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Antitumor Effects of a Novel Sulfonamide, E7010, on Human Ovarian Cancer Cell Lines *in Vitro* and *in Vivo*

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Summary. **We evaluated the antitumor effectiveness of a novel sulfonamide, N-[2-[(4-hydroxyphenyl) amino]-3-pyridinyl]-4-methoxybenzenesulfonamide (E7010), against 10 human ovarian cancer cell lines *in vitro*. The 50% inhibitory concentrations of E7010 for these cell lines ranged from 0.032 μg/ml to 0.48 μg/ml. Those of cisplatin (CDDP) ranged from 0.021 μg/ml to 1.0 μg/ml. No CDDP-resistant cell lines used in this study showed cross-resistance to E7010.**

In mice inoculated with Nakajima and KF cells, administration of E7010 in doses of 200-450 mg/kg every 5 days inhibited tumor growth by 11-79%. Moreover, the E7010-treated mice showed less body weight loss than the CDDP-treated mice at doses exerting equal antitumor effects.

Key words—sulfonamide, E7010, ovarian cancer.

INTRODUCTION

Cisplatin (CDDP)-based chemotherapy has shown excellent effects in patients with ovarian cancer. However, long-term survival rates have hardly improved, because of acquired CDDP resistance in tumors.[1,2] Several CDDP analogues have been developed and shown fewer side effects than CDDP.[3] However, since tumor cells resistant to CDDP show cross-resistance to CDDP analogues,[4] CDDP analogues can not improve the prognosis of ovarian cancer. Therefore, it is necessary to discover new antitumor agents which differ in their cytotoxic mechanisms from CDDP and its analogues.

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Taxol, an antimitotic agent that enhances tubulin polymerization and stabilizes microtubules, has been shown to induce tumor response in patients with platinum-resistant ovarian cancer.[5,6]

The novel sulfonamide N-[2-[(4-hydroxyphenyl) amino]-3-pyridinyl]-4-methoxybenzenesulfonamide (E7010) has good antitumor effects against a variety of tumors, including drug-resistant tumors.[7,8] E7010 is also an antimitotic agent, but has a different cytotoxic mechanism from Taxol. E7010 acts as an inhibitor of tubulin polymerization.[7,8]

In this study, we have estimated the antitumor effect of E7010 against ovarian cancer cell lines *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials

E7010 (for chemical structure, see Fig. 1) was provided by Eisai Co., Ltd. (Tsukuba Research Laboratories, Ibaraki, Japan). CDDP was purchased from Bristol-Myers Squibb Co., Ltd., Tokyo, Japan; doxorubicin (Adriamycin: ADM) and 5-fluorouracil (5-FU) from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan.

Cell lines

Ten human ovarian cancer cell lines were used in this study. Nakajima cells were derived from an endometrioid adenocarcinoma.[9] Nakajima S1 and S2 are CDDP-resistant sublines, established in our laboratory, of Nakajima cells (our unpublished data). TYK was derived from an undifferentiated carcinoma.[10] TYK-R and TYK-R′ are CDDP-resistant sublines of

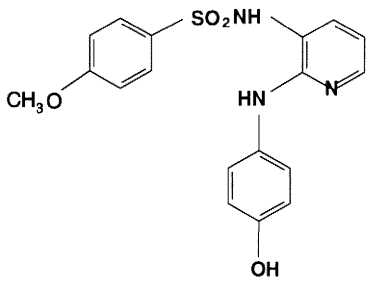

Fig. 1. Structure of E7010, (N-[2-[(4-hydroxyphenyl) amino]-3-pyridinyl]-4-methoxybenzenesulfonamide).

TYK.[11] KK was derived from a clear cell carcinoma.[12] These cell lines were maintained in DMEM supplemented with 10% heat-inactivated fetal calf serum (Bioserum, Victoria, Australia), 100 μg/ml streptomycin and 100 units/ml penicillin. KF was derived from a serous cystadenocarcinoma.[13] KFra and KFrb are CDDP-resistant sublines of KF.[14] KF, KFra and KFrb were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 100 μg/ml streptomycin and 100 units/ml penicillin.

Animals

Six-week-old female BALB/c *nu/nu* athymic nude mice were purchased from Charles River Japan, Inc. (Yokohama, Japan) and maintained under specific-pathogen-free conditions.

Cytotoxicity assay *in vitro*

The cytotoxicities of E7010, CDDP, ADM and 5-FU were determined by the trypan blue dye exclusion test. Cells were plated on 35 mm dishes (Greiner Japan Labortechnik, Tokyo, Japan), 5×10^4 cells per dish. After a 24-hour incubation, various concentrations of the drugs were added. The medium containing the drug was replaced every other day. After 4 days of culture with drugs, the cells were detached by trypsinization and counted with a Coulter counter. Each experiment was performed in duplicate. The survival rate was determined by the following formula:

Survival rate (%)=(No. of treated cells/No. of untreated cells)$\times$100

The 50% inhibitory concentration (IC_{50}) value for each drug was determined from the dose response curve.

