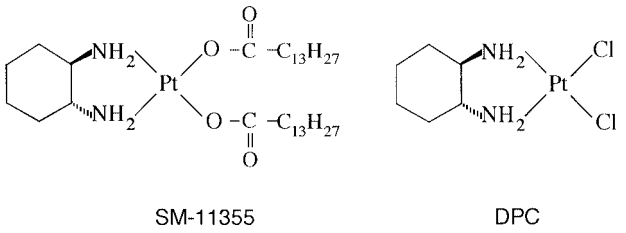

Fig. 1. Chemical structures of SM-11355 and DPC.

Cytotoxicities of drugs suspended in Lipiodol were also assessed using WST-1 assays. H4-II-E and H4-II-E/CDDP cells were seeded into 6-well plates at 5×10^3 cells/2 ml/well and incubated overnight. Falcon cell culture inserts (Becton Dickinson and Co., Franklin Lakes, NJ), equipped with 0.4 μm pore membranes on the bottom, were used to test drugs suspended in Lipiodol. Two milliliters of the specified drug suspended in Lipiodol was added to the Falcon cell culture inserts within the wells. Following a 7-day incubation period, trypsinized cells from each well were collected and seeded into 96-well plates. Then, 20 μl of a solution containing 2 m*M* WST-1 and 0.2 m*M* 1-methoxy PMS was added to each well, followed by an additional 24-h incubation period. The absorbance of each well was measured at 450 nm with a reference wavelength of 650 nm using an Immuno Reader NJ-2001 (Inter Med Japan). IC_{50} was defined as the drug concentration at which a 50% reduction in the cell survival curve of a given cell line was observed.

Glutathione (GSH) measurement To measure GSH content, cells were suspended in 5% *meta*-phosphoric acid at a concentration of 5×10^6 cells/ml. This suspension was sonicated and centrifuged at 3000 rpm for 10 min. The GSH content of the supernatant was determined with a GSH assay kit (Bioxytech GSH-400, Oxis International, Inc., Portland, OR). Cellular GSH content was expressed in nmol GSH/10^6 cells.

Intracellular platinum accumulation and DNA adduct formation within cells exposed to drug solutions DPC and CDDP were dissolved in dimethylformamide or saline and diluted with culture medium to a concentration of 10 μg/ml. Cells were seeded into 6-well plates at 8×10^5 cells/2 ml/well and incubated overnight. Then, 2 ml of the drug solution was added to specified wells. After a 3-day exposure, the cells were collected and washed three times with cold phosphate-buffered saline (PBS(−)). The resulting cell pellets were frozen until required for platinum measurement.

Intracellular platinum accumulation and DNA adduct formation within cells exposed to drugs suspended in Lipiodol Cells were seeded into 6-well plates at 8×10^5

cells/2 ml/well and incubated overnight. Then, 2 ml of the drug suspended in Lipiodol was added to Falcon cell culture inserts within designated wells. Cells were incubated with either 50 μg/ml of SM-11355/Lipiodol or 80 μg/ml of CDDP/Lipiodol. At the indicated time points, the cells were collected and washed three times with cold PBS(−). The resulting cell pellets were frozen until required for platinum measurement.

Degree of DNA repair and DNA damage tolerance Cells (4×10^7) were seeded into 75-cm^2 tissue culture flasks and incubated overnight. Then, 60 μg/ml of either DPC or CDDP was added. After a 2-h exposure, cells were either harvested immediately or incubated in fresh medium for 8 h. The cells were collected and washed three times with cold PBS(−). The resulting cell pellets were frozen until required for platinum measurement.

Determination of platinum uptake in cells Following drug incubation, the cell pellets were lysed using digestion buffer (10 m*M* Tris-HCl, 10 m*M* EDTA, 0.15 *M* NaCl, and 0.5% sodium dodecyl sulfate (SDS)) in the presence of 0.1 mg/ml proteinase K for 1 h at 55°C, then incubated overnight at 37°C. The amount of platinum in each sample was determined by flameless atomic absorption spectrometry (FAAS).

Determination of platinum-DNA adduct formation Following drug incubation, high-molecular-weight DNA was isolated from the cell pellets by a standard procedure as follows. The pellets were lysed at 55°C for 1 h and at 37°C overnight with a digestion buffer in the presence of 0.1 mg/ml proteinase K. DNA was extracted with equal volumes of Tris-EDTA (TE)-saturated phenol and 25:24:1 TE-saturated phenol:chloroform:isoamyl alcohol, and then precipitated with isopropanol. The resultant pellet was dissolved in TE buffer at 37°C overnight. The sample was then treated with 2 μl of 10 mg/ml RNase A at 37°C for 1 h. DNA was again extracted with an equal volume of 25:24:1 TE-saturated phenol:chloroform:isoamyl alcohol and precipitated with isopropanol. The pellet was washed with cold 80% ethanol, and the DNA was dissolved in 2 *N* HCl. The DNA content was assessed spectrophotometrically at 260 nm and the amount of platinum within DNA was determined by FAAS.

