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Original Articles

Antitumor and Cardioprotective Effects of a Ginseng Intestinal Metabolite in Combination with Doxorubicin in Sarcoma-180 Tumor-bearing Mice

Yong Woo Roh, Hyeong Jin Ji, Hee-Youl Chai, Seock-Yeon Hwang[1], Sang-Yoon Nam, Jin Tae Hong[2], Jong Hwan Sung[3], Hyong-Kyu Kim[4], Hyun-Gu Kang, Yun-Bae Kim and Jong-Koo Kang*

College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, Gaeshin-dong, Cheongju 361-763, Korea
[1]Department of Clinical Laboratory Medicine, Chungbuk National University Hospital, Gaeshin-dong, Cheongju 360-240, Korea
[2]College of Pharmacy, Chungbuk National University, Gaeshin-dong, Cheongju 361-763, Korea
[3]Central Research Institute, Ilhwa Co., Ltd., Sutaek-dong, Kuri 471-030, Korea
[4]College of Medicine, Chungbuk National University, Gaeshin-dong, Cheongju 361-763, Korea

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The effects of IH901, a ginseng intestinal metabolite, on the antitumor activity and cardiotoxic adverse effect of doxorubicin were investigated in sarcoma-180 ascitic tumor-bearing mice. To evaluate the enhancing effect on the antitumor activity of doxorubicin, IH901 (50 mg/kg) was orally administered for 28 days, in combination with intraperitoneal injection of doxorubicin (3 mg/kg) on days 5, 12, 19 and 24, to mice intraperitoneally inoculated with 1×10^5 sarcoma-180 cells. The body weights of tumor-bearing mice dramatically increased from 10 days following tumor inoculation, leading to a mean survival time of 17.3 days. In contrast, such an increase in body weights induced by the ascitic tumor growth was markedly attenuated by doxorubicin (3 mg/kg) administration, resulting in a long lifespan of 34.9 days. Interestingly, the body weight gain was further suppressed by the combination of IH901 (50 mg/kg), leading to a normal feature. In addition, the mean survival time was extended by 129.3%, reaching 39.7 days, although IH901 was inactive alone. Separately, for the evaluation of protective effect on the cardiotoxicity of doxorubicin, IH901 (50 mg/kg) was orally administered for 14 days, in combination with intraperitoneal injection of doxorubicin (5 mg/kg) on days 5 and 9 to tumor-bearing mice. Although the heart weight of tumor-inoculated mice decreased to about 75% of normal, such an atrophy was remarkably recovered by coadministration of IH901. Furthermore, IH901 substantially reversed the dramatic decrease in mRNA of myocytic phospholipid hydroperoxide glutathione peroxidase, an antioxidant enzyme, as well as histopathological changes such as cytoplasmic vacuolization, mitochondrial swelling, and loss of myofibrils and intercalated disc induced by doxorubicin. Taken together, it is suggested that IH901 could be a potential adjunct for the potentiation of antitumor activity and reduction of cardiotoxicity of chemotherapeutic agents including doxorubicin.

Key words : Ginseng intestinal metabolite (IH901), doxorubicin, antitumor, cardioprotection, sarcoma-180

*Corresponding author: **Jong-Koo Kang**, College of Veterinary Medicine, Chungbuk National University, 12, Gaesin-dong, Cheongju 361-763, Korea
Tel: 82-43-261-2607
Fax: 82-43-274-9443
E-mail: jkkang@cbu.ac.kr

Introduction

Although surgery and radiotherapy exert successful prognoses in a part of cancer patients, most of cases need chemotherapy for prolongation of survival time. In the 50 years of its development, chemotherapy has yielded important achievements. In spite of effectiveness of chemotherapy, however, there is a limitation in using chemotherapeutic agents[1].

Anthracycline antibiotics such as doxorubicin are one of the most-widely used antineoplastic drugs. Doxorubicin has been proven to be effective in the treatment of acute leukemia, lymphoma and a number of solid human tumors[2,3]. In spite of its high anticancer efficacy, however, the optimal use of doxorubicin is limited by adverse effects, for example, nausea, cardiomyopathy, arrhythmia, congestive heart failure, myelosuppression, and nephrotoxicity. Cardiomyopathy and congestive heart failure, as irreversible chronic side effects, were considered main harmful effects following chronic treatments[4-6].

