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Inhibitory Effects of Ginsenoside Rh_2 on Tumor Growth in Nude Mice Bearing Human Ovarian Cancer Cells

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Ginsenoside Rh_2 (Rh_2), isolated from an ethanol extract of the processed root of *Panax ginseng* CA Meyer, inhibits the growth of B16 melanoma cells. This study was designed to evaluate the ability of Rh_2 to inhibit growth of human ovarian cancer cells (HRA) *in vitro* and in nude mouse. Rh_2 inhibited proliferations of various established human ovarian cancer cell lines in a dose-dependent manner between 10 and 60 μM *in vitro* and induced apoptosis at around the IC_{50} dose. When HRA cells were inoculated s.c. into the right flank of nude mice, all mice formed a palpable tumor within 14 days. Although i.p. administration of Rh_2 alone hardly inhibited the tumor growth, when Rh_2 was combined with *cis*-diamminedichloroplatinum(II) (CDDP) the tumor growth was significantly inhibited, compared to treatment with CDDP alone. When mice were treated p.o. with Rh_2 daily (but not weekly), the tumor growth was significantly ($P<0.01$) inhibited, compared to CDDP treatment alone. When Rh_2 was combined with CDDP, the degree of tumor growth retardation was not potentiated. The survival time was significantly ($P<0.05$) longer than that of medium alone-treated controls or the group treated with CDDP alone. Then, we examined whether p.o. administration of Rh_2 has a dose-dependent inhibitory effect on the tumor growth. I.p. and weekly administration of CDDP had more potent antitumor activity in the order of 1 mg/kg, 2 mg/kg and 4 mg/kg, whereas p.o. and daily administration of Rh_2 (0.4 to 1.6 mg/kg) not only had antitumor activity comparable to that of 4 mg/kg CDDP, but also resulted in a significant increase of the survival. Doses of Rh_2 used in this study did not result in any adverse side-effects as confirmed by monitoring hematocrit values and body weight, unlike 4 mg/kg CDDP, which had severe side-effects. It is noteworthy that p.o. but not i.p. treatment with Rh_2 resulted in induction of apoptotic cells in the tumor in addition to augmentation of the natural killer activity in spleen cells from tumor-bearing nude mice. Thus, particularly in view of the toxicity of CDDP, Rh_2 alone would seem to warrant further evaluation for treatment of recurrent or refractory ovarian tumor.

Key words: Ginsenoside Rh_2 — Human ovarian cancer cell line — Antitumor activity — Apoptosis — Natural killer activity

Cis-diamminedichloroplatinum(II) (CDDP) is considered as one of the most effective antitumor agents for ovarian carcinoma. CDDP-based chemotherapy has clearly improved the response rate in the treatment of ovarian carcinoma, but its clinical potential is often counteracted by intrinsic or acquired resistance and its impact on survival has been only marginal in most cases. Effective chemotherapy for ovarian carcinoma with such CDDP resistance is still not established. Thus, development of antitumor drugs which can be substituted for CDDP is of great importance.

Ginseng is one of the most widely used natural tonics in Oriental countries. Odashima *et al.*[1, 2] have reported that a crude fraction of ginsenosides extracted from roots of *Panax ginseng* CA Meyer induced a phenotypic reverse transformation in cultured Morris hepatoma cells. Ginsenoside Rh_2 (Rh_2) is a plant glycoside with a dammarane skeleton, resembling a steroid skeleton, as an aglycone; it has one sugar at C-3 and the capacity to inhibit the growth of and to stimulate melanogenesis in B16 melanoma cells. Recently, we have also demonstrated that i.p. and p.o. administration of Rh_2 inhibited the tumor growth of human ovarian cancer cells inoculated into nude mice and also had an adjuvant effect with CDDP.[3, 4] In addition, it has been reported that the long-term p.o. administration of red ginseng augmented natural killer (NK) activity not only in mouse, but also in human.[5, 6]

The present study extends our previous *in vitro* and *in vivo* observations. We now report that p.o. administration of Rh_2 is superior to i.p. administration with regard to antitumor activity and has an apoptosis-inducing effect in addition to an augmenting effect on NK activity.