Tumor growth assay *in vivo*

Nakajima and KF cells (1×10^7) were inoculated s. c. into the right flank of nude mice. Administration of E7010 or CDDP was started when the tumor volume reached about 100 mm^3. Three different doses of E7010 (450, 300 and 200 mg/kg/day) suspended in 0.5% methylcellulose were administered p. o. every 5 days for 21 days. In the control group, 0.5% methylcellulose alone was administered in the same manner. Oral administrations were accomplished using a stainless steel gavage tube. CDDP (5 mg/kg) was administered i. p. once a week for 4 weeks. Tumor volumes and body weights were measured just before the administration of each drug and 3 or 4 days and 7 days after the last drug administration. Tumor volume was calculated by using the following standard formula:

Tumor volume (mm^3)=length$\times$(width)^2/2

The animals were sacrificed and tumor weights were measured 28 days after the start of treatment. Antitumor activity was determined by dividing the mean tumor weight or the test groups (T) by that of the control group (C), and was expressed as a percentage (T/C$\times$100).

Statistical analysis

The unpaired *t*-test was used in all statistical analyses.

RESULTS

Cytotoxicity assay *in vitro*

The IC_{50} values of E7010, CDDP, ADM and 5-FU for ovarian cancer cell lines are listed in Table 1.

One report stated that after the administration of E7010 to tumor-bearing mice, the Cmax of E7010 in tumor tissue was 14.4 μg/ml, and the concentrations were maintained above 0.4 μg/ml for 24 h.[15] In our results, the IC_{50} values of E7010 for ovarian cancer cell lines ranged from 0.032 μg/ml to 0.29 μg/ml, levels which were below the concentrations in tumor tissue.

As shown in Table 2, the range of relative resistance (IC_{50} values for resistant sublines/IC_{50} values for parental cell lines) to CDDP in CDDP-resistant cell lines was 2.5-47.6, but the range of relative resistance to E7010 was only 0.4-2.9. For example, TYK-R' was about 50 times more resistant than the parent TYK to CDDP, but only 3 times more resistant to E7010. Nakajima S1 and S2 cells expressed

Table 1. IC_{50} values for drugs in human ovarian cancer cell lines

Cell line	IC_{50} (μg/ml)			
	E7010	CDDP	ADM	5-FU
Nakajima	0.06	0.15	0.004	0.07
Nakajima S1	0.04	0.55	0.001	0.20
Nakajima S2	0.08	0.47	0.001	0.21
TYK	0.08	0.02	0.010	0.50
TYK-R	0.03	0.28	0.018	0.34
TYK-R'	0.24	1.00	0.016	0.48
KF	0.18	0.10	0.003	0.02
KF-ra	0.29	0.35	0.005	0.03
KF-rb	0.28	0.29	0.003	0.04
KK	0.17	0.04	0.003	0.58

cross-resistance to 5-FU, but not to E7010. Thus, E7010 had almost the same activities against the platinum-resistant sublines as those against their parental cell lines *in vitro*.

Tumor growth assay *in vivo*

To determine the *in vivo* antitumor activity of E7010, Nakajima and KF cells, which are able to produce transplant tumors in nude mice, were chosen for this experiment (Fig. 2 and Table 3).

In mice given Nakajima cells, administrations of E7010 every 5 days inhibited the tumor growth in a dose-dependent manner with T/C values of 89.5%, 44.5%, and 21.3% at doses of 200, 300, and 450 mg/kg, respectively. The once-weekly i. p. injection of CDDP inhibited tumor growth with a T/C value of 50.5%.

Table 2. Cross-resistance of CDDP-resistant human ovarian cancer cell lines

Drugs	Relative resistance[a)]					
	Nakajima S1	Nakajima S2	TYK-R	TYK-R'	KF-ra	KFrb
E7010	0.7	1.3	0.4	2.9	1.6	1.6
CDDP	3.7	3.1	13.3	47.6	3.5	2.9
ADM	0.3	0.8	1.8	1.6	1.6	0.9
5-FU	3.0	3.2	0.6	1.0	1.7	2.8

[a)] IC_{50} values for resistant sublines/IC_{50} values for parental cell lines.

Table 3. Antiumor activity against Nakajima and KF cells *in vivo*

Cell lines	Dose (mg/kg/day)	Dead/ Treated	Tumor weight (mg)	T/C (%)	Body weight change (g)
Nakajima					
	Control	0/6	2134±333[a)]	100%	−2.3
E7010 (p.o.)	200	0/6	1909±637	89.5%	−2.0
	300	0/6	949±147[b)]	44.5%	−1.9
	450	0/6	454±249[b)]	21.3%	−3.6
CDDP (i.p)	5	0/6	1078±548[b)]	50.5%	−3.7
KF					
	Control	0/6	4939±937	100%	1.4
E7010 (p.o.)	200	0/6	4211±768	85.3%	0.9
	300	0/6	2926±193[b)]	59.2%	0.9
	450	0/6	1671±418[b)]	33.8%	0.8
CDDP (i.p)	5	0/6	1823±177[b)]	36.9%	−3.3[b)]

[a)] Mean±SD (n=6), [b)] $p<0.01$.

