# Combination effect of AC-7700, a novel combretastatin A-4 derivative, and cisplatin against murine and human tumors *in vivo*

**Yoshihiro Morinaga,[1] Yasuyo Suga,[1] Sumiko Ehara,[2] Katsuhiro Harada,[2] Yukio Nihei[1] and Manabu Suzuki[1]**

[1]Pharmaceutical Research Laboratories and [2]Central Research Laboratories, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki 210-8681

(Received August 9, 2002/Revised November 5, 2002/Accepted November 21, 2002)

**The *in vivo* combination effect of AC-7700, a novel combretastatin A-4 derivative, and cisplatin (CDDP) was examined. The combination of AC-7700 and CDDP increased antitumor activity against murine colon 26 tumor in mice and cured the mice. This combination effect was found over wide dosage ranges of AC-7700 (20–80 mg/kg) and CDDP (2.5–5 mg/kg). Moreover, this combination augmented antitumor activity against murine S180 and M109 tumors, and human LX-1 and LS180 tumor xenografts in mice. The effect was the strongest when AC-7700 and CDDP were administered simultaneously. To study this combination effect, we measured the concentrations of CDDP in tumors, plasma and kidneys of the mice with colon 26 tumor. In the combination with AC-7700, the concentration of CDDP in the tumors increased from 0.5 to 96 h after administration, but did not change or decrease in plasma or kidneys. Against human LS180 xenografts in mice, the combination similarly increased the concentration of CDDP in the tumors. These results suggest that AC-7700 may specifically augment the accumulation of CDDP in tumors, and thus has the potential to be useful in combination chemotherapy with CDDP. (Cancer Sci 2003; 94: 200–204)**

Combretastatin A-4 (CS A-4) is one of a class of compounds, the combretastatins, isolated by Pettit *et al.*[1] from the African shrub *Combretum caffrum*. CS A-4 is a strong tubulin polymerization inhibitor and has strong cytotoxicity *in vitro*.[2] *In vivo*, CS A-4 has produced hemorrhagic necrosis in experimental tumors.

AC-7700, (*Z*)-*N*-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)-vinyl]phenyl]-L-serinamide hydrochloride, is a novel soluble derivative[3] of CS A-4. AC-7700 showed potent antitumor activities against murine tumor and human tumor xenografts in mouse[4] and rat tumor models.[5] It has been suggested that AC-7700 exerts antitumor activity by decreasing tumor blood flow.[5, 6]

In clinical cancer chemotherapy, anticancer drugs are often used in combination. Discovery of useful combination chemotherapy is expected to increase the response rate and the frequency of long-term survival. In treatment with AC-7700, it will be an important issue to determine which drug is most useful for combination chemotherapy.

Cisplatin (CDDP) is one of the most useful clinical anticancer drugs. Therefore, combination chemotherapy of CDDP with other or new anticancer drugs has often been tried. Combination of CDDP with vindesine (VP),[7] etoposide (EP)[8] and cyclophosphamide+adriamycin (CAP)[9] has been investigated in many tumors. Combination with recently developed drugs (taxol,[10] navelbine,[11] CPT-11[12]) has also been studied.

In the present study, we investigated the *in vivo* combination effect of AC-7700 and CDDP on antitumor activity against murine tumor models. In addition, we examined the mechanism of the combination effect by measuring the concentration of CDDP in the tumors.

## Materials and Methods

**Compounds.** AC-7700 was synthesized as described previously.[3] CDDP was purchased from Nippon Kayaku Co., Ltd. (Tokyo). They were dissolved in and diluted with physiological saline on the day of administration.

**Animals and tumors.** Female CDF1, ICR and ICR-*nu*/*nu* mice were purchased from Charles River Japan, Inc. (Yokohama). They were supplied food (CRF-1, Clea Japan, Tokyo) and water *ad libitum*. ICR-*nu*/*nu* mice were maintained under sterile conditions.

