PROTECTIVE EFFECTS OF GINSENOSIDE RC ON TCDD-INDUCED WASTING IN RATS

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Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a contaminant from the synthesis of 2,4,5-trichlorophenoxyacetic acid is well known as an endocrine disruptor. TCDD elicits a broad spectrum of effects including wasting, testicular atrophy, hepatic dysfunction and carcinogenecity[1]. Numerous studies have investigated mechanism of TCDD intoxication in animal breedings and cell cultures. However, little information is available on protective agents against TCDD-induced toxicity. Previously, we reported that water extract of *Panax ginseng* C.A.Meyer protected the wasting symptom and necrotic death of seminiferous vesicle in the guinea pigs exposed to TCDD[2]. In the present study, much of concern was focused on which compound was responsible for the protective effect of ginseng extract against TCDD-induced toxicity. Here, it was demonstrated the administration of ginsenoside Rc from *Panax ginseng* C.A.Meyer effectively inhibited wasting symptom and hepatic cytochrome P450-activation in the rats exposed to TCDD.

Materials and Methods

Preparation of ginsenosides from Korean red ginseng

Korean red ginseng, which is made from fresh ginseng root by steaming and drying, was purchased from Korea Ginseng Corporation (Taejon, Korea). Korean red ginseng was extracted with 10 volumes of distilled water at 85 º, concentrated under a reduced pressure and lyophilized to a dark brownish powder. Resulting powder was dissolved in water and subjected to adsorption chromatography using macroreticular resin (Diaion HP-20). The column was eluted consecutively with water, 25% ethyl alcohol (EtOH) and 100% EtOH. Saponin fraction (SF) was obtained from 100% EtOH eluate and further divided into two subfractions, panaxadiol (PD) and panaxatriol (PT), by silica gel and octadecyl column chramatography. Ginsenoside Rb_1, Rb_2, Rc and Rd were isolated from PD saponins by the method of Sanada[3].

In vivo administration

Male Sprague Dawley rats (180-200 g) were maintained under controlled conditions of 23±1?, 40-60% of relative humidity and 12 hr-light/dark cycle. Rats were allowed free access to chow diet and drink tap water. Experimental groups consisted of normal control, TCDD only and TCDD plus ginseng samples. Rats were given orally either saponin fractions (40 mg/kg) or ginsenosides (40 mg/kg) from day zero to twenty. TCDD was dissolved in acetone/dimethylsulfoxide/corn oil (9:1:790, v/v) and intraperitoneally administered to rats at a single dose of 50 ug/kg on the day seven.

Biological testing

Blood was obtained by cardiac puncture, allowd to clot and centrifuged to obtain serum. Total cholesterol in serum was determined using a diagnostic kit from Sigma. Liver was homogenated in hypotonic buffer containing 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl and 0.05 mM dithiothreitol. Microsomal fraction was obtained from ultracentrifugation (105,000´g) and used for the determination of cytochrome P450 and arylhydrocarbon hydroxylase. Cytochrome P450 was determined by the method of Omura[4] and arylhydrocarbon hydroxylase activity was determined from the method of Nebert[5]. For histological examination, liver tissue was taken and fixed in buffered 10% formalin. Fixed tissue was embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Results and Discussion

One prominent characteristic of the toxic action of TCDD is reduction of body weight and loss of adipose tissue mass. The loss of adipose tissue is accompanied by altered serum lipid profile such as hyperlipidemia. As shown in Fig. 1 and 2, a single administration of TCDD (50 ug/kg) decreased body weight and increased serum cholesterol in the rats. However, TCDD-induced body weight loss and serum cholesterol elevation was reduced in rats administered with saponin fraction (40 mg/kg) together with TCDD. Furthermore, administration of diol saponins (40 mg/kg) suppressed more effectively the TCDD-induced toxic symptom, wasting and elevation of serum cholesterol than total saponin did.

Next, we compared each ginsenoside from diol saponins by measuring body weights after fourteen days of TCDD-administration. Among tested, administration of ginsenoside Rc effectively inhibited the TCDD-induced wasting symptom (Fig. 3). Administration of other ginsenosides from diol saponins such as ginsenodide Rb_1, Rb_2 and Rd was ineffective. To determine whether the mechanism by which ginsenoside Rc inhibited the toxic action of TCDD was dependent on hepatic microsomal monooxygenase system, we analyzed absolute contents and enzymatic activities of cytochrome P450 monooxygenases. Rats administered with TCDD exhibited increased contents of cytochrome P450 (Fig. 4a) and elevated activities of microsomal arylhydrocarbon hydroxylase (Fig. 4b). However, activation of aryl hydrocarbon hydroxylases was suppressed in rats coadministered with ginsenoside Rc and TCDD though contents of cytochrome P450 were not changed in the rats. Histological examination of liver also revealed the TCDD-induced necrotic foci were decreased in the rats coadministered with ginsenoside Rc and TCDD (data not shown). Thus, it is assumed that the protective effect of ginsenoside Rc on the TCDD-induced wasting was mediated in part by inhibition of hepatic microsomal activation. Taken together, it is convinced that ginsenoside Rc might be an effective constituent of ginseng extracts on the protection against TCDD-induced toxicity in rats.

