

## Effects of the Licorice Extract against Tumor Growth and Cisplatin-Induced Toxicity in a Mouse Xenograft Model of Colon Cancer

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**Cisplatin is one of the most effective chemotherapeutic agents and plays a major role in the treatment of a variety of human solid tumors. However, its toxicity limits the clinical use. Recently, the administration of antioxidants has been suggested to protect against cisplatin-induced nephrotoxicity. The present study was designed to estimate the antitumor activity of the licorice extract alone and in combination with cisplatin, and its protective potential against cisplatin-induced toxicity in a mouse xenograft model. The administration of the licorice extract significantly inhibited tumor growth in BALB/C mice inoculated with CT-26 colon cancer cells. The combination of the licorice extract and cisplatin diminished the therapeutic efficacy of cisplatin but promoted considerably antitumor activity of the licorice extract. In mice with cisplatin treatment for 15 d, the serum levels of blood urea nitrogen and creatinine remarkably were increased by kidney damage, and the serum alanine aminotransferase and aspartate aminotransferase levels were elevated by liver damage. The administration of the licorice extract plus cisplatin recovered these functional indices in the kidney and liver to almost the control levels. In addition, the administration of the licorice extract significantly reduced the cisplatin-induced oxidative stress. Taken together, the administration of the licorice extract inhibits the growth of mouse colon carcinoma without any adverse effects, and reduces the cisplatin-induced toxicity. Therefore, the licorice extract may be a candidate for an anticancer and chemopreventive agent. However, cancer patients with cisplatin therapy should avoid the supplementation of the licorice extract.**

**Key words** licorice extract; antitumor activity; cisplatin; nephrotoxicity; hepatotoxicity; oxidative stress

Cisplatin (*cis*-diamminedichloroplatinum II) is a platinum coordinated complex that is widely used as an antineoplastic agent for the treatment of many solid tumors, including cancer of the ovary, testis, lung, bladder, head and neck, cervix and endometrium.<sup>1)</sup> Despite its excellent anticancer activity, the clinical use of cisplatin is often limited by its undesirable side effects, such as severe nephrotoxicity and hepatotoxicity.<sup>2,3)</sup> Although the precise mechanism for this cisplatin-induced toxicity is not well understood, cisplatin is taken up preferentially and accumulates in the human liver and kidney cells,<sup>4)</sup> resulting in the enhanced production of reactive oxygen species (ROS) and the decrease in the antioxidant enzymes.<sup>5,6)</sup> Therefore, antioxidants have been administered before a cisplatin treatment to protect against nephrotoxicity.<sup>7)</sup>

Licorice is an esteemed crude drug in both the Orient and Occident that is originated from the dried roots of several *Glycyrrhiza* species, including *Glycyrrhiza uralensis* FISCHER, *G. glabra* LINNE and *G. inflata* BATALIN.<sup>8,9)</sup> In Chinese traditional medicine, licorice remains one of the most commonly prescribed herbs and has been used in the treatment of various ailments ranging from tuberculosis to peptic ulcers.<sup>10)</sup> Licorice has also been employed as a flavoring and sweetening agent, as well as a demulcent and expectorant in Western countries.<sup>11)</sup> The chemical constituents of licorice include glycyrrhizin and its aglycone, glycyrrhetic acid, which are originally isolated from aqueous extracts and are used in the treatment of hyperlipemia, atherosclerosis, viral diseases, allergic inflammation and hepatotoxicity.<sup>12,13)</sup> The acetone or ethanol extract of licorice has species-specific

flavonoids, such as liquiritin, isoliquiritin and their corresponding aglycones, glabridin, glabrol, glabrene, hispaglabridin A and hispaglabridin B.<sup>14,15)</sup> These flavonoids exhibit antioxidative,<sup>16)</sup> superoxide scavenging<sup>16)</sup> and anticarcinogenic activities.<sup>17)</sup> However, *in vivo* antitumor activity of a licorice extract and its protective activity on cisplatin-induced toxicity have not yet been studied.

In the present study to assess the antitumor activity of a licorice extract and whether it has the potential to serve as a beneficial supplement during cisplatin chemotherapy, we examined the inhibitory effect of the licorice extract alone and in combination with cisplatin on the tumor growth, and its protective effect against cisplatin-induced nephrotoxicity and hepatotoxicity in mice xenografted with mouse colon carcinoma cells.