Murine colon carcinoma colon 26 and human lung carcinoma LX-1 were supplied from the Japanese Foundation for Cancer Research (Tokyo). Murine sarcoma S180 and lung carcinoma M109 were supplied from the National Cancer Center Research Institute (Tokyo). Human colon carcinoma LS180 was obtained from American Tissue Culture Collections (ATCC, Rockville, MD). Colon 26 and M109 tumors were maintained in CDF1 mice subcutaneously. S180 tumor was maintained in ICR mice intraperitoneally. LX-1 tumors were maintained in ICR-*nu*/*nu* mice subcutaneously. LS180 tumor cells were cultured with RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Gibco) in an atmosphere of 5% $CO_2$ in a highly humidified incubator at 37°C.

**Evaluation of antitumor activity.** Tumor fragments, approximately 10–20 mg (colon 26, M109 and LX-1), or tumor cell suspensions, approximately $3\times10^6$–$1\times10^7$ cells (S180 and LS180), were inoculated subcutaneously into the back of mice (6-week-old CDF1 mice for colon 26 and M109, 6-week-old ICR mice for S180, and 5-week-old ICR-*nu*/*nu* mice for LX-1 and LS180) (day 0). On the first day of drug administration, the mice were divided into groups ($n$=6–8) on the basis of tumor volume and body weight. All compounds were administered intravenously.

Tumor volume on day 21 was evaluated as antitumor activity.

Tumor volume was calculated according to the following equation.

$$\text{Tumor volume (mm}^3\text{)} = [\text{length (mm)} \times \text{width (mm)}^2]/2$$

T/C was calculated according to the following equation.

$$\text{T/C (\%)} = \{\text{mean tumor volume (mm}^3\text{) of treated mice} / \text{mean tumor volume (mm}^3\text{) of control mice}\} \times 100$$

Body weight change was calculated according to the following equation.

$$\text{Body weight change (g)} = \text{mean body weight on day 21} - \text{mean body weight on the first day of drug administration}$$

Mice without any tumor detectable by palpation were regarded as tumor-free (cured) mice.

**Measurement of CDDP in tumors, plasma and kidneys.** Mice with a

E-mail: yoshihiro_morinaga@ajinomoto.com

tumor (approximately 100–500 $mm^3$) were administered CDDP (5 mg/kg) or CDDP and AC-7700 (20 mg/kg) simultaneously. Following administration as described in "Results," blood, tumors and kidneys were collected from mice under ethylether anesthesia. The plasma and tissues were stored at −80°C.

The concentrations of CDDP in the tissues were measured as the concentrations of platinum (Pt). Pt was measured by graphite furnance atomic absorption spectrometry (GFAAS).[13] The atomic absorption spectrometry system used was a Hitachi Z-9000 spectrometer with a graphite furnace module equipped with a Zeeman effect background corrector and an autosampler. Pyrolytic graphite tubes were employed for all measurements. Operating conditions were as follows: wavelength, 265.9 nm; lamp current, 10 mA; argon gas flow-rate, 200 ml/min (except during atomization when 0); sample injection volume, 20 $\mu$l. The temperature program is shown in Table 1.

Platinum contents were quantitated by running a calibration curve (0, 0.025, 0.05, 0.1, and 0.2 $\mu$g/ml) immediately before the sample analysis. The concentrations of CDDP were calculated from the concentration of Pt according to molecular weight.

**Statistics.** The differences in the tumor volumes of treated mice vs. control mice on day 21 and the concentrations of CDDP in tumors, plasma, and kidneys of AC-7700 and CDDP-treated mice vs. CDDP-treated mice were statistically analyzed by using the Student's *t* test.

## Results

**Antitumor activity of a combination of AC-7700 and CDDP against murine colon 26 tumor in mice.** Antitumor activities of AC-7700, CDDP and a combination of AC-7700 and CDDP against murine colon 26 tumor in mice were studied (Table 2).

AC-7700 at 10, 20, 40 and 80 mg/kg dose-dependently decreased colon 26 tumor volumes on day 21 (T/C=78, 67, 46 and 28%, respectively). In this study, one mouse (1/6) was dead by day 21 at 80 mg/kg of AC-7700, but in other studies, no death occurred at the same dose and all mice died at 160 mg/kg (data not shown). Therefore, the MTD (maximum tolerable dose) of AC-7700 was regarded as near 80 mg/kg.