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MATERIALS AND METHODS

Agents Rh_2 was kindly supplied by the Korean Tobacco and Ginseng Co., Korea and Seikanshou Co., Japan. The molecular mass and purity of the Rh_2 were 618 Da and more than 99.9%, respectively. Rh_2 was dissolved in absolute ethanol and stored at 4°C CDDP was obtained from Bristol-Myers Squibb Co., Ltd. (Tokyo). These drugs were diluted with medium or saline to the desired concentrations prior to use.

Cell lines HRA cell line was established from ascites of a patient with serous cystadenocarcinoma of the ovary.[7] The KK cells were established from ascites of a patient with clear cell carcinoma of the ovary who did not respond to CDDP-based combination chemotherapy.[8] KF cells were established from tissue of a patient with serous cystadenocarcinoma of the ovary.[9] CDDP-resistant KFr cells were obtained by repeated exposure of the KF cells to escalating doses of CDDP.[10] These cell lines were incubated in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 m*M* glutamine, 100 units penicillin/ml and 100 μg streptomycin/ml (GIBCO, Grand Island, NY) in 5% CO_2 at 37°C. The medium was changed every 3 days, and the cells were passaged when they reached confluence.

***In vitro* treatment** To determine the concentrations of CDDP and Rh_2 required for 50% inhibition (IC_{50}) of HRA, KK, KF and KFr cell proliferations *in vitro*, we seeded 5×10^2 cells for HRA, KF and KFr and 2×10^3 cells for KK in 96-well flat-bottomed microtest-plates (Becton Dickinson Labware, Franklin Lakes, NJ) and incubated them in the absence or presence of CDDP and Rh_2 in a humidified atmosphere of 5% CO_2 at 37°C. After incubation for 5 days, cell viability in each well was measured using a crystal violet staining method.[11] Briefly, an equal volume of 10% formalin phosphate-buffered saline containing 0.2% crystal violet was added to each well and left in room temperature for 20 min. The plates were washed twice with distilled water and dried in room temperature, then the absorbance at 590 nm of stained cells was measured by an automatic microtest-plate reader (Multiscan MCC/340, Titertek, Flow Laboratories Inc., VA). Average absorbance of the control wells in the absence of drugs was regarded as 100%, and the percentage cell growth in each well was calculated. IC_{50} values of CDDP and Rh_2 were calculated from graphical plots.

Flow cytometry Cell cycle phase distribution analysis was performed with a FACSCalibur™ flow cytometer (Nippon Becton Dickinson Co., Ltd., Tokyo) using propidium iodide-stained nuclei of the cells.[12]

DNA fragmentation DNA was analyzed by a gel electrophoresis method as described previously.[13] In this method, DNA fragments of apoptotic cells appear as multimers of 200 bp.

Morphological identification of apoptotic cells To facilitate identification and quantification of apoptotic cells in formalin-fixed, paraffin-embedded sections, we employed a staining method that was developed for tissue sections.[14] This method involves *in situ* nick end labeling (TUNEL) of DNA strand breaks by the incorporation of biotinylated nucleotides with polymerase. It enabled recognition of apoptotic cells by the combination of the TUNEL signals and the morphologic features. For all stainings, slides were counterstained with 1% methyl green. The apoptotic signal was identified by conventional light microscopic examination.

Nude mice Female BALB/c nude mice (about 6 weeks old) were obtained from Japan Clea Laboratories, Tokyo, Japan, and maintained in a pathogen-free environment. The animals were inspected daily and tumor growth was determined by measurement of diameters of the tumor nodule in two dimensions with a caliper. Tumor volume (cm^3) was calculated by use of the following equation; $4/3\times\pi[(r_1+r_2)/2]^3$ where r_1 is longitudinal radius and r_2 is transverse radius. When necessary, the animals were killed and dissected. The tumor tissues were fixed in formalin for histological examination. Large tumors (more than 2 cm in diameter) contained a wide necrotic area in the center. Distant metastases were not observed during the experimental period. Unless treated, all mice died of tumor burden within 100 days after tumor inoculation.

