Protective Effect of Licorice Water Extract against Cadmium-induced Nephro-toxicity in Rats

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Licorice has been used for cure of injuries and for detoxification in East Asia. This study investigated the protective effect of licorice water extract against cadmium ($CdCl_2$, Cd)-induced nephro-toxicity in rats. To induce acute toxicity, Cd (4 mg/kg body weight) was dissolved in normal saline and then, intravenously (i.v.) injected to animals. In experiments, animals were orally administrated with vehicle or licorice water extract (50-100 mg/kg) for 3 days, exposed to a single injection of Cd after 24 h the last licorice/vehicle treatment. Licorice protected kidney injuries by Cd treatment. The number of glomeruli showing vasodilatation and thickening of Bowman's capsule was dose-dependently decreased by licorice. These results suggest that licorice might be a potent preventive protector against Cd-induced nephro-toxicity in rats.

Key words : licorice, cadmium, rat, kidney

Introduction

Licorice, Glycyrrhizae radix, is one of the oldest and most frequently used botanicals in the oriental medicine. Licorice is recommended for life-enhancing properties and cure of injury or swelling as well as for detoxification[1]. Studies have shown that licorice attenuated free radical induced-oxidative damage in the kidney[2], and prevented carcinogenesis induced by toxicants or hormones (eg N-methyl-N-nitrosourea or estradiol)[3]. Licorice contains flavonoids and pentacyclic triterpene saponin including liquiritigenin, liquiritin, isoliquiritigenin, liquiritin apioside, glycyrrhizin and glycyrrhizic acid[4]. Among the components, glycyrrhizin is the major constituent with a content variation from min. 4% to max. 13% the dried root weight[1]. Glycyrrhizin induces apoptosis in stomach cancer and promyelotic leukemia cell lines[5], and inhibits HIV replication in monocytes[6]. In addition, in a recent study we showed that Glycyrrhizae radix and its component, liquiritigenin, an aglycone of liquiritin, had cytoprotective effects against cadmium (Cd)-induced toxicity in cultured cells[7].

Cd, a heavy metal, is widely distributed in the environment due to its use in industry[8,9], and it is well known as a toxic substance due to organ toxicity[10] and long elimination half-life of 10-30 years[11]. Acute exposure of Cd causes dysuria, polyuria, chest pain, fatigue, and headache[12]. Chronic intake of Cd in contaminated food or air produces organ dysfunction as a result of cell death, resulting in pulmonary, hepatic and renal tubular diseases[13]. The pathogenesis of Cd-induced tissue lesions involves the turnover and necrosis of cells[14-16]. Morphologic changes induced by Cd exposure consist of cellular degeneration, inflammatory reaction and fibrosis[17]. Because Cd accumulates largely in the liver and kidney, it is well known as hepatic and renal toxicant.

Previously, We found that licorice protected cell death induced by Cd in H4IIE cells, which is the liver cell line of mouse[7].

In this study, we focused our attention on the effect of licorice in animal model system treated with Cd, not in cell line. We examined the protective effects of licorice pretreatment against Cd-induced nephro-toxicity in rats.

Materials and Methods

1. Preparation of licorice water extract

Licorice water extract was prepared by boiling 600 g of Glycyrrhizae radix (Wolsung, Daegu, Korea) in 5 L of water for 3 h, filtering through a 0.2 μm syringe filter (Nalgene, New York, NY, USA), and storing at -20℃ until use. The amount of licorice water extract was estimated by the dried weight of

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· Received : 2007/03/28 · Accepted : 2007/04/26

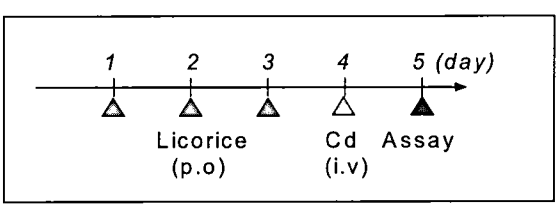
lyophilized water extract of Glycyrrhizae radix. The yield of lyophilized licorice from Glycyrrhizae radix was 13%.

