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Protective Effect of Korean Red Ginseng Against Dichromate Toxicity

Eun Kim, Hak Chul Hyun and Ki Jung Na
Korea Ginseng and Tobacco Research Institute, Taejon 305-345 Korea

Abstract □ The metabolic disturbance and nephrotoxicity induced by sodium dichromate (20 mg/kg, SC) have been diminished by the administration of Korean red ginseng extract (100 mg/kg, PO). Red ginseng has a powerful potency on the blood urea nitrogen (BUN) increment shown in the early 2h after dichromate intoxication. It normalized the dichromate induced hepatic glycogenolysis. The effect of red ginseng on dichroamte induced nephrotoxicity was investigated by hematological analysis, and urinalysis. Ginseng treatment significantly reduced the increases in the urinary excretion of protein and glucose. These effects were dose dependent. Ginseng protected the accumulation of BUN and creatinine in the blood, caused by dichromate intoxication. Unlike CaEDTA, ginseng did not change the urinary excretion of chromium. And it could not convert hexavalent chromium to trivalent chromium. These results suggest that ginseng treatment is effective in decreasing the metabolic disturbance, one of the earliest signs of dichromate toxicity, resulting in the protective effect of dichromate induced renal damage.

Introduction

Ginseng (*Panax ginseng C.A. Meyer*) has diverse pharmacological effects on the human body.[1] The pharmacological activities of ginseng may be related to stimulation of RNA and protein synthesis, and glucose and lipid metabolism.[2,3] In addition, ginsenoside-Rb_2 improved the nitrogen balance of diabetic rats.[4]

There is a close relationship between intracellular metabolism and renal function.[5] The primary function of the kidney is excreting metabolic wastes from the blood in the form of urine. The kidney plays a key role in regulating total body homeostasis.

However, relatively few studies have been done to investigate the pharmacological properties of ginseng on the renal function. In this study, we have investigated the protective effect of Korean red ginseng against the multisystemic toxicity and the renal damage induced by dichromate.

Materials and Methods

Male Sprague-Dawley rats (200 ± 10 g) were used in this study. The sodium dichromate (20 mg/kg, in saline, adjusted pH 7.4 with NaOH) was injected subcutaneously to the rats. Red ginseng extract (100 mg/kg, prepared according to the Kim's method[6]) was dissolved in distilled water and administered orally 30 min prior to sodium dichromate treatment. The corresponding control animals received physiological saline.

In the experimental where attempts were made to document the onset of the nephrotoxicity, the rats were placed in metabolism cage in which they had free access to drinking water and food. Urine was collected on a daily basis. Urine volume and pH were measured quantitatively, and various substances (glucose, protein, ketones, blood, leukocyte, bilirubin, and nitrites) were qualitatively using Combur^9-Test-U (Boehringer). Aliquot was saved for 24 h excretion of protein, glucose, urea, creatinine, and chromium analysis. Blood samples were obtained from heart of thiopental sodium (60 mg/kg, i.p.) anesthetized rats at the requsite time.

Urea was measured using Kyokuto UN-V^R reagents (Tokyo, Japan). Creatinine was measured by reaction with picrate, using Asan Creatinine Set^R reagents (Kyoungki-do, Korea). Glucose was deter-

mined colorimetrically with Asan Glucose Set^{K} reagents utilizing the glucose oxidase and peroxidase reactions. Urinary protein was determined by the Ponceau S method after precipitation of the urinary proteins with trichloroacetic acid.[7] Chromium was analyzed colorimetrically according to the Gooderson's methods.[8] A portion of the liver was digested with 10 m*l* of 1 N NaOH in a boiling water for 60 min and glycogen was precipitated by the addition of 2 m*l* of ethylalcohol and purified according to the methods of Roe and Dailey.[9] Liver glycogen was determined by the anthrone-H_2SO_4 methods, with glucose as the standard.[10] Glycogen content was expressed as mg glucose per 100 g liver.

Results were analyzed using analysis of variance appropriate to the experimental design. Significance of differences between means was tested using the Student's t-test.

Results and Discussion

Fig. 1 represents the typical time courses of nephrotoxicity after a single subcutaneous administration of sodium dichromate (20 mg/kg) on male rat. Red ginseng extract (100 mg/kg) was administered orally 30 min prior to sodium dichromate treatment. Daily urine samples were collected and urinary excretion of protein and glucose were measured. Results are expressed as mg per protein or glucose excreted per day to normalize variations due to polyuria.

Urinary excretion of protein significantly increased at the 1st day after the dichromate injection, reaching maximum level 4 days after treatment. In the 1st day urine sample, increased glucosuria was not detected. Qualitative glucosuria was seen 2 days after dichromate injection, reaching maximum level 3 days after dichromate treatment, and decreased to lower level 6 days after treatment. Proteinuria was persisted longer than glucosuria.