### MATERIALS AND METHODS

**Chemical** Cisplatin, phenylmethylsulfonyl fluoride (PMSF), sodium nitrite, 1,1,3,3-tetramethoxypropane, reduced glutathione (GSH), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), glutathione reductase, reduced nicotinamide adenine dinucleotide phosphate (NADPH), superoxide dismutase (SOD), xanthine oxidase,  $\alpha$ -naphthylamine, sulfanilic acid, xanthine, catalase and 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (purpald<sup>®</sup>) were purchased from Sigma-Aldrich Chemical. The Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA and phosphate-buffered saline (PBS) were obtained from Gibco BRL. All the reagents were of analytical grade.

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### Plant Material and Preparation of the Licorice Extract

The licorice roots (*G. inflata* BATALIN) were purchased from the herbal store in Chunchon, Korea. The authenticity of the plant was confirmed by Emeritus Prof. H. J. Chi, College of Pharmacy, Seoul National University. The botanical origin was confirmed by the presence of high concentration of licochalcone A, a species-specific ingredient of *G. inflata* in HPLC-profile.<sup>8,9</sup> A voucher specimen (060530LE) has been deposited in the Plant Extract Bank, Regional Innovation Center. The licorice slices were ground into a fine powder in a blender (Waring Model 51BL30). The powder (100 g) was then refluxed in 500 ml of 95% ethanol for 3 h to obtain the ethanol extract of licorice. This extraction procedure was repeated 3 times. The solvent was evaporated completely at 50 °C with rotary vacuum evaporator and the extract was freeze-dried. The dried extract was stored at -20 °C until the following experiment.

**Animal** The BALB/c male mice (30±5 g, 5 weeks of age) were purchased from the Central Lab Animal Inc. (Seoul, Korea), and were fed with free access to standard chow diet (Daejong Inc., Seoul, Korea) and water *ad libitum*. All the mice were allowed 1 week to acclimatize before the experiments, and were kept at 25±2 °C, with a relative humidity of 55±5% and a 12 h light–dark cycle. The animal studies were performed in accordance with the experimental protocols of the animal ethics committee of Yonsei University College of Dentistry.

**In Vivo Xenograft Model** Mice were divided into the following 9 groups, each group containing 8 mice: PBS-treated group, CT-26 cell-inoculated group, CT-26 cell-inoculated group with the licorice extract [0.5, 1 and 2 mg/kg body weight (BW)], CT-26 cell-inoculated group with cisplatin, CT-26 cell-inoculated group with the licorice extract (0.5, 1 and 2 mg/kg BW) plus cisplatin. The CT-26 cells (2×10<sup>6</sup> cells in 0.1 ml PBS) cultured in 10% FBS-DMEM were injected subcutaneously into the right flank of the mice. Twenty-four hours later, the BALB/c mice were dosed with the licorice extract in PBS through an oral gavage. Two hours after treatment with the licorice extract, cisplatin (5 mg/kg BW) in PBS was injected intraperitoneally. The licorice extract and cisplatin were administered once daily over a 15 d period. The control group received PBS instead of the licorice extract and/or cisplatin. The tumor volume was measured biweekly using calipers and calculated using the following formula: (length×width<sup>2</sup>)/2. Sixteen hours after the final cisplatin injection, the mice were sacrificed under anesthesia and the individual body weights were measured. The liver and kidneys were excised immediately after blood had been collected from each mouse and weighed.

### Determination of the Serum Biochemical Parameters

The blood samples were left to stand at room temperature for 1 h, and then centrifuged at 3000 rpm for 10 min to obtain the serum. The serum blood urea nitrogen (BUN) and creatinine levels were measured as indicators of the kidney function. The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured to evaluate the liver function. All biochemical assays were performed spectrophotometrically, using commercially available kits (Asan Pharmaceutical, Seoul, Korea). The detection of the serum nitrite and nitrate levels as an index of NO production was based on the Griess reaction.<sup>18</sup> Total nitrite was measured at

540 nm after converting nitrate to nitrite. The nitrite concentration was calculated using a standard curve for sodium nitrite.