***In vivo* treatment** KK cells were not heterotransplantable to nude mice. Transplantability of KFr cells to nude mice was unstable showing a 40–70% success rate. Although both HRA and KF cells showed 100% transplantability, HRA cells were exclusively used for the *in vivo* experiments because the inhibitory effect of Rh_2 on the *in vitro* proliferation of HRA cells was greater than that of KF cells (Table I). When 10^6 HRA cells were inoculated s.c. into the right flank of nude mice, all mice formed a tumor within 14 days. The first experiment was performed to determine the effect of weekly or daily p.o. administration of Rh_2 and its combined effects with CDDP. Treatment

Table I. Effect of CDDP and Rh_2 on the Proliferation of Human Ovarian Cancer Cell Lines

Cells	IC_{50} (μM)	
	CDDP	Rh_2
HRA	0.13±0.01[a)]	30±3
KK	0.51±0.03	45±6
KF	0.21±0.02	40±5
KFr	0.65±0.11	41±5

Cytotoxicity was evaluated in the presence of the indicated agents for 5 days.
a) Mean±SD from four independent experiments.

groups were divided as follows; 1) untreated control, 2) 2 mg/kg CDDP was administered i.p. once a week for 5 weeks from 7 days after tumor inoculation, 3) 0.13 mg/kg Rh_2 was administered p.o. once a week for 5 weeks from 7 days after tumor inoculation, 4) i.p. and weekly administration of 2 mg/kg CDDP was combined with p.o. and weekly administration of 0.13 mg/kg Rh_2 for 5 weeks, 5) 0.4 mg/kg Rh_2 was administered p.o. once a week for 5 weeks from 7 days after tumor inoculation, 6) i.p. and weekly administration of 2 mg/kg CDDP was combined with p.o. and weekly administration of 0.4 mg/kg Rh_2 for 5 weeks, 7) 0.4 mg/kg Rh_2 was administered p.o. every day for 90 days from 7 days after tumor inoculation, and 8) p.o. and daily administration of 0.4 mg/kg Rh_2 for 90 days was combined with i.p. and weekly administration of 2 mg/kg CDDP for 5 weeks. Each group consisted of 10 mice. The second experiment was performed to determine the dose-dependence of the effect of Rh_2 (p.o. and daily administration) alone on the tumor growth, in comparison to that of CDDP alone. Treatment groups were divided as follows; 1) untreated control, 2) 1 mg/kg CDDP was administered i.p. once a week for 5 weeks from 7 days after tumor inoculation, 3) 2 mg/kg CDDP was administered i.p. once a week for 5 weeks from 7 days after tumor inoculation, 4) 4 mg/kg CDDP was administered i.p. once a week for 5 weeks from 7 days after tumor inoculation, 5) 0.4 mg/kg Rh_2 was administered p.o. every day for 90 days from the day of tumor inoculation, 6) 0.8 mg/kg Rh_2 was administered p.o. every day for 90 days from the day of tumor inoculation, and 7) 1.6 mg/kg Rh_2 was administered p.o. every day for 90 days from the day of tumor inoculation. Each group consisted of 10 mice. P.o. administration of Rh_2 was performed using a canula and the volume was 0.4 ml. The final concentration of ethanol in this solution was less than 0.4%. Since tumors larger than 10 cm^3 form an ulcer with a large necrotic area in the center, the tumor size measured in the surviving mice could not be determined exactly. Therefore, the tumor-growth curve was presented until 42 or 49 days after tumor inoculation (Figs. 4 and 5).

NK activity Spleen cells obtained on 7, 14, 21 and 28 days after HRA cell inoculation were used as effector cells against YAC-1 cells. Significant ^{51}Cr-release was observed at 4 h; however, maximum lysis was seen at 18 h. Therefore, for comparative purposes, the longer assay was exclusively used. Target cells were labeled with 100 μCi of ^{51}Cr (New England Nuclear, Boston, MA) for 1 h at 37°C. The ^{51}Cr-release assay was performed at the effector:target ratio of 100:1, 50:1 or 25:1. Cytotoxicity was determined in terms of the amount of ^{51}Cr (cpm) released from target cells, according to the following formula: Specific lysis (%) = (test release − spontaneous release) / (maximum release − spontaneous release) × 100. Spontaneous and maximum releases were from target cells incubated in medium and in medium to which 1 *N* HCl had been added, respectively.

Statistical analysis The significance of differences in survival times among groups was determined by the Mann-Whitney U test. Contingency-table analysis and the χ^2 test were used for comparison of effects of treatment on the tumor growth. NK activity against YAC-1 cells was analyzed using Student's *t* test.