Administration of red ginseng extract reduced the increase in the urinary excretion of protein and glucose. In addition, red ginseng treatment protected both the excretion of leukocyte and hematuria induced by dichromate, and improved the physiological status significantly.

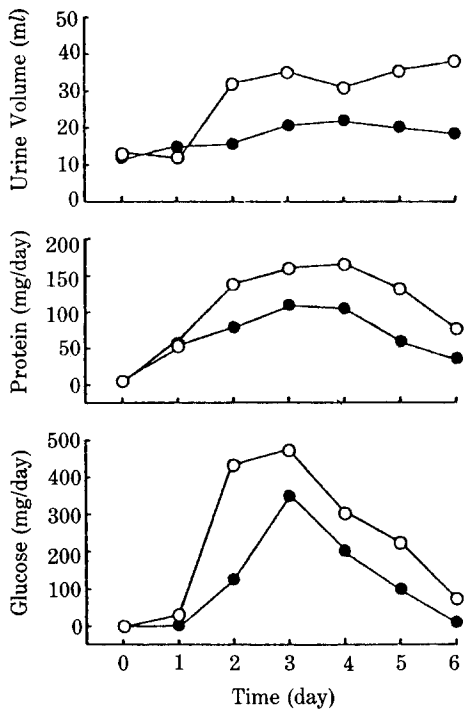

Fig. 1. Effect of red ginseng extract on dirchromate induced nephrotoxicity. Each set of data is from a single rat. The experimental animals received an subcutaneous injection of sodium dichromate. Red ginseng extract was administered orally 30 min prior to sodium dichromate treatment.
○; sodium dichromate (20 mg/kg, s.c.)
●; sodium dichromate+red ginseng extract (100 mg/kg)

Fig. 2 shows the dose effect of red ginseng extract on the nephrotoxicity induced by dichromate 2 days after intoxication. At the ginseng dose of 100 mg/kg, the urinary excretion of protein and glucose were reduced significantly. In contrast to other nephrotoxins such as mercury or cadmium, copious amounts of protein and glucose were found in the urine samples 2 days after dichromate intoxication. Ginseng treatment did not change the electrophoretic patterns of urine proteins (data not shown).

The blood non-protein-nitrogen (NPN) concentra-

tion was determined 3 days after dichromate administration (Table 1). In the dichromate treated group, both the blood urea nitrogen (BUN) and creatinine were increased 2 folds respectively, and uremia was obvious. Ginseng treatment improved the renal clearance of urea and creatinine, and as a results it protected the accumulation of the NPN in the blood. The accumulation of NPN in the blood is the indicator of nephrotoxicity. These results indicate that ginseng treatment protected the renal damage induced by dichromate. Both the degeneration and regeneration of renal tubular cells resulted from administration of potasium dichromate have been studied in the rat.[11,12] These studies showed the development of and recovery from an acute tubular necrosis located exclusively in the proximal tubule. Previously, we reported that red ginseng protects the proximal tubular necrosis caused by intravenous injection of dichromate on rabbits.[13] In the red ginseng treated group, the renal damage is not too extensive and so the kidney structure and function usually return to really normal state within weeks of the onset of injury.

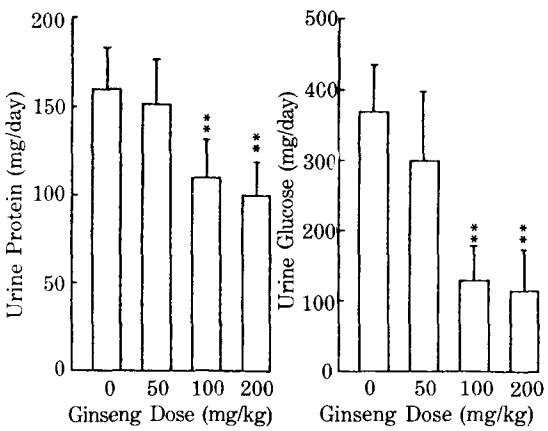

Fig. 2. Effect of red ginseng extract on urinary excretion of protein and glucose in dichromate treated rats. In the urine samples 2 days after a single subcutaneous injection of sodium dichromate (20 mg/kg), the 24 h excretion of urinary protein and glucose were measured. Results are shown as mean ± S.E. of ten animals. The asterisks indicate those values which are significantly different from controls ($p < 0.01$).