CDDP at 2.5 and 5 mg/kg dose-dependently decreased the tumor volumes on day 21 (T/C=52% and 26%, respectively).

**Table 1. Temperature program used for the determination of platinum concentration by GFAAS**

| Step | Temperature (°C) | | Time (s) |
|---|---|---|---|
| | Start | End | |
| Dry | 50 | 200 | 30 |
| Ash 1 | 200 | 1000 | 30 |
| Ash 2 | 1500 | 1500 | 30 |
| Atomization | 3000 | 3000 | 10 |
| Clean | 3000 | 3000 | 5 |

In other studies, all mice were dead at 10 mg/kg of CDDP. The MTD of CDDP was regarded as near 5 mg/kg.

Combinations of AC-7700 and CDDP remarkably decreased the tumor volumes on day 21. In the combination with CDDP at 2.5 mg/kg, AC-7700 at 10 mg/kg decreased T/C (3%) and at 20–80 mg/kg the tumors disappeared (T/C=0%). In the combination with CDDP at 5 mg/kg, the tumors disappeared at 10–80 mg/kg of AC-7700. Body weight changes of mice on day 21 in the combination treatment were not severe. On day 78, all mice were tumor-free in the combination treatment of AC-7700 (20–80 mg/kg) and CDDP (2.5–5 mg/kg).

**Antitumor activity of a combination of AC-7700 and CDDP against murine S180 and M109 tumors and human LX-1 and LS180 tumor xenografts in mice.** Antitumor activities of AC-7700, CDDP and a combination of AC-7700 and CDDP against murine S180 and M109 tumors and human LX-1 and LS180 tumor xenografts in mice were studied (Table 3). Against murine S180 and M109 tumors, the combination of AC-7700 (20 and 80 mg/kg) and CDDP (5 mg/kg) remarkably decreased the tumor volumes. One mouse (1/6) was tumor-free on day 119 in the combination of AC-7700 (20 or 80 mg/kg) and CDDP (5 mg/kg) against S180 tumor. Against human LX-1 and LS180 tumor xenografts, the combination of AC-7700 (20 mg/kg) and CDDP (5 mg/kg) decreased the tumor volumes. Body weight changes of mice with these tumors on day 21 in the combination treatment were not severe.

**Effect of administration schedule on antitumor activity of a combination of AC-7700 and CDDP.** AC-7700 (20 mg/kg) and CDDP (5 mg/kg) were administered simultaneously or in the order of CDDP following AC-7700 at a 6 or 24 h interval or AC-7700

**Table 2. Antitumor activity of a combination of AC-7700 and cisplatin against murine colon 26 tumor model**

| Compounds (mg/kg) | Mice (*n*) | Day 21 | | | | Day 78 |
|---|---|---|---|---|---|---|
| | | Toxic death | Tumor volume (mean±SD; $mm^3$) | T/C (%) | Body weight change (mean; g) | Tumor free |
| Control | 6 | 0/6 | 5294±1179 | 100 | 3.7 | 0/6 |
| AC-7700 (10) | 6 | 0/6 | 4149±811 | 78 | 2.5 | 0/6 |
| AC-7700 (20) | 6 | 0/6 | 3574±542** | 67 | 2.6 | 0/6 |
| AC-7700 (40) | 6 | 0/6 | 2440±412** | 46 | 2.3 | 0/6 |
| AC-7700 (80) | 6 | 1/6 | 1491±543** | 28 | 1.1 | 0/6 |
| CDDP (2.5) | 6 | 0/6 | 2761±326** | 52 | 2.9 | 0/6 |
| CDDP (5) | 6 | 0/6 | 1359±325** | 26 | −1.2 | 0/6 |
| AC-7700 (10)+CDDP (2.5) | 6 | 0/6 | 138±165** | 3 | 0.5 | 3/6 |
| AC-7700 (20)+CDDP (2.5) | 6 | 0/6 | 0±0** | 0 | 0.8 | 6/6 |
| AC-7700 (40)+CDDP (2.5) | 6 | 0/6 | 0±0** | 0 | 0.6 | 6/6 |
| AC-7700 (80)+CDDP (2.5) | 6 | 0/6 | 0±0** | 0 | 0 | 6/6 |
| AC-7700 (10)+CDDP (5) | 6 | 0/6 | 0±0** | 0 | −0.9 | 5/6 |
| AC-7700 (20)+CDDP (5) | 6 | 0/6 | 0±0** | 0 | −0.4 | 6/6 |
| AC-7700 (40)+CDDP (5) | 6 | 0/6 | 0±0** | 0 | −0.7 | 6/6 |
| AC-7700 (80)+CDDP (5) | 6 | 0/6 | 0±0** | 0 | −1.3 | 6/6 |

