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Antitumor activity of a novel ginseng saponin metabolite in human pulmonary adenocarcinoma cells resistant to cisplatin

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Abstract

The in vitro antitumor activity of a novel ginseng saponin metabolite, 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901), was examined against four human cancer cell lines and one subline resistant to cisplatin (CDDP). The growth inhibitory activity of the compound was estimated by MTT tetrazolium assay. The mean concentrations of IH-901 needed to inhibit the proliferation of the cells by 50% (IC_{50}) were 24.3, 25.9, 56.6 and 24.9 μM against human myeloid leukemia (HL-60), pulmonary adenocarcinoma (PC-14), gastric adenocarcinoma (MKN-45) and hepatoma (HepG2) cell lines, respectively. These values are higher than that of CDDP. In the CDDP-resistant PC/DDP cell line, the IC_{50} values of IH-901 and CDDP were 20.3 and 60.8 μM, respectively. These results suggest that IH-901 is not cross-resistant to CDDP in this cell line and could be a candidate for the treatment of CDDP resistant pulmonary cancer. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Ginseng saponin metabolite (IH-901); Antitumor effect; Human cancer cell line; Cisplatin; Cisplatin resistance

1. Introduction

Ginseng radix, the root of *Panax ginseng* C.A. Meyer, has been used as a medicinal plant in Asian countries, and now it is used worldwide for preventive and therapeutic purposes. Among the diverse constituents of ginseng, saponins have been found to be the major components responsible for its biochemical and pharmacological actions. Recently, novel ginseng saponin metabolites formed by the human intestinal bacteria were found and their antitumor activity has been proposed [1]. *Prevotella oris,* which is found in 79% of the human fecal specimens, hydrolyses ginsenoside Rb1 and Rd to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901). Since IH-901 is one of the metabolites detected in blood after the oral administration to mice of ginsenoside Rb1, it is speculated that IH-901 is most likely the major form of protopanaxadiol saponin absorbed from the intestine [2]. The hypothesis that IH-901 may be the active metabolite responsible for the anticarcinogenic effect of ginseng saponins has prompted several groups to investigate the pharmacological effects of IH-901. For instance, Wakabayashi et al. reported that the antimetastatic effects of ginseng saponins are mediated by this metabolite [3]. They also proposed that the induc-

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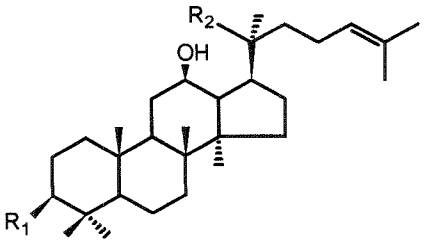
tion of apoptotic cell death of highly metastatic B16-BL6 mouse melanoma cells by the ginseng saponin metabolite probably leads to antimetastatic as well as antitumor activity in vivo [4].

Cisplatin (CDDP) is one of the most effective antitumor agents for the treatment of many types of cancer in the clinic, both as a single agent and in combination with other cytostatics [5]. However, the development of resistance during therapy limits its curative potential. The mechanisms identified in vitro include alterations in cellular drug transport, enhanced DNA repair and an enhanced intracellular detoxification system [6]. A promising way to circumvent CDDP resistance is the use of a resistance modulator [7,8] or the development of non-cross-resistance agents [9].

In the present study, we examined and compared the antitumor effect of IH-901 with that of CDDP in four human cancer cell lines and one subline resistant to CDDP. The main focus of this study was to investigate the effect of IH-901 on acquired resistance to CDDP.



	R_1	R_2
IH-901	OH	O-Glc
Rb1	O-Glc(2→1)Glc	O-Glc(6→1)Glc

Fig. 1. Structural formula of the novel ginseng saponin metabolite 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901).

2. Materials and methods

2.1. Chemicals

IH-901 was prepared by incubating ginseng saponins and intestinal bacteria as described [1]. The structure was confirmed by a series of spectrometric analyses and is shown in Fig. 1 (>99% purity, white powder). IH-901 and ginsenoside Rb1 were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium to the desired concentrations. CDDP was obtained from United Pharmaceutics (Seoul, Korea) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) was a product of Sigma Chemical Co. (St. Louis, MO).