Doxorubicin may exert its antineoplastic effect via free radical-independent mechanisms, including inhibition of the topoisomerase II, binding of drug-iron complex to DNA, and intercalation between DNA base pairs[6]. In comparison, it was suggested that cardiotoxicity of doxorubicin is partially due to the high energy requirement of the heart, showing somewhat different action mechanism from its antineoplastic effect[6,7]. Doxorubicin is well known for its complex cytotoxic mechanisms involving 1) inhibition of enzymes such as topoisomerase II, RNA polymerase, cytochrome c oxidase and others, 2) intercalation into DNA, 3) chelation of iron and generation of reactive oxygen species (ROS), and 4) induction of apoptosis[3]. Such diverse cytotoxic actions demonstrate that the cause of anthracycline-induced cardiomyopathy is probably multifactoral and complex, but most of these changes could be attributed to free oxygen radical production and lipid peroxidation. In the presence of oxygen, redox cycling of doxorubicin-derived quinone-semiquinone yields superoxide radicals which are catalyzed to hydrogen peroxide by superoxide dismutase. Futhermore, hydrogen peroxide subsequently forms hydroxyl radicals which react with polyunsaturated fatty acids, producing lipid peroxides[8]. Moreover, in the presence of transitional metal ions, the chain reaction continues and free iron appears to play a particularly important role in doxorubicin-induced lipid peroxidation[9]. Since doxorubicin-induced cardiotoxicity occurs most likely via an oxidative stresses, moderation of this activity may result in an improved therapeutic index for this compound[10].

Panax ginseng C. A. Mayer (Araliaceae) is a herbal root that has been used in folk medicine of oriental countries for the treatment of psychiatric and neurologic diseases as well as diabetes mellitus. Ginseng saponins (ginsenosides) are regarded as the principal component responsible for the pharmacological activities of ginseng[11]. Ginsenosides such as protopanaxadiol and protopanaxatriol are composed of β-glucosides containing an aglycon with dammarane-type triterpene skeleton. Ginseng β-glucosides are metabolized by neither gastric juice nor intestinal enzymes. Rather, it was reported that orally administered ginsenosides are detected not in as their intact form but in an intestinal bacterial metabolite form in blood[12,13]. Thus, Hasegawa *et al.*[13] demonstrated the biotransformation of ginsenosides by *Prevotella oris*, normal intestinal microflora isolated from human intestinal feces. It has been reported that protopanaxadiol-type ginsenosides such as Rb_1, Rb_2 and Rc are metabolized by intestinal bacteria to 20-O-β-D-glucopyranosyl-20(s)-protopanaxadiol, which is referred to M1, compound K or 1H901[12-15]. Recently, it was also found that the intestinal bacterial metabolites of ginsenosides were absorbed from the intestine to blood and excreted into urine, and that the metabolites were believed to be the primary active principle of ginseng saponins.

1H901 is a major intestinal metabolite formed from ginseng protopanaxadiol saponins and has been shown to possess some pharmacological activities such as the inhibition of glucose uptake by tumor cells[11] and the reversal of multidrug resistance in bacteria and tumor cells[15]. Also, 1H901 exhibited antitumor activities *in vitro* and *in vivo*, displaying prevention of cancer development[15-17]. *In vitro* conditions, 1H901 inhibited the proliferation of the cancer cells such as human myeloid leukemia (HL-60), pulmonary adenocarcinoma (PC-14), gastric adenocarcinoma (MKN-45), hepatoma (HepG2), and CDDP-resistant PC/DDP cell lines[15]. Moreover, it was found that 1H901 has antiplatelet aggregation[15], antiangiogenic[16], antigenotoxic and antiinvasive activities[18]. In case of *in vivo* study on 1H901, both antimetastatic activities on Lewis lung carcinoma[15] and colon 26 cell lines and anticarcinogenic effects in ginseng-hydrolyzing bacteria-colonized mice against 1,2-dimethylhydrazine administration[19] were confirmed.