**Determination of Biochemical Parameters in the Homogenates from the Kidney and Liver Tissues** The kidney and liver tissues were perfused immediately with ice-cold saline (0.9% KCl) to remove the blood. The tissues were cut into small pieces with scissors on ice, and homogenized in 10 volumes of ice-cold 1.15% KCl buffer containing 0.4 mM PMSF. A part of the homogenate was centrifuged at 2000 rpm for 10 min at 4 °C for the determination of malondialdehyde (MDA) and GSH. MDA content was examined in the form of thiobarbituric acid reactive substances (TBARS) at 532 nm.<sup>19</sup> 1,1,3,3-Tetramethoxypropane was used to establish a standard curve, and the final MDA concentration is expressed as nmol MDA per mg tissue protein. GSH was estimated at 412 nm colorimetrically after its complex formation with DTNB.<sup>20</sup> The results were expressed as nmole per mg tissue protein.

The remaining homogenate was centrifuged at 4000 rpm for 30 min at 4 °C for the assay of antioxidant enzymes. Glutathione peroxidase (GSH-Px) activity was determined spectrophotometrically by measuring the oxidized glutathione formed in the glutathione peroxidase reaction by coupling it to the oxidation of NADPH *via* glutathione reductase.<sup>21</sup> GSH-Px activity was expressed in μmol NADP<sup>+</sup> per mg tissue protein. SOD activity was determined according to the method of Elstner and Heupel.<sup>22</sup> The nitrite, resulted from oxidation of hydroxylammonium by superoxide anions generated in the xanthine–xanthine oxidase system, was determined spectrophotometrically by the reaction with sulfanilic acid and α-naphthylamine. SOD activity was expressed as units per mg tissue protein. The determination of catalase activity was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of hydrogen peroxide.<sup>23</sup> The formaldehyde produced was measured with Purpald as a chromogen at 540 nm. Catalase activity was expressed as units per mg tissue protein.

The total protein concentration was determined using a BCA protein assay kit (Pierce Biotechnology, Rockford, Illinois).

**Statistical Analysis** The results are expressed as the mean±S.E. All analyses were carried out using the SPSS statistical program. (version 10.0 software, SPSS Inc. Chicago, Illinois, U.S.A.). The effect of treatment was determined by analyzing the data using one-way ANOVA repeated measures. *p* values <0.05 were considered significant.

## RESULTS

### Inhibitory Effect of the Licorice Extract on the Growth of CT-26 Mouse Colon Carcinoma in Mice

In a xenograft model which murine colon carcinoma CT-26 cells had been inoculated into BALB/c mice, treatment with 0.5, 1 and 2 mg/kg of the licorice extract for 15 d inhibited tumor growth by 38%, 57% and 71%, respectively (Fig. 1). Administration of 5 mg/kg cisplatin also resulted in significant decrease of 92% in tumor growth. The therapeutic efficacy of cisplatin was decreased in the mice receiving the licorice extract plus cisplatin, but treatment with 2.0 mg/kg of the licorice extract did not result in a statistically significant de-

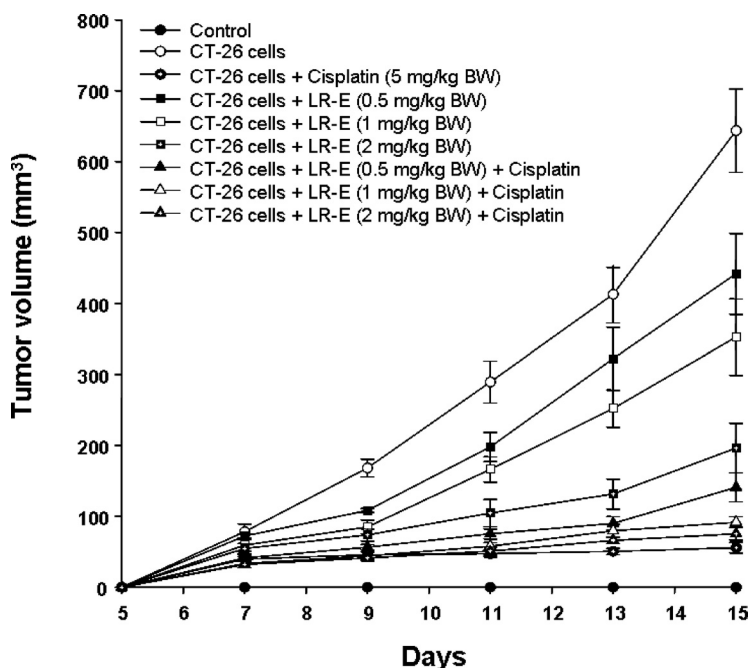


Fig. 1. Inhibitory Effect of the Licorice Extract (LR-E) Alone and in Combination with Cisplatin on the Growth of CT-26 Colon Carcinoma Cells Inoculated into the BALB/c Mice