2. Animals

Animal studies were conducted in accordance with the institutional guidelines for care and use of laboratory animals. Sprague - Dawley rats at 6 weeks of age (140 - 160 g) were provided from Hyochang Science. (Daegu, Korea), and acclimatized for 1 week. Animals were caged under the supply of filtered pathogen-free air, commercial rat chow (Purina, Korea) and water ad libitum at a temperature between 20 and 23℃ with 12 h light and dark cycles and relative humidity of 50%. To induce acute liver injury, Cd (4 mg/kg body weight) was dissolved in normal saline, and intravenously (i.v.) injected to animals. In another set of experiments, animals were orally administrated with licorice water extract (50, 100 mg/kg) for 3 days, exposed to a single injection of Cd after 24 h the last licorice/vehicle treatment. (Table 1, Scheme 1)

Table 1. Group ID used in this study

	Cadmium	Tap water	Licorice
Sham	-	-	-
Vehicle control	+	+	-
Lic I	+	-	+ (50 mg/kg)
Lic II	+	-	+ (100 mg/kg)



Scheme 1. Procedure of this study

3. Histology

Samples from kidney parenchyma was separated and fixed in 10% neutral buffered formalin (NBF), then embedded in paraffin, sectioned (3 ~ 4 μm) and stained with Hematoxylin & Eosin (H&E) stain.

4. Histomorphometrical Analysis.

The number of vasodilated glomerulus was detected as N/100 Glomeruli in kidney parenchyma using automated image analysis under magnification 200 microscopy (Zeiss, Germany) fields (n=6)

5. Statistical Analyses.

Numerical data was calculated as Mean ± S.D. (n=6). Statistical analyses were conducted using Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) with SPSS for Windows (Release 6.1.3., SPSS Inc., USA). In addition, % changes vs vehicle control or sham were detected followings.

% Changes vs vehicle control in cases of test groups (%) = [{(Data of test groups - Data of vehicle control)/Data of vehicle control} × 100]

% Changes vs sham in case of vehicle control (%) = [{(Data of vehicle control - Data of sham)/Data of sham} × 100]

Results

1. Effect on the weight of kidney

Normal rats (Sham) were all survived for the entire experiment period. Four of ten was killed in Cd control group. Rats in the licorice groups showed increased survival rates. The overall survival rates were 100%, 60%, 90% and 100% in sham, Cd control, Lic I and Lic II, respectively. So, we estimated the kidney weight of survival rats. The kidney weight ratio (kidney weight was divided by body weight) was not changed by Cd and/or licorice (Fig. 1).

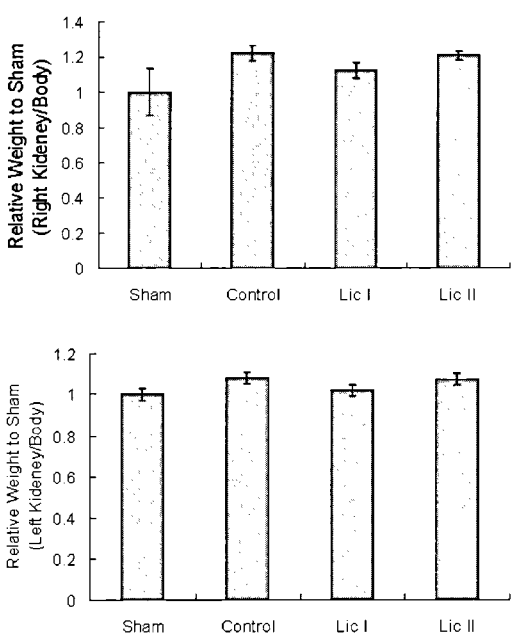


Fig. 1. Weight of kidney after Cd treatment with or without licorice. Weight was recorded 24 h after Cd exposure following the consecutive licorice pretreatment for 3 days. Ten rats per group were used at the beginning. Each kidney weight was divided by body weight measured before sacrifice. Values represent the mean ± SE from the surviving 10 rats in control, 6 rats in Cd alone, 9 rats in Lic I and 10 rats in Lic II groups. Group ID was listed in Table 1.

2. General histological profiles.

The histological profiles of kidney were shown in Fig. 2. Relative well developed Glomeruli, tubules and capsular regions were detected in Sham with normal histological profiles of medullary regions (Fig. 2a, b). However, numerous glomerulus showing vasodilated and thickening of Bowman's capsule so call Cd-intoxicated nephropathy were demonstrated

(Fig. 2c, d). However, these nephropathy induced by Cd were dramatically and dose-dependently decreased in licorice-dosing groups, respectively (Fig. 2e-h).