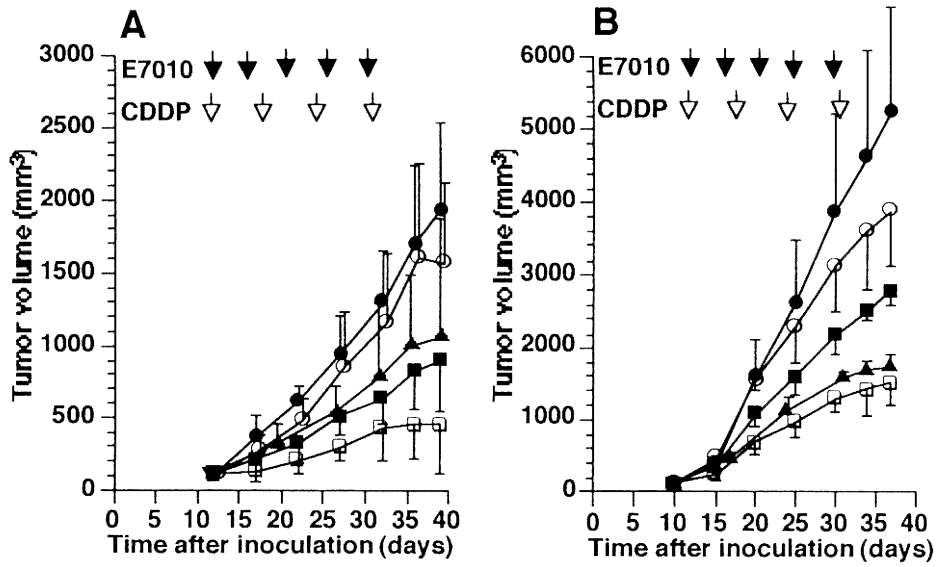

Fig. 2. Growth of Nakajima (**A**) and KF (**B**) ovarian cancer cells treated with E7010 of CDDP. Cells (1×10^7) were inoculated s.c. into the right flank of a nude mouse on day 0. When the tumor volume reached about 100 mm³, the treatment was started. *Arrow*, day of the treatment. *Point*, mean ±SD (n=6); ●, control; ○, E7010 (200 mg/kg); ■, E7010 (300 mg/kg); □, E7010 (450 mg/kg); ▲, CDDP (5 mg/kg).

Since Nakajima cells induced cachexia in the mice, body weight loss was observed even in the control group.

In mice injected with KF cells, treatment with E7010 also inhibited tumor growth in a dose-dependent manner, with T/C values of 85.3%, 59.2%, and 33.8% at doses of 200, 300, and 450 mg/kg, respectively. CDDP treatments inhibited tumor growth as effectively as the p. o. administration of 450 mg/kg of E7010, but the CDDP-treated mice showed a 3.3 g decrease in body weight ($p<0.01$). In contrast, the E7010-treated mice did not show any body weight loss (Table 3).

DISCUSSION

Ovarian cancers are CDDP-sensitive tumors, and chemotherapy including CDDP improves their prognosis. The four-year survival rate of stage III ovarian cancers between 1985 and 1990 was 34.59%, which is significantly higher than that before 1985.[1] The five-year survival rate, however, has not improved,[1] mainly because the effects of the second line chemotherapies on CDDP resistant tumors or recurrent tumors after the first line chemotherapies including CDDP are not satisfactory.[1,2] Taxol is a clinically promising antimicrotubule agent which has been effective against patients with platinum-resistant ovarian cancer.[6] Its antitumor mechanism is the induction of tubulin polymerization.[5] Unlike Taxol, E7010 exerts its cytotoxic action by binding to tubulin, inhibiting tubulin polymerization and arresting cells in the M phase of the cell cycle.[16] Vincristine (VCR) is also known to be an inhibitor of tubulin polymerization. Although E7010 and VCR exert their cell-killing action by binding to tubulin, the binding sites of the two drugs are different. E7010 has been found to show a wider range of activity than VCR.[8,16] Koyanagi et al. reported that E7010 was active against human gastric cancer, colorectal cancer, lung cancer, breast cancer and oral cancer *in vivo*.[7,8] We found that E7010 was also active *in vivo* against two ovarian cancer cell lines, and caused less weight loss in mice than CDDP at doses showing similar T/C values.

In a recent large-scale investigation in Japan, remission induction chemotherapy did not always give rise to an improvement in the long-term prognosis of ovarian cancer. For example, maintenance chemotherapy with orally administered 5-FU or Tegaful was not effective.[2] In our data, E7010 suppressed the growth of CDDP-resistant ovarian cancer cell lines as well as the parent cell line. Moreover,

E7010 has been effective against VCR-, CDDP- and 5-FU-resistant cell lines.[8] Therefore, E7010 may be useful for preventing recurrence after chemotherapies including CDDP for patients with ovarian cancer, instead of 5-FU or other commonly used drugs.

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