Table 1. Effect of the Licorice Extract (LR-E) on the Cisplatin-Induced Nephrotoxicity

Groups	LR-E (mg/kg BW)	Kidney weight (%) <sup>a)</sup>	BUN (mg/dl)	Creatinine (mg/dl)
Control		1.8±0.1	15.6±0.4	1.2±0.04
CT-26 cells		1.8±0.1	17.2±0.4	1.3±0.1
CT-26 cells+LR-E	0.5	1.9±0.04	14.9±0.4	1.3±0.1
CT-26 cells+LR-E	1	1.8±0.1	14.8±0.3	1.3±0.02
CT-26 cells+LR-E	2	1.9±0.1	15.5±0.4	1.5±0.1
CT-26 cells+cisplatin		1.5±0.02 <sup>#</sup>	92.3±3.0 <sup>##</sup>	4.4±0.1 <sup>###</sup>
CT-26 cells+LR-E+cisplatin	0.5	1.6±0.04	77.0±1.7 <sup>**</sup>	3.9±0.1 <sup>*</sup>
CT-26 cells+LR-E+cisplatin	1	1.6±0.03	55.4±1.7 <sup>***</sup>	3.1±0.1 <sup>***</sup>
CT-26 cells+LR-E+cisplatin	2	1.7±0.03 <sup>**</sup>	33.8±1.9 <sup>***</sup>	2.4±0.1 <sup>***</sup>

Values are expressed as mean±S.E. (n=8). <sup>a)</sup> The kidney weight as a percentage of the total body weight. <sup>#</sup>p<0.005, <sup>##</sup>p<0.001, significantly different from the CT-26 cell-inoculated group. \*p<0.05, \*\*p<0.005, \*\*\*p<0.001, significantly different from the CT-26 cell-inoculated group with only cisplatin treatment.

Table 2. Effect of the Licorice Extract (LR-E) on the Cisplatin-Induced Hepatotoxicity

Groups	LR-E (mg/kg BW)	Liver weight (%) <sup>a)</sup>	AST (U/l)	ALT (U/l)
Control		6.1±0.02	12.8±0.5	31.8±1.1
CT-26 cells		6.3±0.02	12.6±0.5	30.7±0.4
CT-26 cells+LR-E	0.5	6.2±0.01	13.9±1.3	33.7±1.3
CT-26 cells+LR-E	1	6.2±0.1	13.4±1.0	34.0±0.9
CT-26 cells+LR-E	2	6.1±0.02	13.8±1.1	32.5±0.9
CT-26 cells+cisplatin		4.1±0.001 <sup>#</sup>	127.9±6.8 <sup>#</sup>	185.3±7.4 <sup>#</sup>
CT-26 cells+LR-E+cisplatin	0.5	4.2±0.002	103.5±3.9 <sup>*</sup>	160.4±3.9 <sup>*</sup>
CT-26 cells+LR-E+cisplatin	1	5.1±0.002 <sup>*</sup>	72.2±2.3 <sup>**</sup>	123.9±5.1 <sup>**</sup>
CT-26 cells+LR-E+cisplatin	2	5.7±0.001 <sup>*</sup>	47.1±3.7 <sup>**</sup>	81.5±4.1 <sup>**</sup>

Values are expressed as mean±S.E. (n=8). <sup>a)</sup> The liver weight as a percentage of the total body weight. <sup>#</sup>p<0.001, significantly different from the CT-26 cell-inoculated group. \*p<0.05, \*\*p<0.001, significantly different from the CT-26 cell-inoculated group with only cisplatin treatment.

crease in the efficacy of cisplatin.