Phospholipid hydroperoxide glutathione peroxidase (PHGPx), known as glutathione peroxidase 4 (GPx4), is an unique intracellular antioxidant enzyme that protects

cells from lipid peroxidation-mediated damage by catalyzing the reduction of lipid peroxides[20-21]. Jolti *et al.*[22] reported that membrane PHGPx might be involved in the cellular mechanisms of adaptation in the heart to the toxic effects of doxorubicin. It was demonstrated that protopanaxadiol ginsenosides Rb1 and Rc enhance glutathione peroxidase activity, and that Rb1 induces superoxide dismutase 1 gene expression. Although IH901, as an active principle of protopanaxadiol ginsenoside, is expected to have an antioxidant activity, it is not yet clarified[23].

In this context, the efficacy of IH901, in combination with doxorubicin, was evaluated on the bases of both tumor-suppressive and side-effect-reducing effects. In the present study, IH901 was coadministered with doxorubicin to check wether it potentiates the antitumor effect and lowers cardiotoxicity of doxorubicin in S-180 tumor-bearing mice.

Materials and Methods

Animals

Male ICR mice (6 weeks old) were obtained from Samtako Co. (Osan, Korea), housed in polycarbonate cages, and used 1 week after acclimation to an environmentally controlled room with temperature 23±2℃, relative humidity 50±10%, frequent ventilation, and 12-hr light cycle. The animals were fed with commercial pellet feed (Samyang Co., Korea) and tap water *ad libitum* throughout the experimental period.

Materials

Doxorubicin hydrochloride was obtained from Ildong Co. (Seoul, Korea), and IH901 (Fig. 1) was a generous gift from Central Research Institute, Ilhwa Co. (Seoul, Korea). Doxorubicin and IH901 were dissolved in sterile physiological saline and Cremophor RH40 immediately before use, respectively.

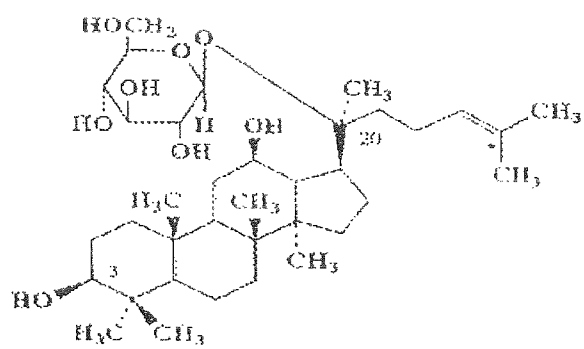

Fig. 1. Chemical structure of IH901.

Experiment 1 (antitumor activity)

The sarcoma-180 (S-180) cell line was maintained by intraperitoneal passage at weekly intervals in mice. For the induction of ascitic tumors, the passaged S-180 cells, 1×10^5 cells/mouse in 0.2 ml RPMI 1640, were inoculated intraperitoneally on day 0. IH901 (50 mg/kg), alone or in combination with doxorubicin (3 mg/kg), was administered into the tumor-bearing mice in a volume of 10 ml/kg. IH901 was orally administered everyday for 28 days 2 hr prior to intraperitoneal administration of doxorubicin on days 5, 12, 19 and 24. The antitumor effect was defined as the percent increase in lifespan (%ILS) calculated according to the following equation[24,25];

$$\%ILS = [(T-C)/C]\times100\%$$

where T and C represent the mean survival days of the treated and control groups, respectively.

Experiment 2 (cardioprotective activity)

Mice were transplanted with S-180 cells as described in antitumor assay. IH901 (50 mg/kg), alone or in combination with doxorubicin (5 mg/kg), was administered into the tumor-bearing mice in a volume of 10 ml/kg. IH901 was orally administered everyday for 14 days 2 hr prior to intraperotoneal injection of doxorubicin on days 4 and 9. At the end of 2-week treatment, mice were sacrificed for the histopathological examination and reverse transcription-polymerase chain reaction (RT-PCR) analysis of PHGPx mRNA expression in the heart.

Light and electron microscopic examination : The heart removed was fixed in 10% formalin solution. For the evaluation of cardiomuscular injuries, paraplast-embedded tissue sections (4 μm in thickness) were stained with hematoxylin and eosin, and examined under a light microscope.