Compounds were administered individually on days 7, 11 and 15, as well as simultaneously in combination.
**$P<0.01$ vs. control.

**Table 3. Antitumor activity of a combination of AC-7700 and cisplatin against murine S180 and M109 tumor models, and human LX-1 and LS180 tumor xenografts**

| Tumors | Compounds (mg/kg) | Mice (*n*) | Day 21 | | | | |
|---|---|---|---|---|---|---|---|
| | | | Toxic death | Tumor volume (mean±SD; $mm^3$) | T/C (%) | Body weight change (mean; g) | Tumor free |
| S180[1)] | Control | 6 | 0/6 | 5832±3318 | 100 | 5.9 | 0/6[4)] |
| | AC-7700 (20) | 6 | 0/6 | 6701±2527 | 115 | 6.3 | 0/6[4)] |
| | AC-7700 (80) | 6 | 0/6 | 3224±819 | 55 | 3.7 | 0/6[4)] |
| | CDDP (5) | 6 | 0/6 | 3517±1707 | 60 | 3.7 | 0/6[4)] |
| | AC-7700 (20)+CDDP (5) | 6 | 0/6 | 492±348** | 8 | 1.5 | 1/6[4)] |
| | AC-7700 (80)+CDDP (5) | 6 | 0/6 | 269±162** | 5 | −0.2 | 1/6[4)] |
| M109[1)] | Control | 6 | 0/6 | 10523±2657 | 100 | 4.7 | 0/6[5)] |
| | AC-7700 (20) | 6 | 0/6 | 5099±959** | 48 | −2.1 | 0/6[5)] |
| | AC-7700 (80) | 6 | 0/6 | 2147±569** | 20 | −1.4 | 0/6[5)] |
| | CDDP (5) | 6 | 0/6 | 6231±829** | 59 | −2.2 | 0/6[5)] |
| | AC-7700 (20)+CDDP (5) | 6 | 0/6 | 222±75** | 2 | −3.5 | 0/6[5)] |
| | AC-7700 (80)+CDDP (5) | 6 | 0/6 | 110±110** | 1 | −2.6 | 0/6[5)] |
| LX-1[2)] | Control | 6 | 0/6 | 1255±477 | 100 | −1.2 | 0/6[6)] |
| | AC-7700 (20) | 6 | 0/6 | 705±195* | 57 | 0 | 0/6[6)] |
| | CDDP (5) | 6 | 0/6 | 652±276* | 51 | −2.9 | 0/6[6)] |
| | AC-7700 (20)+CDDP (5) | 6 | 0/6 | 423±175** | 33 | −1.9 | 0/6[6)] |
| LS180[3)] | Control | 8 | 0/6 | 1929±558 | 100 | 1.3 | 0/6[7)] |
| | AC-7700 (20) | 8 | 0/6 | 1041±505* | 54 | −0.3 | 0/6[7)] |
| | CDDP (5) | 8 | 0/6 | 1482±575 | 77 | −1.2 | 0/6[7)] |
| | AC-7700 (20)+CDDP (5) | 8 | 0/6 | 835±280** | 43 | −1.9 | 0/6[7)] |

*1*) Compounds were administered individually on days 7, 11 and 15, as well as simultaneously in combination.
*2*) Compounds were administered individually on days 11, 15 and 19, as well as simultaneously in combination.
*3*) Compounds were administered individually on days 10, 14 and 18, as well as simultaneously in combination.
$*P<0.05$, $**P<0.01$ vs. control.
Tumor-free mice were evaluated on day *4*) 119, *5*) 55, *6*) 154, or *7*) 38.