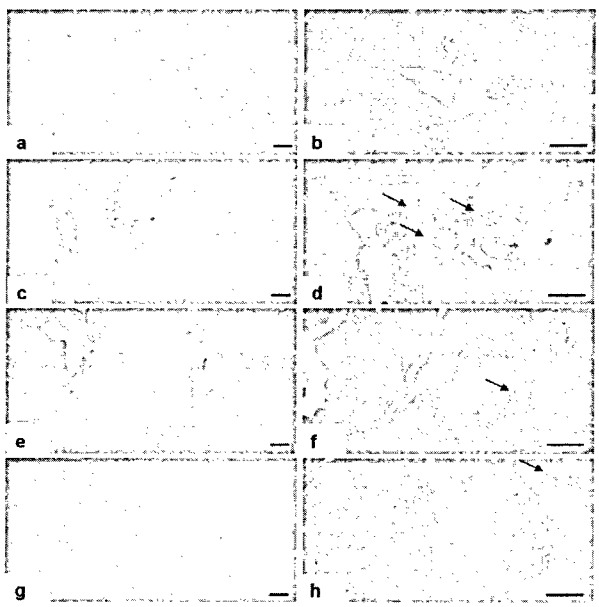


Fig. 2. Histological profiles of the kidney in rats pretreated with licorice prior to Cd exposure. The kidney sections from healthy control rats, sham (a,b), rats treated with Cd, vehicle control (c,d), Cd + licorice (50 mg/kg), Lic I (e,f), and Cd + licorice (100 mg/kg), Lic II (g,h) were stained with H&E (100 or 200). Note that vasodilated glomerulus (arrows) was increased in vehicle control but these Cd-intoxicated nephropathy were dramatically decreased in licorice-doing groups. Group ID was listed in Table 1. Scale bars=20 μm.

3. Number of vasodilated Glomerulus

The number of Glomeruli showing vasodilation was significantly ($p<0.01$) increase as 2658.33% in vehicle control compared to that of sham. However, significantly ($p<0.01$ or $p<0.05$) decreases of vasodilated Glomeruli are demonstrated in Lic I and Lic II compared to that of vehicle control, respectively (Fig. 3, Table 2). They showed clear dose-dependent patterns and they decreased as -41.39 and -56.80% compared to that of vehicle control, respectively.

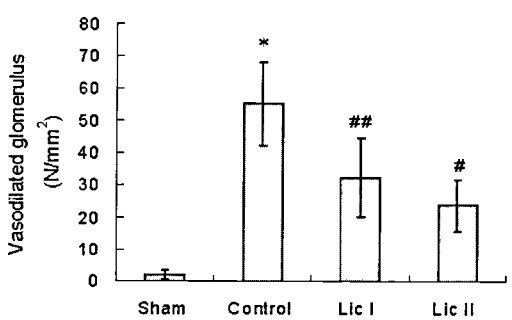


Fig. 3. Effects of licorice on the number of vasodilated glomerulus. The number of vasodilated glomerulus was detected as N/100 Glomeruli in kidney parenchyma. Values represent mean ± SE (n=6, significantly different from vehicle-treated control, *p<0.01; significantly different from Cd alone, #p<0.01, ##p<0.05).

Table 2. Changes on the histomorphometry analysis in kidney of Cd-intoxicated animals

Group	Vasodilated glomerulus (N/mm^2)	Chage of %
Sham	2.00 ± 1.55	-
Control	55.17 ± 12.81*	2658.33
Lic I	32.33 ± 12.23*,##	-41.39
Lic II	23.83 ± 8.01*,#	-56.80

n=6. Mean ± S.D. [% changes vs Sham or Control]. Group ID was listed in Table 1. * p<0.01 compared to that of Sham by MW test; # p<0.01 compared to that of Control by MW test; ## p<0.05 compared to that of Control by MW test.