To examine ultrastructural lesions, a piece of cardiac tissues was fixed in 2.5% glutaraldehyde solution followed by post-fixation in 1% osmium tetroxide solution. Epon-embedded thin sections (40 nm in thickness) were stained with uranyl acetate and lead nitrate, and examined under a transmission electron microscope.

Total RNA isolation and RT-PCR analysis : Total RNA was isolated from the heart using TRIzol® reagent (GIBCO BRL, New York, USA) according to the manufacturer's instructions. Quantity and quality of the total

RNA isolated were determined by a spectrophotometer at 260 nm and also by ultraviolet light visualization on agarose gel stained with ethidium bromide.

The first strand cDNA was synthesized using a power cDNA synthesis kit (Intron Co., Korea). After incubation of RNA with Oligo(dT)$_{12-18}$ primer, enzyme mixture composed of reverse transcription buffer, dithiothreitol, RNase inhibitor, dNTP, and AMV reverse transcriptase was added. Following 60-min incubation at 45°C, the reaction was terminated by increasing temperature to 70°C.

To investigate the gene expression patterns of PHGPx, cDNA was amplified by a thermal cycler (MJ Research, USA) using specific primers (sense primer, 5'-ATGCAC-GAATTCTCAGCCAAG-3'; antisense primer, 5'-GGCAGGCT-TCCCTCTAT-3')[26]. The RT-PCR mixture was made as following; iTaq DNA polymerase, dNTP mixture, PCR buffer containing $MgCl_2$, each sense or antisense primer, and cDNA in ultradistilled water. PCR amplification was carried out in the thermal cycler using a protocol of denaturing, anealing, and extension. The PCR products were run on a 1.5% agarose gel, using β-actin primer as a reference control. The optical densities of PHGPx mRNA from each group were analyzed with AlphaEase Image software (Alpha Innotech Co., USA) and expressed as mean value.

Statistical analysis

Results were presented as mean±S.D. and the statistical significance of difference was evaluated by Student's *t*-test.

Table 1. Effects of doxorubicin (3 mg/kg), IH901 (50 mg/kg) or their combination on the lifespan of S-180 tumor-bearing mice

Treatment	Lifespan	
	Mean (day)	%ILS
S-180 alone	17.3±0.95	0.0
S-180 + doxorubicin	34.9±8.40*	101.7
S-180 + IH901	17.3±2.41	0.0
S-180 + doxorubicin + IH901	39.7±10.86*	129.3

*Significantly different from S-180 alone ($p < 0.05$)

Results

Antitumor activity

The mice treated with vehicle or IH901 alone showed steep ascent in their body weights from days 9-11 following S-180 tumor inoculation (Fig. 2). In contrast, such an increase in body weights of mice induced by S-180 was markedly attenuated by doxorubicin (3 mg/kg) administration on days 5, 12, 19 and 24 after tumor transplantation. Interestingly, the body weight increase was further suppressed by the combination of IH901 (50 mg/kg), leading to a normal profile.

S-180 tumor-bearing mice treated with vehicle survived only for 17.3±0.95 days (Table 1). Also, the mice treated daily with IH901 alone after tumor inoculation, did not survive longer than the animals received vehicle alone. In contrast, the survival time of mice treated with

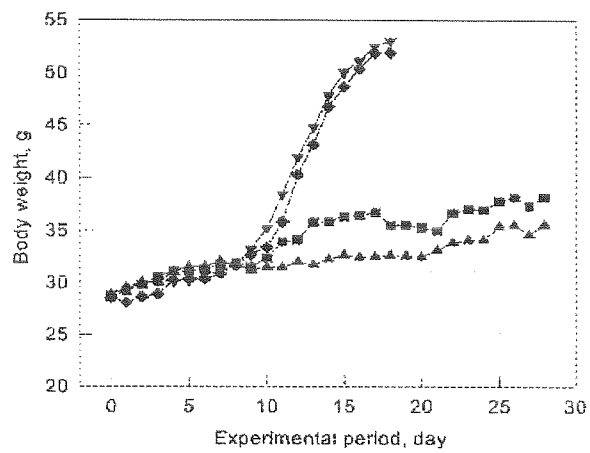

Fig. 2. Effects of doxorubicin (3 mg/kg), IH901 (50 mg/kg) or their combination on the change in mean body weights of S-180 tumor-bearing mice. ▼, S-180 alone; ■, S-180+doxorubicin; ◆, S-180+IH901; ▲, S-180+doxorubicin+IH901.