2.2. Cells

Human pulmonary adenocarcinoma cells (PC-I 4) and the subline resistant to CDDP (PC/DDP) were kindly provided by Dr. W.S. Hong (Asan Medical Center, University of Ulsan, Seoul, Korea). The resistant cell line PC/DDP grows stably in the medium containing 9.25 μg/ml CDDP, and the biochemical properties have been characterized in previous studies [10,11]. Human myeloid leukemia (HL-60), human gastric adenocarcinoma (MKN-45) and human hepatoma (HepG2) cell lines were also used in the experiments. They were maintained in RPMI-1640 medium (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO_2.

2.3. Cell growth inhibition assay

The cell growth inhibitory effect of IH-901 and CDDP was determined using the MTT assay [12]. Cells undergoing exponential growth were suspended in fresh medium at a concentration of 8×10^4 cells/ml and inoculated in a 96-well flat bottomed plate in a volume of 100 μl/well. Cells were stabilized by incubation for 24 h at 37°C and 100-μl aliquots of each drug were added to wells. The plate was incubated at 37°C for 4 days. For assay, 50 μl of MTT (1 mg/ml Dulbecco's phosphate-buffered saline) were added to each well and the plate was incubated for 4 h at 37°C. After the plate was centrifuged at 3000 rev./min for 10 min, the supernatant was aspirated, 150 μl DMSO were added and mixed thoroughly to dissolve the formazan crystals. The optical density was measured at 540 nm on a microplate reader (Molecular Device, Sunnyvale, CA). Each experiment was performed in triplicate and repeated at least twice. Antitumor activity was evaluated using IC_{50} determined by non-linear regression analysis using the GraphPad PRISM™ statistics software package (Ver. 2.0; San Diego, CA).

3. Results and discussion

Four human cancer cell lines known to be sensitive to CDDP and one subline showing resistance to CDDP were used in the present study. The origins of the solid tumor were lung (PC-14), liver (HepG2) and stomach (MKN45) and the human myeloid leukemia cell line was used as a cell line of hematopoietic origin. All of the cells grew in a suspension form while the HepG2 cells grew in an adherent form.

The growth inhibitory effects of IH-901 against four cancer cell lines are shown in Fig. 2 and the mean IC_{50} values were calculated (Table 1). The mean IC_{50} of IH-901 against a total of four tumor cell lines was 32.9 μM. In all lines tested, the mean IC_{50} values for IH-901 were higher than those for CDDP, although statistical significance was observed in three cell lines: HL-60, PC-14 and HepG2. Ginsenoside Rb1, the metabolic precursor of IH-901, showed no cytotoxic effect on any of the cell lines tested. We also examined the antiproliferation effect of IH-901 on the PC/DDP cell line, which is a subline of PC-14 resistant to CDDP [11]. PC/DDP was 10.5-fold resistant to CDDP compared to the parental cell line PC-14. This value is higher than that reported by Hong et al., in which the IC_{50} was measured by the soft agar colony assay [11]. The IC_{50} values of IH-901 and CDDP in PC-14 were 25.9 and 5.8 μM, respectively. In the resistant cell line PC/DDP, the IC_{50} values of IH-901 and CDDP were 20.3 and 60.8 μM, respectively. The relative resistances calculated as the ratio of IC_{50} of the resistant cells to that of the parent cells, were 0.8 for IH-901 and 10.5 for CDDP, suggesting that IH-901 is not cross-resistant to CDDP in this cell line (Table 2).