**Protective Activity of the Licorice Extract on Cisplatin-Induced Nephrotoxicity** The protective effect of the licorice extract against cisplatin-induced nephrotoxicity was assessed by measuring the kidney weight as a percentage of the total body weight, and the levels of BUN and creatinine in the serum of mice in the xenograft model (Table 1). The

mice treated with the licorice extract alone for 15 d did not show any kidney dysfunction. In contrast, cisplatin administration for 15 d caused the significant damage to the renal function in the mouse xenograft model, which was characterized by a decrease in the kidney weight and increases in the serum BUN and creatinine levels. Treatment with the licorice extract plus cisplatin for 15 d remarkably reversed dose-de-

Table 3. Effect of the Licorice Extract (LR-E) on NO Level in Serum and MDA and GSH Levels in the Kidney and Liver Tissues of the Cisplatin-Treated Mice

Groups	LR-E (mg/kg BW)	NO in serum ( $\mu\text{M}$ )	Kidney		Liver	
			MDA (nmol/mg)	GSH (nmol/mg)	MDA (nmol/mg)	GSH (nmol/mg)
Control		7.5 $\pm$ 1.2	12.9 $\pm$ 0.8	4.3 $\pm$ 0.5	10.0 $\pm$ 0.3	4.7 $\pm$ 0.1
CT-26 cells		8.8 $\pm$ 0.5	13.3 $\pm$ 0.4	5.1 $\pm$ 0.5	10.5 $\pm$ 0.9	4.1 $\pm$ 0.1
CT-26 cells+LR-E	0.5	8.6 $\pm$ 0.6	12.2 $\pm$ 1.3	4.3 $\pm$ 0.5	11.3 $\pm$ 0.2	4.0 $\pm$ 0.1
CT-26 cells+LR-E	1	8.4 $\pm$ 0.4	13.6 $\pm$ 0.8	4.1 $\pm$ 0.7	11.8 $\pm$ 0.2	4.0 $\pm$ 0.2
CT-26 cells+LR-E	2	7.4 $\pm$ 0.7	13.2 $\pm$ 0.6	4.1 $\pm$ 0.5	11.4 $\pm$ 0.6	4.1 $\pm$ 0.2
CT-26 cells+cisplatin		103.9 $\pm$ 7.5 <sup>#</sup>	58.2 $\pm$ 2.4 <sup>#</sup>	1.6 $\pm$ 0.1 <sup>#</sup>	44.2 $\pm$ 1.6 <sup>#</sup>	0.6 $\pm$ 0.1 <sup>#</sup>
CT-26 cells+LR-E+cisplatin	0.5	89.7 $\pm$ 5.9	48.7 $\pm$ 5.2	2.0 $\pm$ 0.3	36.8 $\pm$ 1.6*	0.7 $\pm$ 0.1
CT-26 cells+LR-E+cisplatin	1	75.4 $\pm$ 6.5*	33.6 $\pm$ 1.8**	2.6 $\pm$ 0.3*	24.3 $\pm$ 3.2**	1.4 $\pm$ 0.2**
CT-26 cells+LR-E+cisplatin	2	49.3 $\pm$ 2.9**	22.8 $\pm$ 1.0**	3.5 $\pm$ 0.1**	16.5 $\pm$ 0.8**	2.8 $\pm$ 0.4**

Values are expressed as mean $\pm$ S.E. ( $n=8$ ). <sup>#</sup> $p<0.001$ , significantly different from the CT-26 cell-inoculated group. \* $p<0.05$ , \*\* $p<0.005$ , significantly different from the CT-26 cell-inoculated group with only cisplatin treatment.

Table 4. Effect of the Licorice Extract (LR-E) on Antioxidant Enzymes in the Kidney and Liver Tissues of the Cisplatin-Treated Mice