RESULTS

Effects of CDDP and Rh_2 on human ovarian cancer cell proliferation *in vitro* As shown in Table I, HRA cells had the highest sensitivity to CDDP and Rh_2 among

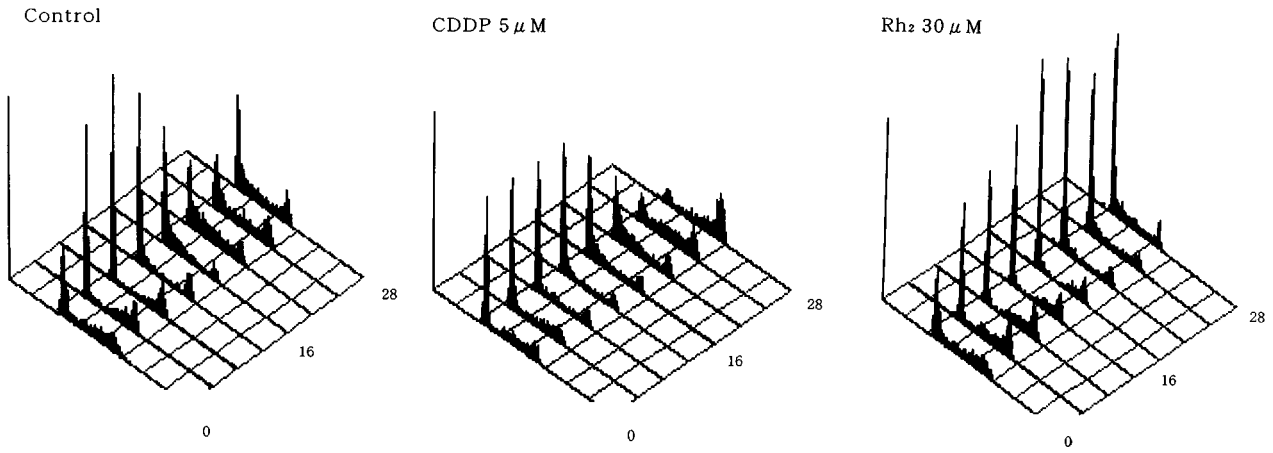

Fig. 1. Effects of Rh_2 on the cell cycle phase of HRA cells. Preconfluent cells were incubated for the indicated time with concentrations based on the IC_{50} of Rh_2 and CDDP to HRA cells, then harvested and washed 3 times with fresh medium. Preincubated cells and incubated cells were analyzed by flow cytometry. Numbers indicate hours.

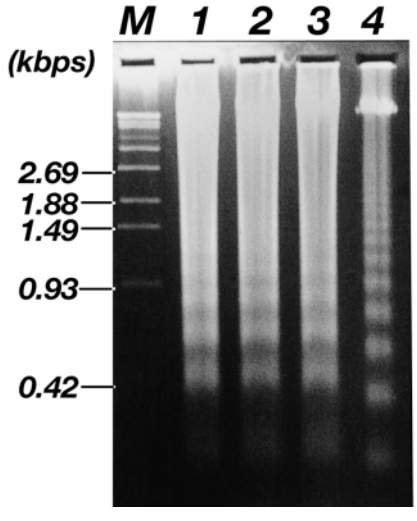

Fig. 2. Induction of DNA fragmentation by Rh_2. Preconfluent HRA cells were incubated for 6 days with 30 μM Rh_2, then harvested and washed 3 times with fresh medium. DNA fragmentation of apoptotic cells in the incubated cells was determined by a gel electrophoresis method. Lanes 1–3: HRA cells incubated with 30 μM Rh_2 for 6 days. Lane 4: apoptosis positive control in HL-60 cells.

various human ovarian cancer cell lines examined in this study, while the CDDP-resistant KK and KFr cells had similar Rh_2 sensitivity to that of the CDDP-sensitive KF cells. The IC_{50} dose of CDDP resulted in accumulation of HRA cells in the G_2-M phase, while that of Rh_2 caused accumulation in the G_1 phase (Fig. 1).

Effects of Rh_2 on apoptosis of HRA cells *in vitro* and *in vivo* Rh_2 induced apoptosis of HRA cells around the IC_{50} dose *in vitro* (Fig. 2). To determine the effects of Rh_2 on apoptosis of HRA cells *in vivo*, i.p. or p.o. administration of Rh_2 to nude mice with an approximately 1 cm diameter tumor was performed after HRA cell inoculation. At 7 days after i.p. or p.o. treatment with 0.4 mg/kg of Rh_2, the tumor was stained as described in "Materials and Methods." P.o. treatment with Rh_2 induced significantly more apoptotic cells in the tumors than did i.p. treatment (Fig. 3).

