BUPLEURUM FALCATUM-INDUCED ALTERATIONS OF BIOCHEMICAL ACTIVITIES.

SUK-IN LIM*

INTRODUCTION

According to oriental medicinal textbook, Bupleurum falcatum release exterior conditions(1). This herb had been used for constrained Liver Qi with symptoms as dizziness, vertigo, chest and flank pain, emotional instability. And also,it had been used for alternating chills and fever, accompanying bitter taste in mouth, irritability, vomiting, and a sensation of constriction in the chest(2).

Recently, Buplenum radix has been reported to possess a wide range of biological activities including inhibition of hepatic damage induced by immunological factor(3, 4), inhibition of CNS activity(5), inhibition of inflammation(6), and antifever effect. And, it is reported that Bupleurum radix decreased the GPT level in CCl4-intoxicated rats(7).

In CCl_4 induced liver toxicity, hepatocytic membrane is disrupted by trichloromethyl radical generated at cytochrome, and which is evoked by lipid peroxidation(8). In cells, lipidperoxide is eliminated by a various antioxidative systems such as GSH/NADPH redox cycle including GSH peroxidase, superoxide dismutase, and catalase(9, 10).

* 大田大學校 韓醫科大學 藥理學敎室

The purpose of the present study was to characterize the effects of BFE on lipidperoxidation, glutathione status ,and on the activities of those enzymes altering glutathione homeostasis.

EXPERIMENTAL METHODS

DRUG PREPARATION

The Buplerum falcatum was disintegrated and extracted with hot MeOH in reflux extraction apparatus for 6 hours. An aquous fraction was obtained in each separation steps those were added consequently with hexane, $CHCl_3$, and EtOAc. And finally, BuOH fraction was obtained from aquous fraction, and concentration and drying was done with evaporator and freeze dryer.

ANIMALS AND TREATMENT

Spraque-Dawley rats (200-250g) and ICR mice (about 20g) were housed three and five per plastic cage on hard wood chips and acclimatized for at least 7 days prior to use. The animal room temperature was maintained at 20-24°C, relative humidity at 50-60%, and controlled lightning interval. Rats were fed an unrefinded diet and tap water *ad libitum*. After 1 weeks of acclimatization, the rats and mice were divided into four

groups: control group, CCl_4 group, high dose of extracts and CCl_4 group, low dose of extracts and CCl_4 group. The extract was administered orally for 4 days at the dose of 100mg/kg and 20mg/kg, and CCl_4 (0.5ml/kg) was administered orally once at third day in each group with the exception of control.

MICROSOME AND CYTOSOL FRACTION

Six rats from each group were killed by decapitation at 4 hours after final administeration. Livers were perfused *in situ* with ice cold 1.15% KCL containing 0.1 mM EDTA. Whole liver homogenates were prepared by mincing and then homogenizing with Ultra-Turrax. The whole homogenate was centrifuged at 3,000 x g for 10 min. The supernatant was centrifuged again at 10,000 x g for 20 min, and the supernatant fraction centrifuged once more 105,000 x g for 1 hour in ultracentrifuge. Supernatant was used as the cytosolic fraction and pellet was resuspended in PBS solution. All procedure was done below 4°C.

MDA CONTENTS

MDA contents measured using liver homogenates and microsome fraction according to previously described(12). In briefly, liver homogenates and sodium lauryl sulfate were mixed and incubated for 30 minutes. 0.1 N of HCL and TBA were added then, heated at 95°C for 1ours. After centrifugation, reaction products were measured. Protein was determined by the method of Lowry et al(11).

GLUTATHIONE CONTENTS

After the addition of 0.5% picric acid to the washed liver cells, the cells were collected. And then, protein was removed by centrifugation at 12,000 x g, 10 min. The supernatant was withdrawn for the determination of glutathione. Total glutathione and oxidized glutathione were measured as previously described (13).

ENZYME ASSAY

The following enzymic activities were measured using the cytosolic fraction. Glutathione peroxidase activity was assayed according to the procedure of Paglia et al(14). Enzyme activity is defined as n mol per mg protein per minute at 25°C. The standard assay mixture contained 0.1 mM Tris HCl, 1 mM glutathione, 0.2mM NADPH, 2U glutathione reductase, 0.25 M hydroperoxide and cytosol fraction. Glutathione S-transferase activity was assayed according to Habig's method(15).