Groups	LR-E (mg/kg BW)	Kidney			Liver		
		GSH-Px ( $\mu\text{mol/mg}$ protein)	SOD (Unit/mg protein)	Catalase (Unit/mg protein)	GSH-Px ( $\mu\text{mol/mg}$ protein)	SOD (Unit/mg protein)	Catalase (Unit/mg protein)
Control		53.5 $\pm$ 2.8	8.0 $\pm$ 0.3	18.2 $\pm$ 0.3	35.5 $\pm$ 1.2	7.2 $\pm$ 0.3	19.8 $\pm$ 0.5
CT-26 cells		55.4 $\pm$ 2.8	8.2 $\pm$ 0.2	18.9 $\pm$ 0.6	35.8 $\pm$ 0.2	7.5 $\pm$ 0.2	20.1 $\pm$ 0.3
CT-26 cells+LR-E	0.5	54.9 $\pm$ 1.2	8.0 $\pm$ 0.5	18.9 $\pm$ 0.4	33.8 $\pm$ 2.3	7.2 $\pm$ 0.4	19.6 $\pm$ 0.3
CT-26 cells+LR-E	1	53.5 $\pm$ 2.5	8.1 $\pm$ 1.1	18.4 $\pm$ 0.5	36.1 $\pm$ 2.4	7.4 $\pm$ 0.2	20.2 $\pm$ 0.4
CT-26 cells+LR-E	2	55.2 $\pm$ 0.8	8.0 $\pm$ 0.3	19.1 $\pm$ 0.5	35.4 $\pm$ 5.3	7.3 $\pm$ 0.5	19.8 $\pm$ 0.2
CT-26 cells $\pm$ cisplatin		16.5 $\pm$ 0.8 <sup>#</sup>	3.3 $\pm$ 0.3 <sup>#</sup>	6.0 $\pm$ 0.1 <sup>#</sup>	12.2 $\pm$ 0.5 <sup>#</sup>	2.7 $\pm$ 0.3 <sup>#</sup>	9.8 $\pm$ 0.4 <sup>#</sup>
CT-26 cells+LR-E+cisplatin	0.5	18.8 $\pm$ 0.6	3.7 $\pm$ 0.2	9.6 $\pm$ 0.3**	12.6 $\pm$ 0.7	2.9 $\pm$ 0.2	10.0 $\pm$ 0.7
CT-26 cells+LR-E+cisplatin	1	28.7 $\pm$ 0.5**	4.8 $\pm$ 0.6*	12.0 $\pm$ 0.1**	17.7 $\pm$ 1.4*	3.7 $\pm$ 0.2*	12.1 $\pm$ 0.8*
CT-26 cells+LR-E+cisplatin	2	41.3 $\pm$ 1.5**	6.7 $\pm$ 0.6**	15.3 $\pm$ 0.2**	26.5 $\pm$ 1.4**	5.0 $\pm$ 0.4**	14.3 $\pm$ 0.9**

Values are expressed as mean $\pm$ S.E. ( $n=8$ ). <sup>#</sup> $p<0.001$ , significantly different from the CT-26 cell-inoculated group. \* $p<0.05$ , \*\* $p<0.005$ , significantly different from the CT-26 cell-inoculated group with only cisplatin treatment.

pendently the cisplatin-induced decrease in the kidney weight and the cisplatin-induced increase in the serum BUN and creatinine levels.

**Protective Activity of the Licorice Extract on Cisplatin-Induced Hepatotoxicity** As shown in Table 2, oral administration of the licorice extract itself for 15 d did not affect the liver function, but cisplatin remarkably impaired the liver function in the mouse xenograft model. In all the mice that had received cisplatin, the liver weight was decreased as a percentage of the total body weight and the serum ALT and AST levels were increased. The licorice extract effectively blocked the cisplatin-induced changes in the liver weight and the serum ALT and AST levels in the mice treated with the licorice extract plus cisplatin for 15 d.

**Inhibitory Effect of the Licorice Extract on Cisplatin-Induced Oxidative Stress** Treatment with the licorice extract alone did not alter the serum nitrite level as an indicator of NO production, the tissue MDA level as a measure of lipid peroxidation and the tissue GSH level as the main determinant of the intracellular redox status, in the mice with colon cancer xenografts. The serum nitrite and tissue MDA levels were significantly higher in mice treated with cisplatin for 15 d in the xenograft model, whereas the tissue GSH levels had decreased considerably. The cisplatin-mediated increases in the serum nitric oxide and tissue MDA levels were prevented by the treatment with the licorice extract plus cis-

platin. The decrease in the tissue GSH levels by cisplatin was also recovered to almost the control levels (Table 3). Furthermore, the activities of GSH-Px, SOD and catalase were examined in kidney and liver tissues of mice treated with the licorice extract, cisplatin and their combination. Administration of the licorice extract together with cisplatin blocked the cisplatin-induced reduction in the activities of these antioxidant enzymes, as shown in Table 4.