Acknowledgments

This work was supported by the research grant, K01-GP-304, from Korea Tobacco and Ginseng Corporation.

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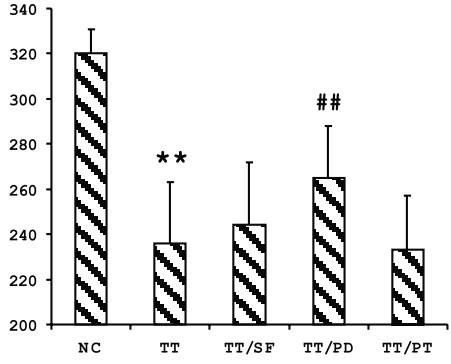

Figure 1. Effect of saponin fraction on body weight
NC: normal control; TT: 50 ug/kg TCDD-treated; TT/SF: 50 ug/kg TCDD plus 40 mg/kg saponin fraction-treated; TT/PD: 50 ug/kg TCDD plus 40 mg/kg diol saponin-treated; TT/PT: 50 ug/kg TCDD plus 40 mg/kg triol saponin-treated. **: significant at P< 0.01 as compared to NC; ##: P< 0.01 as compared to TT

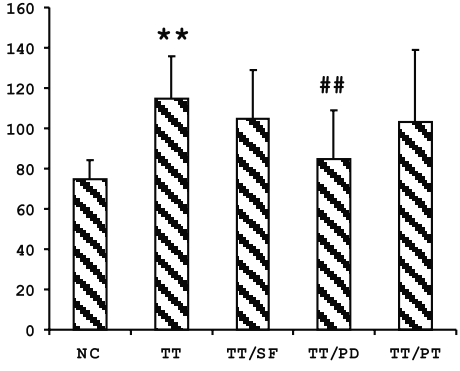

Figure 2. Effect of saponin fraction on the level of serum total cholesterol
NC: normal control; TT: 50 ug/kg TCDD-treated; TT/SF: 50 ug/kg TCDD plus 40 mg/kg saponin fraction-treated; TT/PD: 50 ug/kg TCDD plus 40 mg/kg diol saponin-treated; TT/PT: 50 ug/kg TCDD plus 40 mg/kg triol saponin-treated. **: P< 0.01 as compared to NC; ##: P< 0.01 as compared to TT.

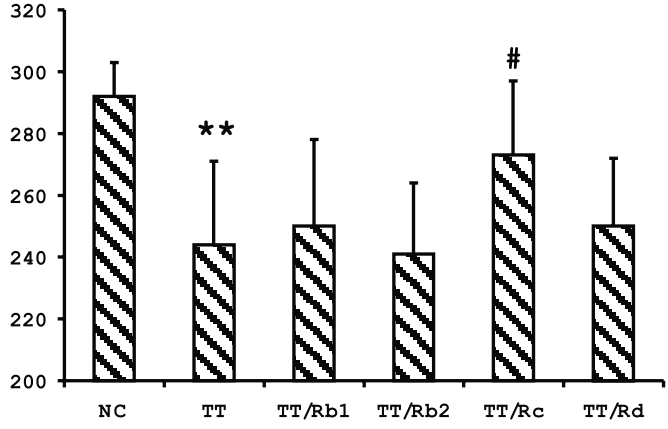

Figure 3. Effect of ginsenosides on body weight
NC: normal control; TT: 50 ug/kg TCDD-treated; TT/Rb_1 (Rb_2, Rc, Rd): 50 ug/kg TCDD plus 40 mg/kg ginsenoside-treated. **: significant at P< 0.01 as compared to NC; #: P< 0.05 as compared to TT.

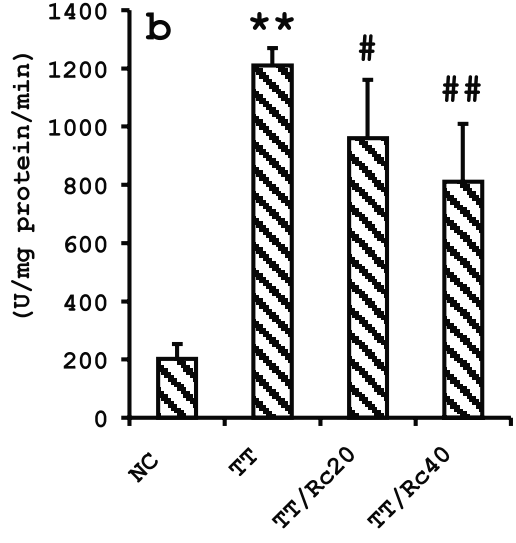

Figure 4. Effect of ginsenoside Rc on cytochrome P450 and arylhydrocarbon hydroxylase activity
NC: normal control; TT: 50 ug/kg TCDD-treated; TT/Rc20 (or 40): 50 ug/kg TCDD plus 20 (or 40) mg/kg Rc-treated. **: significant at P< 0.01 as compared to NC; #, ##: P< 0.05 and P<0.01 as compared to TT.