STATISTICAL ANALYSIS

Student's t-test was employed to asscess the statistical significance. Values which differ from contrl over $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

In the case of CCl_4 induced liver toxicity, the basic sequence of events involves initial generation of the trichloromethyl radical at cytochrome locus of the monooxygenase system(16). These initial events are accompanied by

covalent binding of CCl_4 cleavage product largely to lipid and protein of liver cell ER(17) and by the initiation of lipid peroxidation(8). Once reactive metabolites are formed in liver, Protection and defense mechanism may bring about their rapid removal and inactivation. Toxicity then depends on the balance between th rate of metabolite formation and the trate of removal.

In this study, the increase in hepatic lipid peroxide level and decrease in intracellular glutathione level observed after CCl_4 treatment were ameliorated by pretreatment with Bupleurum radix extract(Table I, Table II, Table III). This results imply the possibility that BFE possess some radical scavenging component as antioxidant, and affect the activities of some enzymes such as glutathione peroxidase, glutathione-S-transferase, and glutathione reductase (18, 22). In cells, the reduced glutathione converted into the oxidized glutathione to detoxify the endogeneous hydrogen peroxide or lipid peroxides. And the redox status of glutathione can be maintained by NADPH/NADP sytem, glutathione reductase and glutathione peroxidase(19, 20). Consideration that toxicity depends on the balance between the rate of metabolite formation and the rate of removal, and liver injury may be prevented by some compounds which stimulate GSH-production and/or scavenge the radical intermediates(21, 22), the level of glutathione is very important parameter in estimation of liver toxicity or evaluation of hepatoprotective agents.

Glutathione-S-transferase is regarded as detoxifying enzyme, which catalyzed the first step in mercapuric acid formation. The marked increases in glutathione -S-transferase activities in conjuction with the increased glutathione levels suggest that BFE pretreatment may increase glutathione-S-transferase mediated conjugation of electrophilic agents and, thus, produce protective effects against some hepatotoxicants (Table IV, Table V).

In conclusion, the decreased level of hepatic glutathione and glutathione S-transferase activities induced by hepatotoxicant were ameliorated by BFE treatment. These changes might exhibit the protective effect of BFE on hepatic lipidperoxidation.

Table I. Effects of BFE on indics (MDA) of lipid peroxide concentrations in liver homogenates.

GROUP	MDA (nmol/mg protein)
Control	1.62 ± 0.54
CCl_4	5.72 ± 1.47
CCl_4 + BFE(100mg/kg)	3.83 ± 1.23*
CCl_4 + BFE(20mg/kg)	5.53 ± 1.53

BFE: Bupleurum falcatum extracts
*: Significant, $p<0.05$

Table II. Antiperoxidative effect of BFE in liver microsome fraction.

GROUP	MDA(nmol/mg protein)
Control	2.63 ± 0.73
CCl_4	6.58 ± 1.35
CCl + BFE(100mg/kg)	4.34 ± 0.96*

BFE: Bupleurum falcatum extracts
*: significant, $p<0.05$

Table III. Effect of BFE on intracellular glutathione level in rat liver homogenates.

GROUP	glutathione(nmol/mg protein)
Control	10.56 ± 1.46
CCl_4	5.84 ± 1.19
CCl_4 + BFE(100mg/kg)	8.09 ± 2.01*

BFE: Bupleurum falcatum extracts
*: Significant, $p<0.05$

Table IV. Effect of BFE on hepatic glutathione-S-transferase(GSH-S-Tx) activities.

GROUP	GSH-S-Tx(nmol/min/mg protein)
Control	187.4 ± 13.6
CCl_4	105.8 ± 11.5
CCl_4 + BFE(100mg/kg)	155.5 ± 15.9*

GSH-S-Tx activities are expressed as nmoles product formed/min/mg protein.
BFE: Bupleurum falcatum extracts
*: Significant, $p<0.05$

Table V. Effect of BFE on hepatic glutathione peroxidase (GSH-Px) activities.

GROUP	GSH-Px (nmol/min/mg protein)
Control	327.8 ± 38.6
CCl_4	187.8 ± 21.5
CCl_4 + BFE(100mg/kg)	278.5 ± 50.3*

GSH-Px activities are expressed as nmoles NADPH oxidized/min/mg protein.
BFE: Bupleurum falcatum extracts
*: Significant, $p<0.05$

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