Platinum content determination The platinum content was analyzed quantitatively at 265.9 nm by FAAS (Z-9000, Hitachi, Tokyo). The limit of quantification was 5 ng/ml and the sample volume was 0.01 ml. Argon gas was used to purge the furnace.

Table I. Resistance Profile of H4-II-E/CDDP

Drug	H4-II-E IC_{50} (μg/ml)	H4-II-E/CDDP IC_{50} (μg/ml)	Resistance factor[a)]
CDDP	0.17±0.01	1.5±0.15[b)]	8.8
CBDCA	1.4±0.08	4.8±0.91[b)]	3.4
DPC	0.081±0.020	0.16±0.10	2.0
5-FU	0.43±0.13	0.15±0.08[b)]	0.4
MTX	0.006±0.001	0.014±0.012	2.3
MMC	0.0052±0.0006	0.0069±0.0028	1.3
DOX	0.35±0.03	0.37±0.05	1.1
VP-16	1.3±0.10	1.4±0.15	1.1
VCR	0.26±0.01	0.27±0.02	1.0

Cells were seeded and incubated overnight. Then, drug solutions were added to the cell suspensions. Following incubation for 7 days, cytotoxicities were estimated using WST-1 assay. Data represent the mean±SD of three or four individual experiments.
a) Resistance factor = IC_{50} (H4-II-E/CDDP)/IC_{50} (H4-II-E).
b) Significantly different from the corresponding IC_{50} value of H4-II-E ($P<0.05$, Student's *t* test).

RESULTS

Resistance to CDDP The cytotoxic effects of CDDP to H4-II-E and the derived cell line, H4-II-E/CDDP, as determined by 7-day exposure WST-1 assays, are shown in Table I. H4-II-E/CDDP was 8.8-fold more resistant to CDDP after a 7-day exposure. The level of resistance observed for H4-II-E/CDDP remained stable in the absence of further maintenance doses of CDDP for at least 3 months. The morphology of CDDP-resistant H4-II-E/CDDP was nearly identical to that of the parental cell line, and no differences were observed in their population doubling times (15.0 h for H4-II-E and 14.5 h for H4-II-E/CDDP).

Cross-resistance to other antitumor drugs Table I shows the sensitivity profiles of H4-II-E and H4-II-E/CDDP to carboplatin (CBDCA), DPC, 5-fluorouracil (5-FU), methotrexate (MTX), mitomycin C (MMC), doxorubicin (DOX), etoposide (VP-16) and vincristine (VCR) using 7-day exposure WST-1 assays. H4-II-E/CDDP was 3.4-fold more resistant to CBDCA, while there were no differences in H4-II-E or H4-II-E/CDDP sensitivities to DPC, which has a different carrier ligand than CDDP and CBDCA. No cross-resistance was observed to any non-platinum agent. Collateral sensitivity was observed to 5-FU in H4-II-E/CDDP.

Intracellular GSH levels To assess the possible role of increased intracellular detoxification of platinum in H4-II-E/CDDP, intracellular GSH levels were measured. Total GSH levels were increased 2.9-fold in H4-II-E/CDDP compared to H4-II-E (4.7±0.23 vs. 1.6±0.23 nmol/10^6 cells, respectively; values are means±SD of 3 measurements). This increase was statistically significant ($P<0.01$).

Intracellular platinum accumulation and platinum-DNA adduct formation The levels of intracellular platinum accumulation and of platinum-DNA adducts in H4-II-E and H4-II-E/CDDP following 3-day exposure to CDDP, are shown in Table II. Intracellular platinum levels were

Table II. Intracellular Platinum Accumulation and Platinum-DNA Adduct Formation Following Treatment with CDDP and DPC

Drug	Cell line	Intracellular Pt (ng/10^6 cells)	Pt-DNA adducts (pg/μg DNA)
CDDP	H4-II-E	64.8±37.6	57.5±28.5
	H4-II-E/CDDP	10.4±3.1	9.6±6.6
DPC	H4-II-E	8.2±8.3	15.5±9.3
	H4-II-E/CDDP	9.0±3.1	26.2±32.2

Cells were seeded and incubated overnight. Then, drug solutions were added to the cell suspensions. Following incubation for 3 days, the cells were collected. Data represent the mean±SD of four individual experiments.