**Table 4. Effect of administration schedule on antitumor activity of a combination of AC-7700 and cisplatin against murine colon 26 tumor model**

| Compounds (mg/kg) | Interval (h) | Day 78 |
|---|---|---|
| | | Tumor free |
| AC-7700 (20)+CDDP (5)[1)] | 0 | 5/5 |
| AC-7700 (20)→CDDP (5)[2)] | 6 | 2/5 |
| AC-7700 (20)→CDDP (5)[2)] | 24 | 1/5 |
| CDDP (5)→AC-7700 (20)[2)] | 6 | 1/5 |
| CDDP (5)→AC-7700 (20)[2)] | 24 | 1/5 |

*1*) Compounds were administered simultaneously on days 7, 11 and 15.
*2*) The first compound was administered on days 7, 11 and 15, and the second compound was administered following the first compound after an interval.

following CDDP at a 6 or 24 h interval to mice with colon 26 tumor (Table 4). When AC-7700 and CDDP were administered simultaneously, all of the mice were cured (tumor-free 5/5), but the numbers of tumor-free mice decreased (2/5 and 1/5) when AC-7700 and CDDP were administered separately at intervals.

**Body weight change of normal mice treated with a combination of AC-7700 and CDDP.** Body weight change following administration of AC-7700, CDDP, and a combination of AC-7700 (20 mg/kg) and CDDP (5 mg/kg) was studied in normal mice. AC-7700 did not decrease body weight. CDDP and AC-7700+CDDP tended to decrease body weight slightly, but not significantly, on day 2, and the values recovered by day 4. The combination of AC-7700 and CDDP did not cause severe decrease in body weight in normal mice.

**Effect of the combination of AC-7700 and CDDP on the concentration of CDDP in murine colon 26 tumor in mice.** The concentrations

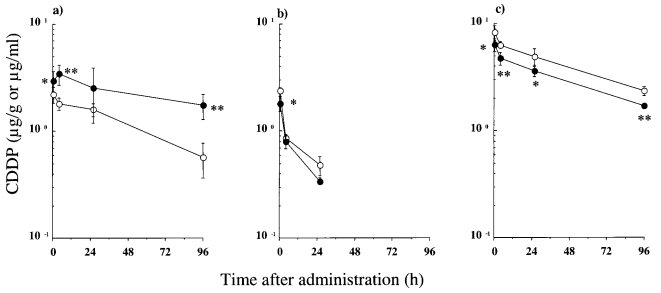


**Fig. 1.** Effect of a combination of AC-7700 and CDDP on accumulation of CDDP in a) tumors, b) plasma, and c) kidneys of mice with colon 26 tumor. AC-7700 (20 mg/kg) and CDDP (5 mg/kg) were administered individually on day 10, as well as simultaneously in combination. Tumors, plasma, and kidneys were collected at the indicated time after administration, and CDDP was measured as described in "Materials and Methods." The values for the concentration of CDDP are given as the mean±SD ($n=5$). * $P<0.05$, ** $P<0.01$. ○ CDDP alone, ● combination (AC-7700+CDDP).

of CDDP in colon 26 tumors, plasma, and kidneys after administration were measured (Fig. 1).

In the tumors (Fig. 1a), CDDP in the combination treatment increased at 0.5 h after administration (CDDP: 2.21±0.36 μg/g vs. AC-7700+CDDP: 2.98±0.64 μg/g; $P<0.05$). This increase was greatest at 4 h (CDDP: 1.82±0.24 μg/g vs. AC-7700+CDDP: 3.44±0.72 μg/g; $P<0.01$) and continued through 96 h (CDDP: 0.57±0.21 μg/g vs. AC-7700+CDDP: 1.75±0.46 μg/g; $P<0.01$) after administration.