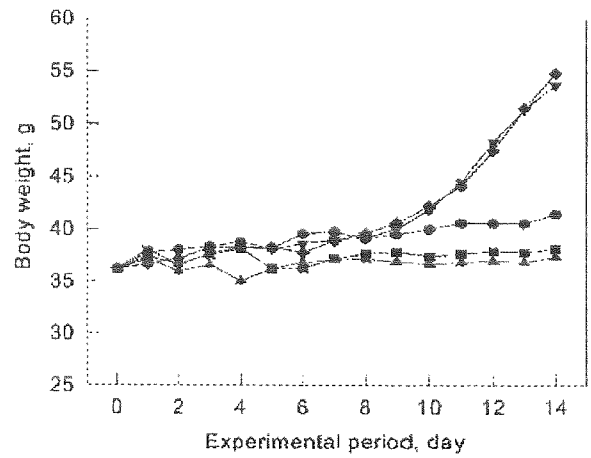

Fig. 3. Effects of doxorubicin (5 mg/kg), IH901 (50 mg/kg) or their combination on the change in mean body weights of S-180 tumor-bearing mice. ●, Normal control; ▼, S-180 alone; ■, S-180 + doxorubicin; ◆, S-180 + IH901; ▲, S-180 + doxorubicin + IH901.

Table 2. Absolute and relative liver weights of mice treated with doxorubicin (5 mg/kg) and/or IH901 (50 mg/kg)

Treatment	Normal	S-180 alone	S-180 + doxorubicin	S-180 + IH901	S-180 + doxorubicin + IH901
Absolute liver weight (g)	0.226±0.033	0.170±0.083	0.168±0.037	0.184±0.034	0.206±0.020[c]
Relative liver weight (%)	0.549±0.076	0.320±0.045[a]	0.440±0.088[b]	0.345±0.095	0.550±0.032[bc]

[a]Significantly different from normal control ($p < 0.05$).
[b]Significantly different from S-180 alone ($p < 0.05$).
[c]Significantly different from S-180 + doxorubicin ($p < 0.05$).

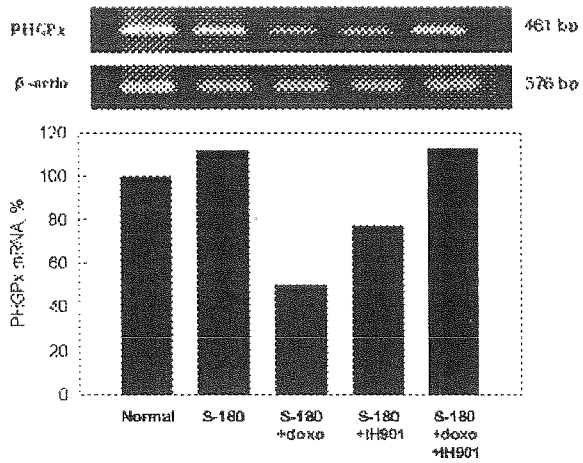

Fig. 4. Reverse transcription-polymerase chain reaction (RT-PCR) analysis for phospholipid hydroperoxide glutathione peroxidase (PHGPx) mRNA expression in the heart of S-180 tumor-transplanted mice treated with doxorubicin (doxo, 5 mg/kg) and/or IH901 (50 mg/kg); upper, The 461 and 376 bp fragments represent PHGPx transcript and β-actin, an internal standard, respectively; lower, Relative levels of PHGPx mRNA expression was presented compared to the values for β-actin mRNA.

doxorubicin was extended to 34.9±8.40 days in spite of withdrawal of the drug on day 25, resulting in 101.7 of %ILS. Moreover, the effect of doxorubicin was remarkably enhanced by coadministration with IH901, leading to 39.7±10.86 days of survival time and 129.3 of %ILS.