Effects of Rh_2 on tumor growth and survival of nude mice with HRA cell tumor To evaluate the *in vivo* effects of Rh_2 on the tumor growth and survival of the tumor-bearing mice, we performed two experiments using nude mice. First, to examine the effect of p.o. treatment with Rh_2, weekly or daily administration was compared. P.o. and weekly (but not daily) administration of 0.13 and

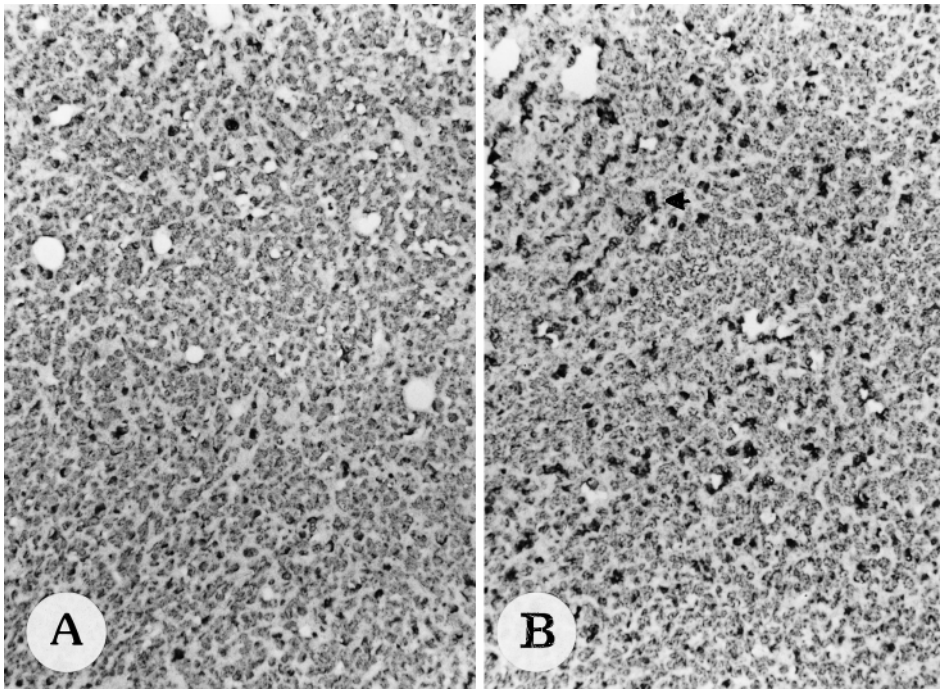

Fig. 3. Apoptotic cells in tumor tissues from nude mice treated i.p. or p.o. with Rh_2. Nude mice with about 1 cm diameter tumor nodule were treated i.p. or p.o. every day for 7 days with 0.4 mg/kg Rh_2, then the tumor was removed. Apoptotic cells in the tumor were stained by a method involving *in situ* nick end labeling (TUNEL) of DNA strand breaks ("Materials and Methods"). TUNEL signals increased as shown by the arrowhead. A, i.p. treatment; B, p.o. treatment.