As shown in Fig. 3, ginseng treatment reduced

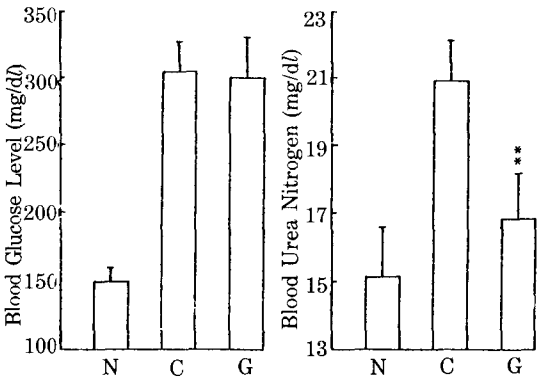

Fig. 3. Effect of red ginseng extract on serum metabolites after 30 min of sodium dichromate intoxication. Blood samples were obtained from the hearts of thiopental sodium (60 mg/kg, i.p.) anesthetized rats. Results are shown as mean ± S.E. of ten animals. The asterisks indicate those values of which are significantly different from control ($p < 0.01$).
N; normal group
C; dichromate (20 mg/kg, s.c.) treated control group
G; ginseng (100 mg/kg, p.o.) treated experimental group

Table 1. Effect of red ginseng extract on various parameters of renal function[a].

	BUN[b]	Creatinine	B/C ratio	U/P_{urea}	U/P_{creat}	C_{urea}[c]	C_{creat}
Normal	15.1 ± 1.9	0.49 ± 0.05	31.5 ± 2.5	106.2 ± 12.5	160.4 ± 9.2	0.92 ± 0.11	1.40 ± 0.17
Control	35.9 ± 4.1	1.01 ± 0.06	34.2 ± 2.1	17.8 ± 5.5	45.2 ± 8.2	0.43 ± 0.10	1.10 ± 0.10
Ginseng	25.8 ± 1.2[d]	0.83 ± 0.07[d]	31.1 ± 2.3	42.4 ± 11.9[d]	78.1 ± 11.1[d]	0.68 ± 0.08[d]	1.22 ± 0.38

a; Values present the mean (± S.E.) of ten animals.
b; Serum NPN concentration is expressed as mg per d*l*.
c; Renal clearance of NPN is expressed as m*l* per min.
d; Significantly different from control ($p < 0.05$).

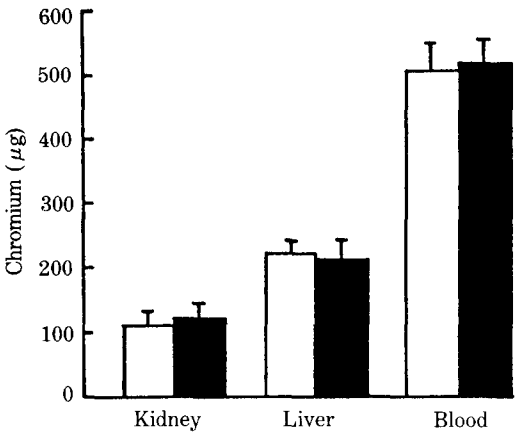

Fig. 4. Effect of red ginseng extract on the distribution of chromium. The chromium contents were determined 30 min after a single subcutaneous injection of sodium dichromate (40 mg/kg).
Red ginseng extract (100 mg/kg) was administered orally 30 min prior to dichromate treatment.
□ ; dichromate treated control group
■ ; ginseng treated experimental group

the increase in blood urea nitrogen concentration, which occurred 30 min after dichromate administration. However, ginseng treatment did not affect the hyperglycemia induced by dichromate. Dichromate showed the largest metabolic disturbance only in the early period after treatment. Dichromate possesses a characteristic dual feature on cellular metabolism, which might be related to the metabolic fate of dichromate.[14]

The liver content of glycogen was very significantly depleted by dichromate intoxication. In the first 2h period, liver glycogen was more reduced by 1300 mg glucose per 100 g liver than that of normal after the administration of dichromate. In the ginseng treated group, liver glycogen was more reduced by 1000 mg glucose per 100 g liver than that of normal. Ginseng treatment protected the accelerated glycogenolysis induced by dichromate consistently. Dichromate was transported very rapidly, and really 50% were distributed in 3 organs, blood, liver, and kidney 2h after injection. Ginseng treatment did not affect the chromium distribution (Fig. 4).

Many attempts have been done to detoxify the chromium intoxication. Hemodialysis and peritoneal dialysis have been used clinically in chromium poisonings, however, the benefit has not been fully demonstrated in chromium clearance and toxicity.[15-17] In the animal experiment, the treatment of hexavalent chromium poisonings by chelating agents or by reducing agents has some advantage in protection renal damage.[18,19] In the preliminary experiments, it was found that reduction and chelation were helpful to prolong the life span of rabbits intoxicated by intravenous injection of sodium dichromate. Both the ascorbic acid and CaEDTA produced an increase in urinary chromium excretion.

In the clinical case reports, it was observed that patients die from an initial multisystem shock followed by renal failure after hexavalent chromium intoxication.[16,17] It seems that metabolic disturbance may be one of the earliest signs of hexavalent chromium toxicity, and the initial factor of renal damage. Red ginseng extract has not reducing activity and chelating power against hexavalent chromium compounds. Although not fully studied, the powerful potency of ginseng to calm the initial factor may be helpful to overcome the later renal toxicity.

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