## DISCUSSION

Oxidative stress and mitochondrial oxidative damages have been implicated in the etiology of several toxic effects causing by many anticancer drugs.<sup>24)</sup> Cisplatin is one of the most active cytotoxic agents in the treatment of cancer, and its therapeutic activity is dose dependent. However, its use is limited mainly by its nephrotoxicity.<sup>25)</sup> In addition, other less frequent toxic effects, such as hepatotoxicity, ototoxicity and neuropathy, can occur and adversely affect patients given high doses of cisplatin.<sup>26)</sup> Cisplatin induces mitochondrial dysfunctions, particularly inhibition of the electron transfer system, resulting in the enhanced production of superoxide anions, hydrogen peroxide and hydroxyl radicals.<sup>27)</sup> There are evidences suggesting that cisplatin-induced renal and liver damage is closely associated with the increase in lipid peroxidation and GSH depletion by these reactive oxygen species

(ROS), and the decrease in the antioxidant enzymes.<sup>5,6)</sup> In addition, recent studies suggest that dietary antioxidants and some foods, such as black grapes and *Brassica rapa*, can attenuate cisplatin-induced nephrotoxicity and hepatotoxicity.<sup>28,29)</sup> The present study was designed to estimate the protective potential of the licorice extract, including species-specific antioxidative flavonoids, against cisplatin-induced nephrotoxicity and hepatotoxicity and whether its use influences the therapeutic efficacy of cisplatin, as well as its antitumor activity in an animal model.

We first examined the effect of the licorice extract on the tumorigenicity and the therapeutic efficacy of cisplatin in CT-26 cells-inoculated BALB/c mice. The administration of the licorice extract significantly inhibited the growth of tumors. The combination of the licorice extract and cisplatin diminished the therapeutic efficacy of cisplatin but promoted considerably the antitumor activity of the licorice extract. The combination of the licorice extract at 2 mg/kg BW and cisplatin did not result in a statistically significant decrease in the inhibition of tumor growth as compared to cisplatin treatment.

Next, our results show that the repeated administration of cisplatin for 15 d in the xenograft model causes significant renal and liver dysfunction. The cisplatin-induced kidney damage was characterized by a decrease in kidney weight as a percentage of the total body weight and increases in the serum creatinine and BUN levels. The cisplatin-induced liver damage was estimated by a decrease in the liver weight as a percentage of the total body weight and increases in the serum ALT and AST levels. The administration of the licorice extract in combination with cisplatin repaired these functional indices in the kidney and liver to the control levels.

The effect of the licorice extract on cisplatin-induced oxidative stress was also evaluated by determining the level of lipid peroxidation, GSH depletion and the activities of antioxidant enzymes in the kidney and/or liver tissues, as well as the serum nitric oxide level. The results showed a significant increase in the serum nitric oxide and tissue MDA levels, whereas the tissue GSH content and antioxidant enzymes such as GSH-Px, SOD and catalase were remarkably declined in the mice given the consecutive treatment of cisplatin for 15 d. The balance between oxidants and antioxidants is critical for maintenance of the biological integrity of the tissues. The depletion of GSH and decreased activities of antioxidant enzymes is an early and key event during cisplatin-induced lipid peroxidation and subsequent toxicity. Cisplatin treatment also causes a significant increase in the activity of the calcium-independent nitric oxide synthase (NOS) in rat kidney and liver, leading to an increase in serum NO level as well as in tissue NO formation.<sup>30,31)</sup> Peroxynitrite, generated by the reaction between nitric oxide and superoxide anion, oxidizes biomolecules such as DNA, proteins and lipids. In the present study, the oral administration of the licorice extract in combination with the cisplatin treatment prevented the increases in the serum nitric oxide and tissue lipid peroxidation levels, and significantly recovered the tissue GSH level and antioxidant enzyme activities in the cisplatin-treated mice.

In conclusion, oral administration of the licorice extract significantly inhibited the growth of tumor without any ad-

verse effect in a xenograft model of CT-26 mouse colon carcinoma. The licorice extract revealed potent protective activity against the cisplatin-induced toxicity. Therefore, the licorice extract may be a candidate for an anticancer and chemopreventive agent. However, cancer patients with cisplatin therapy should avoid the supplementation of the licorice extract, and possibly even limit their exposure to licorice-containing foods because of the interference of the licorice extract in the therapeutic efficacy of cisplatin.

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