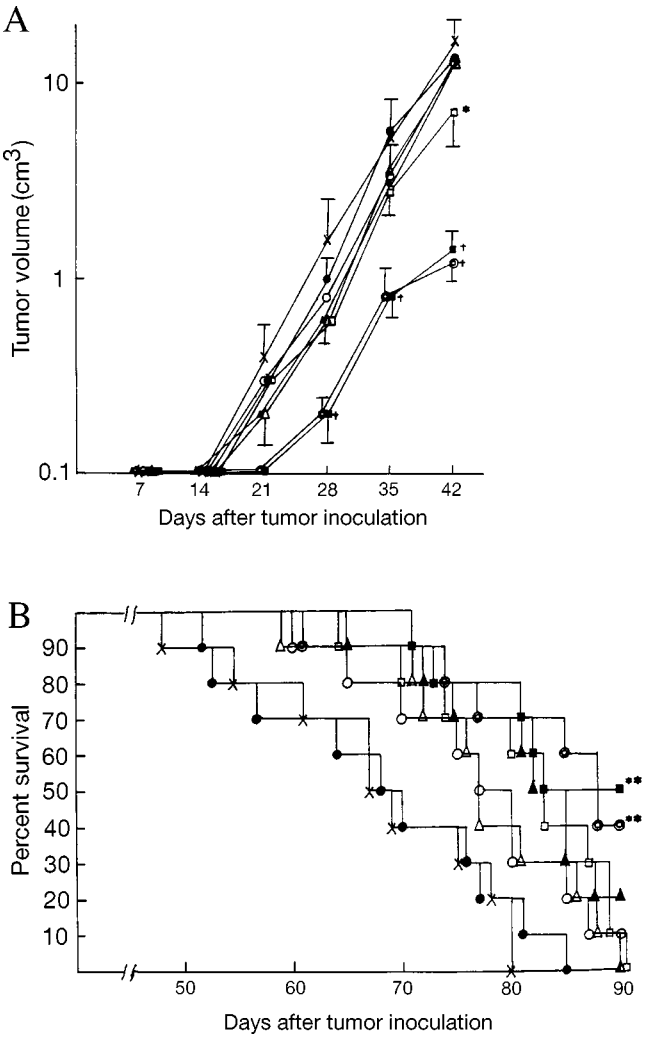

Fig. 4. Effects of weekly p.o. and daily p.o. administration of Rh_2 on the tumor growth and survival rate. A, effects on the tumor growth; B, effects on the survival rate. Scales for the tumor volume are logarithmic. × untreated, ○ 2 mg/kg CDDP alone, ● 0.13 mg/kg Rh_2 alone (weekly), △ 2 mg/kg CDDP plus 0.13 mg/kg Rh_2 (weekly), ▲ 0.4 mg/kg Rh_2 (weekly), □ 2 mg/kg CDDP plus 0.4 mg/kg Rh_2 (weekly), ■ 0.4 mg/kg Rh_2 (daily), ◎ 2 mg/kg CDDP plus 0.4 mg/kg Rh_2 (daily). * P<0.05, compared to untreated group. † P<0.01, compared to CDDP alone-treated group. ** P<0.05, compared to CDDP alone-treated group.

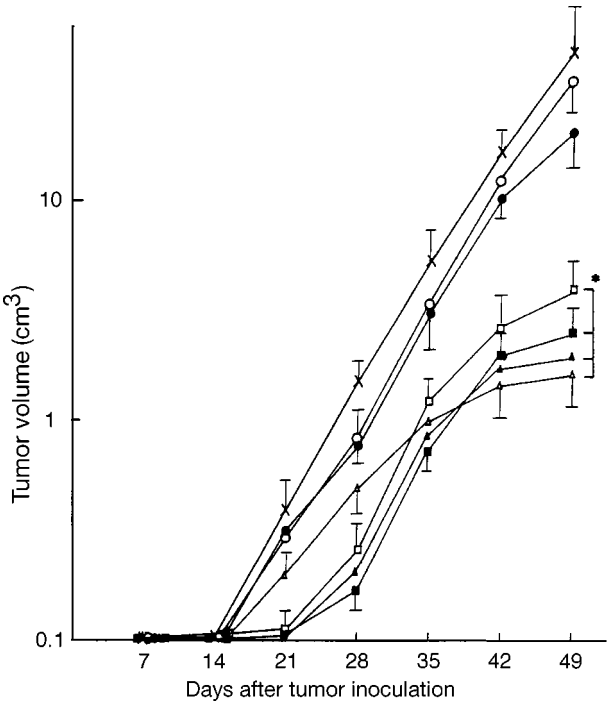

Fig. 5. Inhibitory effects of p.o. administration of Rh_2 on the tumor growth in comparison to i.p. administration of CDDP. Scales for the volume are logarithmic. × untreated, ○ 1 mg/kg CDDP, ● 2 mg/kg CDDP, △ 4 mg/kg CDDP, ▲ 0.4 mg/kg Rh_2, □ 0.8 mg/kg Rh_2, ■ 1.6 mg/kg Rh_2. * P<0.05, compared to 2 mg/kg CDDP alone-treated group.