In the plasma (Fig. 1b), CDDP in the combination treatment decreased at 0.5 h after administration (CDDP: 2.38±0.14 μg/ml vs. AC-7700+CDDP: 1.82±0.29 μg/ml; $P<0.05$). This de-

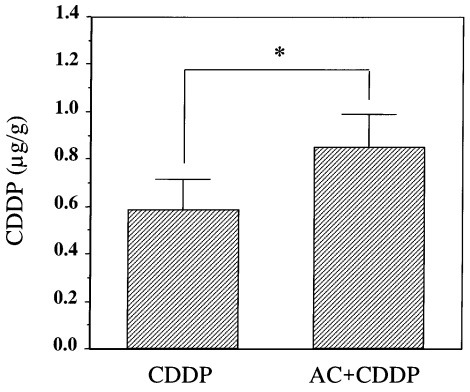


**Fig. 2.** Effect of a combination of AC-7700 and CDDP on accumulation of CDDP in tumors of nude mice with human LS180 tumor. AC-7700 (20 mg/kg) and CDDP (5 mg/kg) were administered individually on day 10, as well as simultaneously in combination. Tumors were collected at 24 h after administration, and CDDP in the tumors was measured as described in "Materials and Methods." The values for the concentration of CDDP are given as the mean±SD ($n$=5). * $P$<0.05.

crease was not evident at 4 and 26 h. CDDP was not detectable at 96 h.

In the kidneys (Fig. 1c), CDDP in the combination treatment decreased at 0.5 h after administration (CDDP: 8.28±1.32 μg/g vs. AC-7700+CDDP: 6.37±0.89 μg/g; $P$<0.05). The decrease continued through 96 h (CDDP: 2.34±0.23 μg/g vs. AC-7700+CDDP: 1.69±0.10 μg/g; $P$<0.01) after administration.

**Effect of the combination of AC-7700 and CDDP on the concentration of CDDP in human LS180 tumor in mice.** The concentration of CDDP in human LS180 tumors at 24 h after administration was measured (Fig. 2). The combination of AC-7700 and CDDP increased CDDP in the tumor (CDDP: 0.59±0.13 μg/g vs. AC-7700+CDDP: 0.85±0.14 μg/g; $P$<0.05).

## Discussion

The combination of AC-7700 and CDDP augmented antitumor activity *in vivo* against murine and human tumors in mice. In particular, the combination cured mice with colon 26 tumor. The MTD of AC-7700 or CDDP in mice with colon 26 tumor was near 80 mg/kg or 5 mg/kg, respectively. Each compound alone at the MTD did not cure the mice. In the combination with CDDP, AC-7700 at 1/8–1 MTD cured the mice. Therefore, this combination effect was considered a strong synergistic effect. Moreover, the combination did not cause toxic death in spite of combining the compounds at their respective MTDs. These results suggest that the combination of AC-7700 and CDDP is a chemotherapy with a high therapeutic index. The combination of AC-7700 and CDDP did not severely decrease body weight in mice with or without tumors. It is possible that this combination augments antitumor activity without augmenting side effects.

Tubulins in tumor cells are regarded as a target of AC-7700 *in vitro*.[3] Vindesine is an antitumor drug with antitubulin activity.[14] We compared AC-7700 with vindesine in combination with CDDP by using A549 human tumor cell and colon 26 cell *in vitro*. The *in vitro* combination effect of AC-7700 and CDDP was an additive and partially antagonistic effect (data not shown), which was the same as that of vindesine or other antitubulin drugs (vincristine, vinblastine) and CDDP.[15] The *in vivo* combination effect of AC-7700 and CDDP may be similar to the *in vitro* combination effect of vindesine and CDDP. However, the combination of vindesine and CDDP did not cure mice with colon 26 tumor *in vivo* (data not shown). These data suggest that AC-7700 has different mechanisms from vindesine in combination with CDDP *in vivo*.