6.2-fold lower in H4-II-E/CDDP compared to the parental line. Also, contents of platinum-DNA adducts were 6.0-fold lower in H4-II-E/CDDP. Similar results were obtained after a 2-h exposure to CDDP, platinum levels being reduced in H4-II-E/CDDP (Table III). In contrast, after a 3-day exposure to DPC (Table II), there were no significant differences in either intracellular platinum level or platinum-DNA adduct level between H4-II-E and H4-II-E/CDDP. However, after a 2-h exposure to DPC, platinum levels were lower in H4-II-E/CDDP compared to the parental cell line.

DNA repair and DNA damage tolerance The extent of removal of platinum-DNA adducts was determined by incubating cells for 2 h with either CDDP or DPC, followed by an 8-h incubation in drug-free medium to allow for repair (Table III). The percentages of platinum removed from DNA after CDDP exposure were 45% and 14% for H4-II-E and H4-II-E/CDDP, respectively. Thus, the efficiency of repair of DNA damage caused by CDDP was not elevated in the CDDP-resistant line. Total platinum-bound DNA levels following DPC exposure were not reduced in either cell line. To measure DNA damage tolerance, AUCs (areas under the adduct versus time curves) were calculated to estimate the degree of exposure to cytotoxic DNA lesions. The AUC for lesions produced by CDDP in H4-II-E was 5.6-fold greater than that for H4-II-E/CDDP. At the IC_{50} drug concentration, the AUC provides an indication of DNA damage tolerance. This value represents the level of exposure to DNA lesions that will kill 50% of exposed cells. The results indicate that there were no differences in tolerance to DNA damage induced by CDDP or DPC between H4-II-E/CDDP and the parental line.

Cytotoxic activity of SM-11355/Lipiodol The effects of CDDP/Lipiodol on inhibiting cell growth in H4-II-E and H4-II-E/CDDP are shown in Fig. 2. The IC_{50} values were 0.57 and 9.6 μg/ml, respectively. Thus, there was a significant difference in the sensitivities of the two cell lines. In contrast, there was no difference in their sensitivities to SM-11355/Lipiodol (IC_{50}; 3.1 and 4.6 μg/ml for H4-II-E

Table III. DNA Repair and DNA Damage Tolerance Following Treatment with CDDP or DPC

	CDDP		DPC	
	H4-II-E	H4-II-E/CDDP	H4-II-E	H4-II-E/CDDP
IC_{50} (μg/ml)[a]	0.41	2.2	0.27	0.19
Intracellular Pt (ng/10^6 cells)				
at 2 h[b]	24.3	3.0	13.0	6.8
at 10 h[c]	11.8	3.4	7.9	4.3
DNA-Pt adducts (pg/μg DNA)				
at 2 h[b]	98.9	15.1	25.5	13.1
at 10 h[c]	54.7	13.1	33.1	15.9
AUC of DNA-Pt adducts[d] (pg×h/μg DNA)	713	128	260	129
DNA damage tolerance[e] (pg×h/μg DNA)	4.9	4.6	1.1	0.41

Cells were seeded and incubated overnight. Then, drug solutions were added to the cell suspensions. Drug exposure lasted for 2 h. Data represent the mean of two or three individual experiments.
a) IC_{50}s were determined 7 days after 2-h drug exposure.
b) The cells were collected immediately after 2-h exposure.
c) After 2-h exposure, the cells were incubated in fresh medium for 8 h and then collected.
d) AUCs were determined by the trapezoidal rule from the beginning of 2-h drug exposure to 8 h post-exposure.
e) DNA damage tolerance was defined as the AUC of the DNA-Pt adduct curve, extrapolated to the drug's IC_{50} obtained using 2-h drug exposure.