Table II. Effect of Rh_2 on the Survival of Tumor-bearing Nude Mice

Treatment	50% survival (days)	% ILS
Untreated	60	
1 mg CDDP	70	16.7
2 mg CDDP	75	25.0[a)]
4 mg CDDP	65	8.3
0.4 mg Rh_2	85	41.6[a,b)]
0.8 mg Rh_2	83	38.3[a,b)]
1.6 mg Rh_2	84	40.0[a,b)]

ILS, increase of life span.
a) P<0.05, compared to untreated.
b) P<0.05, compared to 2 mg CDDP.

0.4 mg/kg Rh_2 alone did not show any inhibitory effect on the tumor growth. When 0.4 mg/kg Rh_2 was daily administered, Rh_2 showed more marked (P<0.01) inhibitory effect on the tumor growth than did i.p. treatment with 2 mg/kg CDDP. However, even if p.o. and daily administration of Rh_2 was combined with CDDP, inhibition of the tumor growth was not augmented (Fig. 4A). P.o. and daily treatment with 0.4 mg/kg Rh_2 and its combination with CDDP resulted in a significant prolongation of the survival time (P<0.05), compared to treatment with CDDP alone (Fig. 4B). Next, we examined whether p.o. and daily administration of Rh_2 has a dose-dependent inhibitory effect on the tumor growth. When mice were treated

Table III. Effects of Rh_2 on the NK Activity of Spleen Cells from Tumor-bearing Nude Mice

Treatment	NK activity (%)	
	Day 21	Day 28
Intact nude mice	3.5 ± 2.0[a)]	
Untreated nude mice	8.5±1.6[b)]	8.0±1.3[b)]
2 mg/kg CDDP treated	7.2±1.9	6.6±2.1
0.4 mg/kg Rh_2 treated	19.1±2.7[c)]	20.2±2.0[c)]

Nude mice were killed 21 and 28 days after tumor inoculation and NK activity was measured as described in "Materials and Methods." Since no significant increase in the NK activity was observed on Days 7 and 14, the values on Days 21 and 28 are presented. Each result shows percent cytotoxicity obtained at the effector:target ratio of 50:1.
a) Mean±SD from 5 mice.
b) $P<0.05$, compared to intact nude mice.
c) $P<0.05$, compared to untreated and 2 mg/kg CDDP-treated nude mice.

with 4 mg/kg CDDP, the tumor growth was markedly inhibited while about a half of the mice died as a result of loss of body weight. On the other hand, p.o. and daily treatment with 0.4 mg/kg Rh_2 had an inhibitory effect on the tumor growth comparable to that of 4 mg/kg CDDP, without any adverse side-effect such as weight loss or lowering of hematocrit (Fig. 5), consequently resulting in a significant increase of life span (ILS) (Table II). However, the tumor-inhibitory effect was not enhanced at higher doses (0.8 and 1.6 mg/kg) of Rh_2.

Effects of Rh_2 on the NK activity of spleen cells in tumor-bearing nude mice We measured the NK activity of spleen cells obtained from untreated, 2 mg/kg CDDP alone-, and 0.4 mg/kg Rh_2 alone-treated nude mice. Spleen cells from untreated nude mice showed a significant increase in the NK activity ($P<0.05$) at 21 and 28 days after tumor inoculation, compared to that of intact nude mice. Treatment with CDDP alone hardly affected the NK activity. In contrast, p.o. and daily administration of Rh_2 resulted in a significant ($P<0.05$) increase in the NK activity, compared to treatment with CDDP alone (Table III).