Tubulin-binding reagents have antivascular activity in animal tumors,[16, 17] but they function only at levels close to their MTDs. We examined whether AC-7700 decreased tumor blood flow at effective antitumor dosages by using the Evans blue method on murine tumor models[6] and by using the hydrogen clearance method on rat tumor models.[5] Decrease of tumor blood flow is considered to decrease the quantity of drug that arrives at the tumor and to inhibit the excretion of drugs distributed to the tumor. In this study, we showed that the combination of AC-7700 and CDDP increased the concentration of CDDP and kept it at high level in the tumors and that the simultaneous administration of AC-7700 and CDDP was most effective (the combination effect disappeared if there was a 6 h interval between administration of the two drugs). As the decrease of tumor blood flow by AC-7700 appears at 30 min after administration,[5] it is suggested that AC-7700 may inhibit the excretion of CDDP distributed to the tumor by decreasing tumor blood flow. CDDP is a type I anticancer drug and its cytotoxicity is dependent on the area under the curve (AUC).[18] The AUC of CDDP in colon 26 tumor increased about 2 times in the combination of AC-7700 and CDDP (data not shown). Therefore, we speculate that increased accumulation of CDDP in the tumor was related to the augmentation of *in vivo* antitumor activity against tumors in mice and the cures of mice with the combination of AC-7700 and CDDP.

Recently, a combination treatment of combretastatin A4 phosphate (CA4DP) and CDDP was reported.[19–21] The combination of CA4DP and CDDP enhanced tumor response activities (tumor surviving fraction) and the greatest enhancement was observed when CA4DP was given 15 min–1 h after administration of CDDP. We obtained cures of mice with the combination of AC-7700 and CDDP in the present examinations. Our data indicated a decrease of the combination effect of AC-7700 and CDDP by a 6 h interval between the two treatments, but we consider that our data do not conflict with the data for CA4DP and CDDP. We consider that enhancement might also be observed when AC-7700 is given 15 min–1 h after administration of CDDP based on our mechanism. The combination treatment with AC-7700 and CDDP may have greater potential than that with CA4DP and CDDP with regard to the cure of patients, based on our data.

T/C of CDDP alone at 5 mg/kg against LS180 was 77%. The LS180 tumor is a CDDP-insensitive tumor. The concentration of CDDP in LS180 was much lower than that in colon 26 when CDDP alone was administered. However, with the combination of AC-7700 and CDDP, the concentration of CDDP in the tumor increased and chemotherapy was effective (T/C=43%). Therefore, this combination is suggested to be useful against not only CDDP-sensitive tumors, but also CDDP-insensitive tumors, by enhancing the accumulation of CDDP in tumors.

In combination with AC-7700, other anticancer drugs may also be accumulated in tumors by a decrease of tumor blood flow. Therefore, AC-7700 may be useful in wide-ranging combination chemotherapy.

1. Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garia-Kandall D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A4. *Experientia* 1989; **45**: 205–11.
2. El-Zayar AAE, Degen D, Drabek S, Clark GM, Pettit GR, von Hoff DD. *In vitro* evaluation of the antineoplastic activity of combretastatin A-4, a natural product from *Combretum caffrum* (arid shrub). *Anticancer Drugs* 1993; **4**: 19–25.
3. Ohsumi K, Nakagawa R, Fukuda Y, Hatanaka T, Morinaga Y, Nihei Y, Ohishi K, Suga Y, Akiyama Y, Tsuji T. Novel combretastatin analogues effective against murine solid tumors: design and structure-activity relation-