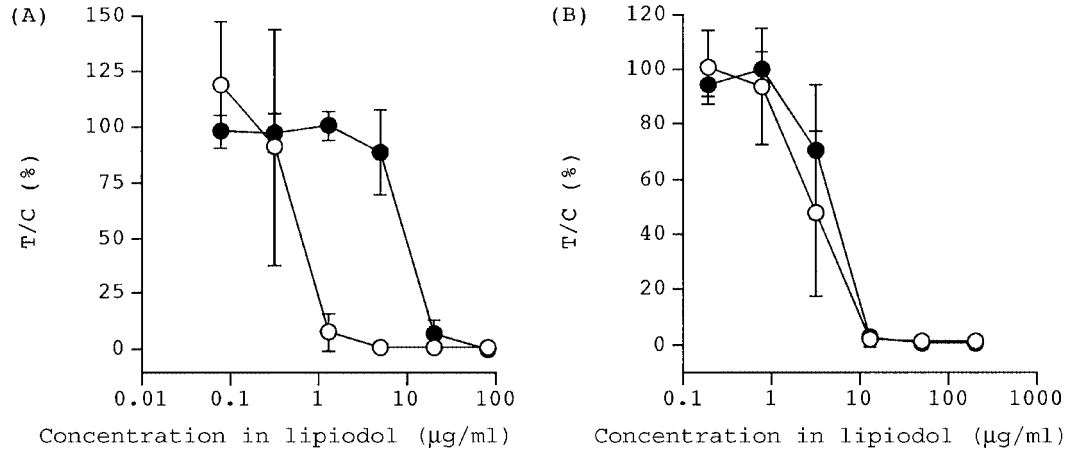

Fig. 2. Cytotoxic activities of CDDP/LPD (A) and SM-11355/LPD (B) against H4-II-E (○) and H4-II-E/CDDP (●). Cytotoxicity was expressed as T/C (%) relative to absorbance of the Lipiodol-treated control group after 7 days of drug exposure. Data represent the mean±SD of three or four experiments.

Table IV. Intracellular Platinum Accumulation and Platinum-DNA Adduct Formation Following Treatment with CDDP/LPD and SM-11355/LPD

Drug	Cell	Intracellular Pt (ng/10^6 cells)	Pt-DNA adducts (pg/μg DNA)
CDDP/LPD	H4-II-E	37.9±30.2	118.6±81.9
	H4-II-E/CDDP	8.1±3.2	7.0±1.6
SM-11355/LPD	H4-II-E	86.1±39.1	24.5±18.0
	H4-II-E/CDDP	43.8±11.2	14.6±3.7

Cells were seeded and incubated overnight. Then, CDDP/Lipiodol (80 μg/ml) and SM-11355/LPD (50 μg/ml) were added to Falcon cell culture inserts. At the indicated time points, the cells were collected. Data represent the mean±SD of three individual experiments.

and H4-II-E/CDDP, respectively) (Fig. 2). Intracellular platinum accumulation and the formation of platinum-DNA adducts in H4-II-E and H4-II-E/CDDP after exposure to CDDP/Lipiodol and SM-11355/Lipiodol are shown in Table IV. After a 3-day exposure to CDDP/Lipiodol, intracellular platinum levels were 4.7-fold lower in H4-II-E/CDDP compared to the parental line, and those of platinum-DNA adducts were 17-fold lower in H4-II-E/CDDP. In contrast, after a 7-day exposure to SM-11355/Lipiodol, there was no significant difference in either intracellular platinum (P=0.15) or platinum-DNA adduct levels (P=0.40) between H4-II-E and H4-II-E/CDDP.

DISCUSSION

Previous studies suggested that the lipophilic platinum complex, SM-11355, has an antitumor effect when suspended in Lipiodol. Furthermore, SM-11355 is better suited to Lipiodol formulation than CDDP. SM-11355/Lipiodol shows sustained release, while CDDP/Lipiodol shows extremely rapid release.[20, 21] In a rat hepatic tumor model, SM-11355/Lipiodol showed a more selective antitumor effect and less hepatic toxicity than CDDP/Lipiodol.[18] The selective antitumor effect of SM-11355/Lipiodol may be attributable to the sustained release of its active compound. SM-11355/Lipiodol releases DPC as its active platinum compound(s) that binds to nuclear DNA and mediates SM-11355/Lipiodol cytotoxicity.[21] The purpose of the present study was to clarify the mechanism of SM-11355/Lipiodol cytotoxicity and to evaluate the efficiency of SM-11355/Lipiodol in inhibiting the growth of CDDP-resistant tumors, using the rat hepatoma cell line H4-II-E and its CDDP-resistant subline H4-II-E/CDDP.

H4-II-E/CDDP with acquired resistance to CDDP was established by continuous exposure of H4-II-E to CDDP over a period of 12 months. Compared with the parental cell line, this cell line exhibited an 8.8-fold increase in resistance to CDDP and was not cross-resistant to DPC. Other studies have also found that other CDDP-resistant lines are not cross-resistant to DPC. Recent investigations