DISCUSSION

In the present study, we confirmed that Rh_2 inhibited proliferation of not only HRA cell line, but also other human ovarian cancer cell lines (Table I). Although the precise mode of action of Rh_2 is still unclear, it has been reported that Rh_2 not only arrests the cell cycle of B16 melanoma cells at the G_1/G_0 phase, but also stimulates melanogenesis in these cells.[15, 16] Flow-cytometric analysis revealed that when HRA cells were incubated with 0.15 μM CDDP or 30 μM Rh_2 for 3 days, CDDP blocked the cell cycle in the G_2-M phase, while Rh_2 caused G_1-arrest with induction of apoptosis (Fig. 1). In addition, Rh_2 seemed to increase the cell adhesiveness to a plastic surface and the cell agglutinability (data not shown), in accordance with previous observations.[16] As shown in Fig. 2, Rh_2 induced apoptosis in HRA cells more easily at around the IC_{50} dose than did CDDP. Apoptosis is a late event in cell death, resulting from a number of distinct pathways.[17] The pathway which is involved in induction of apoptosis by Rh_2 should be addressed. We have previously demonstrated that i.p. administration of Rh_2 had an adjuvant effect to CDDP and improved the survival rate of tumor-bearing nude mice.[4] Red ginseng containing Rh_2 has been used as a preventive medical agent for more than 2000 years in Asian countries, especially in Korea, Japan and China. The effect of i.p. pretreatment with Rh_2 on the tumor growth was examined to test whether it can be effective for cancer treatment. I.p. administration of Rh_2 alone did not cause any significant inhibition on the tumor growth. Even if Rh_2 was injected i.p. before tumor inoculation, no inhibitory effect was observed. Only when Rh_2 was combined with CDDP, a growth-inhibitory effect on the tumor growth was observed ($P<0.05$, compared to untreated, CDDP alone- and Rh_2 alone-treated groups).[3] These results suggest that i.p. administration of Rh_2 is ineffective.

Thus, in the first experiment we investigated the effect of p.o. administration of Rh_2 on tumor growth and survival (Fig. 4). When Rh_2 alone was administered p.o. once a week, no inhibitory effect on the tumor growth was observed (Fig. 4A). However, daily p.o. administration of 0.4 mg/kg Rh_2 resulted in a significant ($P<0.01$) tumor growth retardation, showing a greater antitumor activity than weekly i.p. administration of 2 mg/kg CDDP. Moreover, the survival time was significantly ($P<0.05$) prolonged and 5 out of 10 nude mice survived during the experimental period (Fig. 4B). Next, we determined whether daily p.o. administration of Rh_2 shows a dose-dependent effect on the tumor growth (Fig. 5). Although Rh_2 did not show a dose-dependent inhibitory effect between 0.4 mg/kg and 1.6 mg/kg, the degree of inhibition by these doses of Rh_2 was significantly ($P<0.05$) higher than that by 2 mg/kg CDDP. In the previous study, we found that 0.02 mg/kg Rh_2 showed significantly less inhibitory effect on the tumor growth than 0.3 mg/kg Rh_2.[4] Thus, 0.4 mg/kg Rh_2 seemed to be an effective and optimal dose with regard to inhibition of the tumor growth. When 4 mg/kg CDDP was administered i.p. once a week, the strongest tumor growth inhibition was obtained. However, 5 out of 10 mice treated with this dose died owing to loss of body weight and a rapid fall of hematocrit during the treatment course (data not shown). In this case, prolongation of the survival time could not

be obtained (Table II). On the other hand, a high dose of Rh_2 (1.6 mg/kg) administered p.o. daily had no adverse effect.

The precise mode of action of Rh_2 is still unknown. Previously, we suggested that NK activity was modulated by Rh_2.[18] Therefore, we examined the time course of NK activity in the spleen cells from nude mice treated with Rh_2. Although spleen cells from intact nude mice showed very weak NK activity, those from tumor-bearing nude mice at Day 21 and 28 showed a significant increase in the NK activity. Treatment with 2 mg/kg CDDP alone hardly affected the NK activity, while daily p.o. administration of 0.4 mg/kg Rh_2 alone remarkably augmented the NK activity (Table II), suggesting the relevance of Rh_2 to the enhancement of the NK activity. In addition to enhancement of NK activity by Rh_2, p.o. administration of Rh_2 induced a higher degree of apoptosis in the tumor than did i.p. administration (Fig. 3), and consequently when Rh_2 was administered p.o., but not i.p., the tumor growth was markedly inhibited. It is well-known that protopanaxadiol (PPD), an aglycone formed from Rh_2 as a result of intracellular deglycosylation, inhibits the cell growth more strongly than Rh_2 itself. Therefore, the marked inhibition of the tumor growth by p.o. administration of Rh_2 may be attributable to more efficient conversion of Rh_2 to PPD in the case of p.o. administration compared to i.p. administration. The Rh_2 and PPD contents in the tumor should be examined.

In conclusion, we have demonstrated that p.o., but not i.p., administration of Rh_2 shows a potent antitumor effect comparable or even superior to i.p. administration of CDDP, and can be safely done without adverse side-effects. Considering the toxicity of CDDP, the use of Rh_2 alone would seem to warrant further clinical evaluation.

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