Cardioprotective activity

All the animals survived throughout the experimental period of 14 days (Fig. 3). The body weights of S-180 tumor-bearing mice treated with IH901 (50 mg/kg) or its vehicle alone greatly increased, above those of normal tumor-free rats, from 10 days following tumor inoculation. Such an increase in body weights induced by tumor growth was completely blocked by administration with doxorubicin (5 mg/kg) on days 5 and 9, leading to oversuppression below the normal body weight gain. No further suppression of body weights was induced by coadministration of IH901.

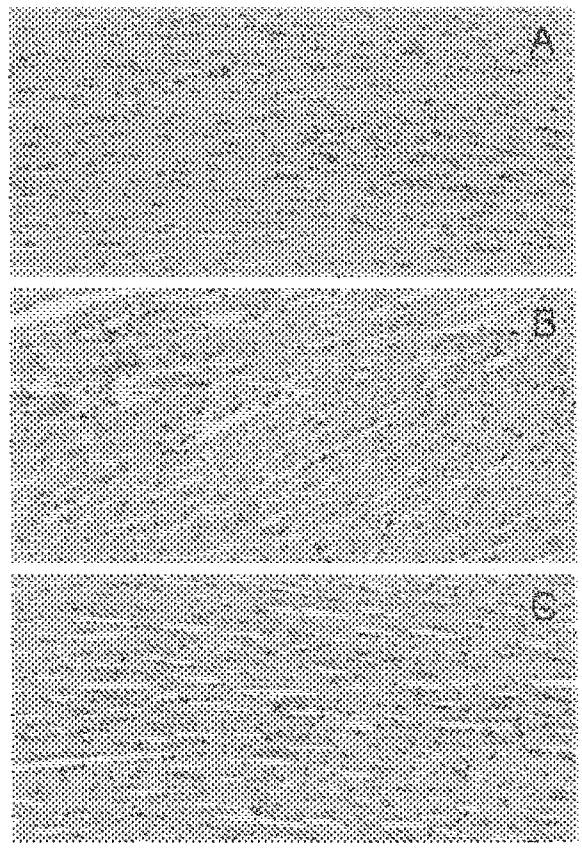

Fig. 5. Representative longitudinal sections of cardiac muscles. A, normal; B, S-180 + doxorubicin (5 mg/kg); C, S-180 + doxorubicin (5 mg/kg) + IH901 (50 mg/kg). Note cytoplasmic vacuolization and loss of myofibrils in B, in comparison with mild lesions in C and normal features in A. Hematoxylin & eosin, ×200.

The absolute heart weights of tumor-bearing mice reduced to 80% of that of normal animals, implying a cardiac atrophy (Table 2). However, such a decrease in heart weights was reversed by the combinational treatment of IH901 with doxorubicin. In comparison, a marked decrease in relative heart weights caused by tumor inoculation was attenuated by doxorubicin, but not by IH901,

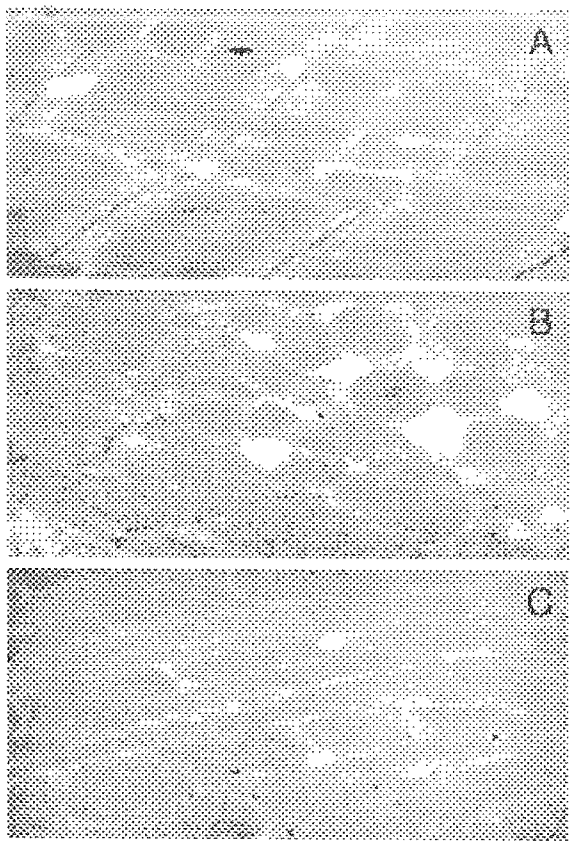

Fig. 6. Representative cardiac muscle cells. A, normal; B, S-180 + doxorubicin (5 mg/kg); C, S-180 + doxorubicin (5 mg/kg) + 1H901 (50 mg/kg). Note swollen mitochondria and loss of myofibrils and intercalated disc in B, in comparison with mild lesions in C and normal features in A. Uranyl acetate & lead nitrate, ×15,000.