alluded to a multifocal basis for CDDP resistance, involving one or more of the following properties: reduced intracellular accumulation (reduced uptake and/or increased efflux) of platinum, increased cytosolic detoxification of platinum, increased DNA repair and/or an increased tolerance to DNA damage caused by platinum.[2–6] Intracellular GSH levels were increased approximately 3-fold in H4-II-E/CDDP compared to H4-II-E. Although GSH inactivates DPC as well as CDDP, H4-II-E/CDDP shows no cross-resistance to DPC. Therefore, increased GSH levels cannot be responsible for the resistance to CDDP in H4-II-E/CDDP. No cross-resistance was observed to non-platinum antitumor agents (5-FU, MTX, MMC, DOX, VP-16 and VCR) in H4-II-E/CDDP, which suggests that H4-II-E/CDDP is not multidrug-resistant (MDR). After a 2-h or 3-day exposure to CDDP, intracellular platinum accumulation and levels of platinum bound to DNA were significantly lower in H4-II-E/CDDP compared to H4-II-E. As H4-II-E/CDDP does not show multidrug resistance, the existence of P-glycoprotein and/or multidrug resistance associated protein (MRP)[1, 27, 28] cannot play a role in the CDDP resistance of H4-II-E/CDDP. Thus, reduced intracellular platinum levels in H4-II-E/CDDP may be associated with a reduction in influx, not an increase of efflux. In H4-II-E/CDDP, intracellular accumulation of DPC was reduced compared to the parental cell line after a 2-h exposure, but not after a 3-day exposure. There was no difference in sensitivity to DPC between H4-II-E and H4-II-E/CDDP. Although the mechanism responsible for the difference in intracellular accumulation after 2-h exposure to DPC is not clear, there may be differences in the time course of drug influx of DPC between H4-II-E and H4-II-E/CDDP. After 2-h exposure to CDDP and DPC, no difference was observed in the alterations of platinum-DNA adduct levels between the paired cell lines. This suggests that DNA repair is not the mechanism by which H4-II-E/CDDP acquires resistance. It was recently reported that increased DNA damage tolerance is associated with a mismatch repair deficiency.[5, 6, 29, 30] To measure DNA damage tolerance, the AUCs of the changes in platinum-DNA adduct levels were calculated. This was done by extrapolating the curve to the drug's IC_{50}, which was calculated following 2-h drug exposure. At the IC_{50} drug concentration, the AUC provides a level of exposure to DNA lesions that will kill 50% of exposed cells. The results indicate that H4-II-E/CDDP can tolerate the same level of exposure to DNA lesions, generated by CDDP, as the parental line. This suggests that the dominant factor governing CDDP resistance in H4-II-E/CDDP may be reduced uptake of CDDP into cells, resulting in a reduction of platinum-DNA adduct formation.

There was no difference between H4-II-E and H4-II-E/CDDP in their sensitivities to SM-11355/Lipiodol. However, H4-II-E/CDDP was resistant to CDDP/Lipiodol, as well as CDDP. Levels of intracellular platinum accumulation and platinum-DNA adduct formation within H4-II-E/CDDP after SM-11355/Lipiodol exposure did not differ from those of H4-II-E. However, these levels were significantly reduced in H4-II-E/CDDP compared to H4-II-E following CDDP/Lipiodol exposure. Therefore, SM-11355/Lipiodol showed equivalent antitumor effects in H4-II-E/CDDP and H4-II-E at the same IC_{50} level. Since these results were consistent with those observed after exposure to DPC, the effects of SM-11355/Lipiodol on CDDP-resistant H4-II-E/CDDP may be attributable to DPC released from SM-11355/Lipiodol. This observation is consistent with previous reports involving the rat hepatoma cell line AH-109A.[20–22] SM-11355/Lipiodol required a higher level of intracellular platinum accumulation to acquire the same level of platinum-DNA adducts achieved by CDDP and DPC. This result is also consistent with a previous report involving AH-109A. Although the reason for this has not been identified, it may be due to a preferential uptake of platinum compounds with weaker activity released from SM-11355/Lipiodol. Alternatively, SM-11355/Lipiodol may directly influence cell membrane function.

In summary, a newly established H4-II-E/CDDP cell line was approximately 10-fold more resistant to CDDP, but showed no cross-resistance to DPC. The major mechanism of CDDP resistance in H4-II-E/CDDP is reduced intracellular platinum accumulation that may be due to the inactivation, or reduced function, of the active CDDP transport mechanism. There was no difference in the levels of intracellular platinum accumulation or platinum-DNA adduct formation after exposure to SM-11355/Lipiodol between H4-II-E/CDDP and the parental cell line. These results suggest that the activity of SM-11355/Lipiodol is not affected by the acquisition of CDDP resistance in H4-II-E. Thus, SM-11355/Lipiodol can be expected to exert antitumor activity against CDDP-resistant tumors in the clinical setting. However, the efficiency of SM-11355/Lipiodol in inhibiting a variety of CDDP-resistant cell lines needs to be examined, due to the multifocal nature of CDDP resistance.

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