STUDIES ON BIORESPONSES OF PHELLODENDRI CORTEX

林錫麟·康坼林[1)]

I. INTRODUCTION

The body is exposed to a huge number of foreign chemicals in a variety of circumstances, and these compounds is intaked into body through ingestion, inhalation, or absorption. Liver is the primary organ occuring biosynthesis, degradation, and metabolic alteration of foreign compounds (1). The liver cells may be easily encountered with numerous of chemicals and biological agents, such as plant products, fungal products, bacterial metabolites, medicinal agents, pesticides, and industrial by-products, and taken as food contaminants or as accidental inhalation or ingestion (2,3). Alteration of xenobiotics to highly reactive intermediates occurs to such as extent that many of these chemicals are transformed to hepatotoxicants, and such are involved in the etiology and pathogenesis of liver disorder. Accordingly, lots of efforts have been made to protect the liver injury induced by various toxicants. (4,5).

Phellodendri Cortex (Phellodendron amurense) which drains Damp Heat in the Lower Burner, quells kidney Fire, drains Fire and detoxifies Fire Poison,

1 大田大學校 韓醫學科 藥理學敎室

has been used for Damp Heat leukorrhea with diarrhea or dysentery, swollen and painful knees,legs. And its decoction has also been used for Damp Heat dysentery-like disorder or abdominal pain (6,7). In modern medicinal studies, this herb has been reported to show the antibiotic effects, and anti CNS effects. It has also been reported that Phellodendri Cortex lowered blood pressure, but increased the secretion of the pancreas (8). But the information is lack about the effect bof Phellodendri Cortex on liver injury induced by hepatotoxicants.

II. EXPERIMENTAL METHODS

1. EXPERIMENTAL ANIMALS

Spraque-Dawley rats (200-250 g) and ICR mice (about 20 g) 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 ℃, relative humidity at 50-60 %, and controlled lightning interval. Rats were fed an unrefinded diet and tap water *ad libitum*.

2. SAMPLE PREPARATION AND TREATMENT

Phellodendri Cortex were purchased from Kyung-dong Korean market in Seoul. The 0.5 kg of this herb was disintegrated and extracted in boiling water for 6 hours, and then concentrated to the volume of 500 ml using evaporator. 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 decoction was administered orally for 4 days at the dose of 10 ml/kg and 25 ml/kg, and CCl_4 (0.15 ml/kg) was administered orally once at third day in each group with the

exception of control.

3. SLEEPING TIME

On fourth day, mice was fed with hexobarbital sodium (50 mg/kg) intraperitoneally. The duration of sleep was measureed.

4. SERUM BIOCHEMICAL FACTORS

The rats were anesthetized with ethyl ether, and whole blood was withdrawn with heartpuncture using plastic syringe. After standing in dark room, tubes containing blood was centrifuged at 3,000 rpm for 30 minutes in order to get serum. The level of AST and ALT was measured by enzymatic method (9)

5. MDA CONTENTS

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. MDA contents measured using liver homogenates according to Pryor et al.(10). 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)

6. 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.

Ⅲ. RESULTS AND DISCUSSION

A numerous toxicants, such as halogenated hydrocarbon, pesticides, medicinal compounds, industrial pollutants, have been reported to produce liver necrosis (12,13). The exact mechanism involved in the cytotoxicity of those chemicals to liver cells are not understood entirely, but recognized as the result of any or combination of a variety of biochemical alterations in the cell. Membrane damage, which is the direct cause of cell death, can influence the intracellular or membrane associated protein, such as receptor, transporters, and the other enzymes. Toxic agents may reacteither with the protein or lipid components and significantly alter transport function and thus cellular integrity. These effect may disrupt a variety of transport or permeability mediated physiological and biochemical functions and result in a wide specrum of toxic events. 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(14). These initial events are accompanied by covalent binding of CCl_4 cleavage product largely to lipid and protein of liver cell ER (15) and by the initiation of lipid peroxidation (16). Breakdown of the cell membrane by covalent binding with free-radical causes the disturbance of the function of those membrane bound enzymes to the extracelluar fluid. The leakage of cytoplasmic enzyme, AST, ALT, and lipid peroxidation are known as good signs of membrane damages. Therefore, evaluation parameters of hepatic membrane injury in this study were assessed by AST and ALT activity, BUN in the serum, and MDA contents in liver homogenates.

Table I. Effect of PCE on the duration of sleeping time induced hexobarbital in CCl_4-intoxicated mice.

Group	duration of sleep (min)
Control	32.72 + 1.25
CCl_4	52.36 + 1.91
CCl_4 + PCE(25ml/kg)	41.59 + 1.79*
CCl_4 + PCE(10ml/kg)	47.38 + 1.64*

PCE : Phellodendri Cortex extracts
* : Significance, $p<0.05$

Table II. Effects of PCE on AST activities in CC_4 intoxicated rats.

GROUP	AST activities (IU/L)
Control	128.63 + 10.68
CCl_4	244.18 + 14.47
CCl_4 + PCE(25ml/kg)	185.82 + 16.39*
CCl_4 + PCE(10ml/kg)	204.25 + 11.86*

LCE: Phellodendri Cortex Extracts
*: significance, $P<0.05$

Table III. Effects of PCE on ALT activities in CCl_4 intoxicated rats.

GROUP	ALT activities (IU/L)
Control	38.75 + 5.28
CCl_4	127.04 + 21.9
CCl_4 + PCE(25ml/kg)	81.28 + 14.26*
CCl_4 + PCE(10ml/kg)	98.35 + 9.33

PCE: Phellodendri Cortex Extracts
*: significant, $p<0.05$

Table IV. Effects of PCE on indics (MDA) of lipid peroxide concentrations.

GROUP	MDA (nmol/mg protein)
Control	0.28 + 0.04
CCl_4	0.71 + 0.08
CCl_4 + PCE(25ml/kg)	0.46 + 0.07*
CCl_4 + PCE(10ml/kg)	0.57 + 0.08

PCE: Phellodendri Cortex Extracts

*: Significant, $p<0.05$

As shown in the results, PCE decreased ALT and AST activities which is increased by CCl_4 toxicities in serum. And, it also showed a protective effect on MDA production induced by CCl_4 intoxication. This results imply the possibility that PCE possess some radical scavenging components as antioxidants. These antioxidants affects the protection system, such as glutathione peroxidase (18, 19), glutathione-S-transferase (20, 21), glutathione reductase (22), superoxide dismutase(23), and catalase(24). 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. Glutathione is the most important and widely occuring nonprotein thiol in living system that plays a major role in many redox and detoxification reaction in the liver (25). The availability of GSH may be the factor stimulating the excretion of the reactive and radical intermediate through conjugation reaction in Phase II. In cells, GSH (reduced glutathione) converted into GSSG (oxidized glutathione) to detoxify the endogeneous hydrogen peroxide or lipid peroxides. And the redox status of glutathione can be maintained by NADPH/NADP sytem and glutathione reductase(22, 26)

The tetrachlorocarbon is converted into reactive compounds, trichloromethyl radical and trichloromethylperoxy radical, in liver microsome, and those attack membrane and/or deplete GSH. Therefore, Liver injury may be prevented by some compounds which stimulate GSH-production and/or scavenge the radical

intermediates. In this study, PCE showed a protective effect on liver damage induced by tetrachlorocarbon. Though precise mechanism is not clear, it is supposed that PCE may act on, at the least, one of defense system mentioned above.

IV. REFERENCES

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