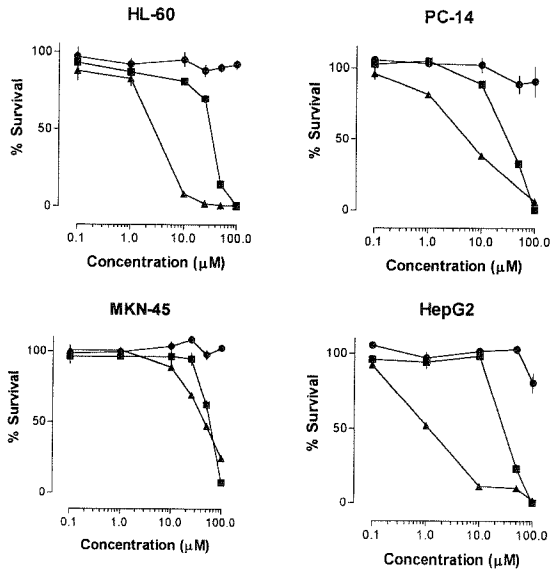


Fig. 2. Antitumor activity of IH-901 (■), CDDP (▲) and ginsenoside Rb1 (●) against human myeloid leukemia (HL-60), human pulmonary adenocarcinoma (PC-14), human gastric adenocarcinoma (MKN-45) and human hepatoma (HepG2) cell lines Each point represents the mean and standard error of three experiments.

Table 1
Tumor-specific IC_{50} values for IH-901 and CDDP using the MTT assay

Tumor cell	IC_{50} (μM)[a]		*P* value	Significance
	IH-901	CDDP		
HL-60	24.3	2.3	0.0009	Y
PC-14	25.9	5.8	0.02	Y
MKN-45	56.6	47.4	0.67	N
HepG2	24.9	1.1	0.0004	Y

[a] IC_{50} was calculated by non-linear regression analysis of the data obtained from three experiments. Results are expressed as mean ± SEM.

Ginseng has been traditionally used for the prevention and treatment of various diseases including cancer. Among the diverse constituents of ginseng, saponins have been found to be the major components responsible for its biochemical and pharmacological action [13]. For example, ginsenoside Rh2 has been suggested to possess growth-inhibitory effects in vitro

Table 2
Cytotoxicity of IH-901 and CDDP against human pulmonary adenocarcinoma cell line PC-14 and its subline resistant to CDDP

	IC_{50} (μM)[a]		Relative resistance[b]	
	IH-901	CDDP	IH-901	CDDP
PC-14	25.9 ± 1.5[c]	58 ± 1.1	1.0	1.0
PC/DDP	20.3 ± 14[c]	60.8 ± 1.2	0.8	10.5

[a] IC_{50} was calculated by non-linear regression analysis of the data obtained from three experiments. Results are expressed as mean ± SEM.

[b] The relative resistance was calculated as the ratio of the IC_{50} value of the resistant cells to that of the parent cells.

[c] Significantly different from the corresponding value obtained from the treatment of CDDP ($P < 0.01$).

and in vivo [14,15] and to induce differentiation and apoptosis in various cancer cells [16,17]. IH-901 is a novel ginseng saponin metabolite formed by intestinal bacteria after oral administration of ginseng extract in humans and rats [1]. Although the mechanism by which IH-901 exerts its cytotoxic activity on the tumor cells is largely unknown, it is reported that IH-901 inhibits tumor cell growth by suppressing glucose uptake [18]. IH-901 possesses antimetastatic effects in vitro as well as in vivo [3,19]. Apoptosis might be the mode of cell death induced by IH-901, which leads to the antitumor and antimetastatic activity in vivo [4]. In this study, we have found that the antitumor activity of IH-901 is not superior to CDDP in all cancer cell lines tested. However, in a CDDP-resistant subline of PC-14, IH-901 was about 3-fold more effective than CDDP. We have previously reported that IH-901 possesses antigenotoxic effects against chemical carcinogens, such as benzo[*a*]pyrene [20]. Taken together with the antimetastatic, antigenotoxic and apoptosis-inducing activities of IH-901, it might have potential clinical use in the prevention and the treatment of cancer, especially with acquired resistance to CDDP.

In conclusion, the present results suggest that IH-901 could be a candidate for the treatment of CDDP-resistant pulmonary cancer. The potential value of this compound is further stressed by its antimetastatic activity in vivo.

Acknowledgements

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