which might be due to an effect on body weight changes. However, it is of interest to note that the combination of 1H901 and doxorubicin further recovered the relative heart weights to normal level, instead of similar body weights to those of rats given doxorubicin alone.

The PHGPx mRNA level analyzed in the heart after the termination of the experiment, using β-actin mRNA level as an internal standard, greatly decreased following doxorubicin treatment (Fig. 4). Interestingly, however, such a reduction was fully recovered by coadministration of 1H901, although 1H901 alone somewhat suppressed the mRNA expression.

Light microscopic examination showed moderate cytoplasmic vacuolization and loss of myofibrils in the heart of mice treated with doxorubicin (Fig. 5B). However, 1H901 remarkably attenuated the doxorbicin-induced myocardial lesions, especially the focal degeneration and disorganization of myofibrils (Fig. 5C). Separately, the heart tissue of rats administered with 1H901 alone did not show any specific lesions (data not shown), similar to the features of normal liver (Fig. 5A).

In the electronic microscopic examination of the heart tissues, doxorubicin treated rats showed swollen mitochondria, and loss of myofibrils and intercalated disc (Fig. 6B). Meanwhile, 1H901 markedly attenuated the myocardial lesions induced by doxorubicin, leading to normal features (Fig. 6C). In addition, 1H901 alone did not cause any specific lesions in the heart (data not shown), as shown in normal animals (Fig. 6A).

Discussion

In cancer treatment, chemotherapy is still one of the most promising measures because chemotherapeutic agents show superior efficacy to surgery and radiotherapy. In spite of the efficacy, there are serious problems using the agents in terms of side effects and the appearance of resistant cells[23]; that is, one of the major limiting factors in chemotherapy is narrow therapeutic window to kill neoplastic cells without influencing normal cells[2], resulting in serious harmful effects in patients. Thus, in addition to careful dosing, strategy to diminish the side effects of anticancer drugs with preservation of their efficacy are necessary[27].

In order to overcome the multidrug resistance and dose limitation, combinational and alternative therapies have been applied as one of the considerable approaches in treating cancers[27,28]. Combinational therapy usually utilizes diverse cytotoxic agents which are different in action mechanism or adopt specific dosing schedule to reduce side effects and to achieve a synergistic efficacy. However, there is a possibility that the combination of chemotherapeutic agents rather substantially increase the toxicity[27,29]. Pierga *et al.*[30] tried to evaluate the combinational efficacy of doxorubicin with etoposide, 5-fluorouracil or cisplatin. Although combination of the chemotherapeutic agents was effective in metastatic endometrial carcinoma, the enhanced toxicity was unacceptable.

In this context, diverse combinations of a chemotherapeutic agent with alternative or adjunctive agents have been studied[31]. Singal *et al.*[7] proposed that such combinations could be successful to broaden the antineoplastic spectrum as a strategy for the prevention of doxorubicin-induced cardiotoxicity. Alternative therapies include agents mainly derived from plants or animals. Among them,

herbal products represent a specialized subset of alternative therapies, since many people have traditionally experienced diverse plants and their extracts[27]. For example, *Astragalus*, *Essiac*, ginseng, green tea, garlic, and *Iscador* have commonly been used in cancer prevention and treatment[32].

The present study was designed to explore the possible synergistic application of doxorubicin in combination with IH901, a major intestinal metabolite formed from ginseng panaxadiol saponins. *In vivo* antitumor assay, doxorubicin successfully suppressed the increase in body weights following ascitic tumor growth, and thereby prolonged the survival time of mice, showing 101.7% increase in lifespan. Interestingly, synergistic antitumor effects were achieved by combination of IH901 with doxorubicin, leading to further suppression of body weight gain and prolongation of lifespan (129.3% ILS).