Discussion

Cadmium (Cd) has been well known as an environmental or occupational toxin. Nowaday, Cd, highly toxic element is a ubiquitous environmental pollutant of increasing concern. Food crops grown on Cd-containing soils or on soils naturally rich in this metal constitute a important source of environmental exposure to Cd other than exposure from smoking[18,19].

In Korea, according to previous study, Cd intake was exclusively from food, both in children and mothers who live in Busan, their daily Cd intake was 0.457 μg[20]. In special, as compared with the values reported in the study, Pb exposure levels among Asian populations are similar to the levels in Europe and in the United States, whereas Cd exposure levels seem to be higher in Asia than in Europe. The contribution of the Pb absorption by the route of diet was variable and was related to the extent of air pollution, but Cd intake was almost exclusively via the dietary route with little contribution of the respiratory uptake[21].

As an increase of rate of soil-to-plant transfer of Cd, there is a predict that human exposure to dietary Cd will gradually increase in the next 10~20 years[18].

In 1989, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) set the provisional tolerable weekly intake (PTWI) of Cd at 1 μg/kg body weight/day[22]. But, Cd-linked nephro-toxicity occurred in higher than expected frequencies in human populations whose dietary Cd intakes were within the limit of PTWI[18].

The effects of exposure to cadmium in laboratory animals include renal tubular damage, placental and testicular necrosis, liver damage, osteomalacia, testicular tumors, teratogenic malformations, anemia, hypertension, pulmonary edema, chronic pulmonary emphysema, and induced deficiencies of iron, copper, and zinc[23].

Some of these effects have been observed in human after exposures to cadmium oxide fumes, diet of cadmium contaminated rice and are characteristic of the syndrome described in Japan as Itai Itai disease. A combination of osteomalacia and kidney injury (Itai-Itai disease) was first reported in Japan in the late 1940s. At the same period, severe

bone damage occurred in cadmium-exposed workers in some European factories[23-25].

Kjellstrom T reported the possible mechanism of cadmium-induced bone effects: (1) interference with parathyroid hormone (PTH) stimulation of Vit D production in kidney; (2) reduced activity of kidney enzymes activating Vit D; (3) increased excretion of Ca in urine; (4) reduced absorption of calcium from intestines; (5) direct interference with Ca incorporation into bone cells; and (6) direct interference with collagen production in bone cells[25].

In this study, we demonstrated that the protective effects of licorice against Cd-induced nephro-toxicity in rats.

There are about 35 heavy metals exposed to our environment. Heavy metals become toxic when they are not metabolized and accumulate in organs. Almost organs are involved in heavy metal toxicity.

Kidney is important target organ in Cd-induced toxicity. Sometimes, increase in organ weight is considered a sign of organ toxicity. However, no changes in the ratio of kidney weight to body weight were found in our study. Entire experiment period was 5 days, and Cd was exposed to rats for 1 day. Therefore, it seems that a day is too short to make the change in the kidney weight. But we found that Cd could induce severe renal toxicity in Ca-intoxificated rats by means of histological analysis.

Long term exposure of Cd causes renal tubular diseases[26-28]. Although protective activity of Japanese hepatoprotective drug containing glycyrrhizin has been founded in chronic Cd-induced nephrotoxicity[29], there are no reports about the effects of licorice in acute or chronic kidney injuries induced by Cd. So, we showed that the protective effect of licorice against Cd-induced nephrotoxicity, in this study. The number of glomeruli showing vasodilatation by Cd was decreased in licorice pretreated groups.

In general, toxicants tend to induce apoptosis at low level of exposure, whereas they cause necrosis at high level of exposure[30], Cd induces both apoptotic and non-apoptotic cell death[31]. Caspase inhibitors attenuated Cd-induced apoptosis. Previously, we investigated the cytoprotective effects of licorice against heavy metal-induced toxicity in cultured mouse liver cell line H4IIE[7]. We already found that licorice prevented Cd-induced cell death and inhibited the translocation of Bad from cytosol to mitochondrial membrane[32]. In the present study, we assessed the in vivo effects of licorice against Cd-induced injuries, and clearly demonstrated the protective effects of licorice. The mechanism on the protective effect of licorice in Cd-induced nephro-toxicity remains to be established in the future.

Acknowledgments

This study was supported by a grant of Oriental Medicine R&D project, Ministry of Health & Welfare, Republic of Korea. (B050035)

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