ships. *J Med Chem* 1998; **41**: 3022–32.
4. Nihei Y, Suga Y, Morinaga Y, Ohishi K, Okano A, Ohsumi K, Hatanaka T, Nakagawa R, Tsuji T, Akiyama Y, Saito S, Hori K, Sato Y, Tsuruo T. A novel conbretastatin A-4 derivative, AC-7700, shows marked antitumor activity against advanced solid tumors and orthotopically transplanted tumors. *Jpn J Cancer Res* 1999; **90**: 1016–25.
5. Hori K, Saito S, Nihei M, Suzuki M, Sato Y. Antitumor effects due to irreversible stoppage of tumor tissue blood flow: evaluation of a novel combretastatin A-4 derivative, AC-7700. *Jpn J Cancer Res* 1999; **90**: 1026–38.
6. Nihei Y, Suzuki M, Okano A, Akiyama Y, Hori K, Saito S, Sato Y. Anti-vascular effects of AC-7700 on solid tumors; comparison with other tubulin binding agents. *Proc Am Assoc Cancer Res* 1998; **39**: 167.
7. Shinkai T, Saijo N, Tominaga K, Eguchi K, Shimizu E, Sasaki Y, Fujita J, Futami H. Comparison of vindesine plus cisplatin or vindesine plus mitomycin in the treatment of advanced non-small cell lung cancer. *Cancer Treat Rep* 1985; **69**: 945–51.
8. Johnson BE, Salem C, Nesbitt J. Limited stage small cell lung cancer treated with concurrent hyperfractionated chest radiotherapy and etoposide/cisplatin. *Lung Cancer* 1993; **9**: 215–65.
9. Belinson JL, McClure M, Ashikaga T, Krakoff IH. Treatment of advanced and recurrent ovarian carcinoma with cyclophosphamide, doxorubicin, and cisplatin. *Cancer* 1984; **54**: 1983–90.
10. Bonomi P, Kim K, Kusler J, Johnson D. Cisplatin/etoposide vs paclitaxel/cisplatin/G-CSF vs paclitaxel/cisplatin in non-small-cell lung cancer. *Oncology (Huntingt)* 1997; **11**: 9–10.
11. Wozniak AJ, Crowly JJ, Balcerzak SP, Weiss GR, Spiridonidis CH, Baker LH, Albain KS, Kelly K, Taylor, SA, Gandara DR, Livingston RB. *J Clin Oncol* 1998; **16**: 2459–65.
12. Masuda N, Fukuoka M, Kudoh S, Kusunoki Y, Matsui K, Nakagawa K, Hirashima T, Tamanoi M, Nitta T, Yana T. Phase I study of irinotecan and cisplatin with granulocyte colony-stimulating factor support for advanced non-small-cell lung cancer. *J Clin Oncol* 1994; **12**: 90–6.
13. Wood SA, Vlassopoulos D, Mucci A. Effects of concentrated matrices on the determination of traces of platinum and gold in aqueous samples using solvent extraction-Zeeman effect graphite furnace atomic absorption spectrometry and inductively coupled plasma-mass spectrometry. *Anal Chim Acta* 1990; **229**: 227–38.
14. Himes RH, Kersey RN, Heller-Bettinger I, Samson FE. Action of the vinca alkaloids vincristine, vinblastine, and desacetyl vinblastine amide on microtubules *in vitro*. *Cancer Res* 1976; **36**: 3798–802.
15. Lee K, Tanaka M, Kanamaru H, Hashimura T, Yamamoto I, Konishi J, Kuse F. *In vitro* antagonism between cisplatin and vinca alkaloids. *Br J Cancer* 1989; **59**: 36–41.
16. Hill SA, Lonergan SJ, Denekamp J, Chaplin DJ. Vinca alkaloid: anti-vascular effects in a murine tumour. *Eur J Cancer* 1993; **29A**: 1320–4.
17. Chaplin DJ, Pettit GR, Parkins CS, Hill SA. Antivascular approaches to solid tumor therapy: evaluation of tubulin binding agents. *Br. J. Cancer* 1996; **74** Suppl 27: S86–S88.
18. Ozawa S, Sugiyama Y, Mitsuhashi Y, Kobayashi T, Inaba M. Cell killing action of cell cycle phase-non-specific antitumor agents is dependent on concentration-time product. *Cancer Chemother Pharmacol* 1988; **21**: 185–90.
19. Chaplin DJ, Pettit GR, Hill SA. Anti-vascular approaches to solid tumour therapy: evaluation of combretastatin A4 phosphate. *Anticancer Res* 1999; **19**: 189–96.
20. Li L, Rojiani AM, Siemann DW. Preclinical evaluations of therapies combining the vascular targeting agent combretastatin A-4 disodium phosphate and conventional anticancer therapies in the treatment of Kaposi's sarcoma. *Acta Oncol* 2002; **41**: 91–7.
21. Siemann DW, Mercer E, Lepler S, Rojiani AM. Vascular targeting agents enhance chemotherapeutic agent activities in solid tumor therapy. *Int J Cancer* 2002; **99**: 1–6.