Meanwhile, IH901 alone neither suppressed tumor growth nor increased the life span in ascitic tumor-bearing mice. Whereas, it has been reported that IH901 exerted *in vivo* antineoplastic activities on Lewis lung carcinoma[15] and colon 26 cell lines[19], and cytotoxicity in human myeloid leukemia (HL-60), pulmonary adenocarcinoma (PC-14), gastric adenocarcinoma (MKN-45), hepatoma (HepG2) and CDDP-resistant PC/DDP cell lines[13,15]. Moreover, it was demonstrated that IH901 induced apoptosis of HL-60 cells through activation of caspase-3 protease, which occurred via mitochondrial cytochrome c release independently of bcl-2 modulation[17]. In the present study, the results suggest that the *in vivo* antitumor activity of IH901 was not caused by direct cytotoxicity to S-180 ascitic tumor cells.

Next, we tried to clarify whether IH901 attenuates cardiotoxicity induced by doxorubicin. Morphologically, the most characteristic ultrastructural features of doxorubicin-induced cardiomyopathy include loss of myofibrils, dilatation of sarcoplasmic reticulum, cytoplasmic vacuolization, swelling of mitochondria, and increased number of lysosomes. These structural alterations have been noticed in a variety of experiment animal models such as rabbits, mice and rats[6,33]. In the present study, pathological evidence of myocardial damage was also observed in S-180-transplanted mice treated with a relatively high dose (5 mg/kg) of doxorubicin. The changes included disruption of several subcellular elements, such as mitochondrial swelling, disorganization of myofibrils, loss of intercalated disc, and cytoplasmic vacuolization. Interestingly, such lesions were greatly attenuated by IH901.

As an action mechanism, it is well known that doxorubicin induces lipid peroxidation resulting from ROS generated via redox cycling[34]. Sequentially, secondary products such as hydroxy fatty acids produced during peroxidation of polyunsaturated fatty acids are highly cytotoxic. They react with different kinds of biomolecules, and thereby induces cell membrane injuries, leading to fatty acid decomposition[35]. Futhermore, the ROS induces rapid depletion of antioxidant enzymes in the heart possessing relatively low levels of antioxidant defense system. Therefore, the heart is the most sensitive target organ of anthracycline anticancer agents including doxorubicin.

PHGPx is a selenium-containing antioxidant that interacts directly with peroxidized phospholipids and cholesterol in biomembranes[21]. Accordingly, PHGPx are primarily depleted under oxidative stresses during body-protective action. In our RT-PCR analysis, doxorubicin decreased the PHGPx mRNA expression in the heart of S-180 tumor-bearing mice. However, IH901 reversed the PHGPx expression to the control level. Kang *et al.*[36] suggested that doxorubicin directly affect spermatogenic cells by triggering the reduction of PHGPx mRNA level in the testes, and that ginseng intestinal metabolite-1 may enhance the transcription of PHGPx, and thereby protect the spermatogenic cells against oxidative damages. However, it is unlikely that IH901 exert cardioprotective activity through same mechanism, since IH901 alone rather reduced the PHGPx expression. Therefore, it is suggested that IH901 might have another action mechanism such as its antioxidant function, displaying activity under oxidative stresses.

To date, there have been a number of studies that reported attenuation of doxorubicin toxicity using various natural and artificial compounds such as vitamin E[37], ascorbic acid[38], reduced glutathione[39], SOD[40] and an iron chelator ICRF-187[41]. However, most of the antioxidants and iron chelators failed to show substantial protection against doxorubicin-induced cardiomyopathy, and it still remains whether their combination maintains or increases the antitumor activity of doxorubicin in tumor-bearing animals. While IH901 did not show an antitumor property in S-180 tumor-bearing mice, its combination with doxorubicin showed noticeable enhancement of antitumor efficacy as well as reversal of cardiotoxic adverse effects.

Based on the results, it is believed that IH901 exert antitumor-enhancing effects by not suppressing tumor growth, but protecting body from oxidative damages induced by doxorubicin. Although the exact action mech-

anisms remained to be clarified, IH901 was found to be a potential candidate as an adjunct to chemotherapeutic agents.

Acknowledgement

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