ROLE OF BAICALEIN COMPOUNDS AS ANTIOXIDANT IN THE TRADITIONAL HERBAL MEDICINE

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ABSTRACT

Antioxidant activity of some flavonoids in a traditional Sino-Japanese herbal medicine and its active ingredients was analyzed. Sho-saiko-to the crude drug used against hepatitis protected rat liver microsomes from iron-mediated lipid peroxidation. Among the components of Sho-saiko-to, wogon the extract of *Scutellaria* root showed an inhibitory effect on the lipid peroxidation of microsomes. Baicalein and baicalein glycoside the principal flavonoids in the *Scutellaria* root extract also inhibited microsomal lipid peroxidation potently. Antioxidant action of baicalein compounds in the *Scutellaria* root extract can explain the protective effect of Sho-saiko-to on liver function.

Reactive oxygen species including superoxide, hydrogen peroxide and hydroxyl radical are associated with many pathological conditions including ischemia-reperfusion, inflammation and aging (3). Liver injury induced by various hepatotoxin such as carbon tetrachloride, bromobenzene and D-galactosamine is closely related to the oxygen toxicity, in that free radicals and reactive oxygen species are involved (3, 7, 11, 15). Several traditional Sino-Japanese medicines have been used by clinicians to treat chronic hepatitis. Sho-saiko-to the crude drugs, a mixture of several plant extracts, shows a protective effect on the experimental rat liver injury induced by D-galactosamine (1). However, the mechanism of the protection by this crude drug has remained obscure. In this paper, we examined the effect of Sho-saiko-to (TJ-9, Tsumura Pharmaceutical) and its active ingredients on the iron-induced lipid peroxidation of microsomes. Sho-saiko-to and wogon the extract of *Scutellaria* root inhibited the lipid peroxidation of microsomes effectively. Baicalein and the extract of *Scutellaria* root inhibited the lipid peroxidation of microsomes effectively. Baicalein and baicalin the principal

flavonoids in wogon play a central role in antioxidant action of Sho-saiko-to.

MATERIALS AND METHODS

Materials

Baicalein and baicalin were obtained from Aldrich (Tokyo, Japan). Sho-saiko-to (TJ-9) and the following extracts consisting of TJ-9 were supplied from Tsumura Pharmaceutical (Tokyo, Japan): wogon (*Scutellaria* root), *Bupleurum* root, ginger, jujube fruit, *Pinellia* tuber, licorice root (*glycyrrhiza*) and ginseng.

Preparation of Liver Microsomes

Microsomes were prepared from the livers of adult male rats by standard differential centrifugation techniques (9). Microsomes were suspended in 0.25 M KCl at a protein concentration of about 15 mg/ml, and were stored at -80°C .

Lipid Peroxidation of Liver Microsomes

Two types of lipid peroxidation processes in microsomes can be distinguished under the *in vivo* and *in vitro* conditions; a non-enzymatic and enzymatic processes. The non-enzymatic process can be

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Table 1 *Effects of Crude Drugs on the Microsomal Lipid Peroxidation*

Addition*	Thiobarbituric acid-reactive substances (A_{532}) formed	
	Enzymatic	Non-enzymatic
None	0.753	1.56
Sho-saiko-to (TJ-9)	0.151	0
<i>Scutellaria</i> root (wogon)	0	0
<i>Bupleurum</i> root	0.717	1.556
Ginger	0.71	0.89
Jujube fruit	0.623	1.497
<i>Pinellia</i> tuber	0.73	1.538
Licorice root	0.70	0.09
Ginseng	0.756	1.588

*Water extract of crude drugs was added to the reaction systems of FeCl_3 and ADP with NADPH (enzymatic system), and of ascorbic acid with FeCl_3 (non-enzymatic system) at the concentrations of 0.07 mg/ml and 0.12 mg/ml, respectively.

induced by Fe^{3+} /ascorbic acid, and the enzymatic process is catalyzed by NADPH-dependent cytochrome P450 reaction (6).

(A) *Non-enzymatic lipid peroxidation of microsomes* Reaction mixture of 1 ml contained 60 mM Tris-HCl buffer (pH 7.5), 10 μM FeCl_3 , 0.5 mM ascorbic acid, microsomal fraction of 0.2 mg protein, and the additives, the extract of crude drug or flavonoid. The mixture was incubated at 37°C for 20 min, and the reaction was stopped by addition of 100% trichloroacetic acid. Lipid peroxides produced were determined as the thiobarbituric acid-reactive substances (2, 4).

(B) *Enzymatic lipid peroxidation of microsomes* Lipid peroxidation was carried out by adding NADPH plus Fe^{3+} -ADP (14). The reaction mixture contained 60 mM Tris-HCl buffer (pH 7.5), 0.24 mM FeCl_3 , 2.4 mM ADP, microsomal fraction (0.15 to 0.2 mg protein), the additives including several extracts of crude drug or flavonoid, and 0.12 mM NADPH in a final volume of 0.83 ml. Reaction was started by addition of NADPH, and stopped by adding 100% trichloroacetic acid after incubation at 37°C for 10 min. Lipid peroxides produced were determined as described above.

Antioxidant activity of following compounds was determined: baicalein, baicalin, Sho-saiko-to, wogon (*Scutellaria* root extract), *Bupleurum* root extract, ginger extract, jujube fruit extract, *Pinellia* tuber extract, licorice root (*glycyrrhiza*) extract, and ginseng extract. Baicalein and baicalin were

dissolved in dimethylsulfoxide. Other plant extracts were suspended in water, and the supernatant obtained by centrifugation at $10,000 \times g$ for 20 min was used for the microsomal lipid peroxidation assay.

RESULTS

We examined the effect of TJ-9 and its ingredients on the enzymatic and non-enzymatic lipid peroxidation of microsomes from rat liver. TJ-9 itself and only wogon as an ingredient of TJ-9 inhibited enzymatic lipid peroxidation of microsomes (Table 1). We further examined the effects of higher concentration of various crude drugs on non-enzymatic lipid peroxidation induced by ascorbic acid plus FeCl_3 . TJ-9 and wogon completely inhibited the formation of thiobarbituric acid reactive substances. Lipid peroxidation was also inhibited by licorice root extract and to a lesser extent by ginger extract (Table 1).

Effect of increasing concentrations of TJ-9 and wogon on the enzymatic and non-enzymatic lipid peroxidation was analyzed. The concentrations required for 50% inhibition of non-enzymatic lipid peroxidation by ascorbic acid plus FeCl_3 were 10 $\mu\text{g/ml}$ and 2 $\mu\text{g/ml}$ for TJ-9 and wogon, respectively (Fig. 1A). TJ-9 and wogon also inhibited the enzymatic lipid peroxidation: the concentrations required for 50% inhibition were 50 $\mu\text{g/ml}$ and 25 $\mu\text{g/ml}$ for TJ-9 and wogon, respectively (Fig. 1B).

Wogon the extract of *Scutellaria* root contains baicalein and its glycoside baicalin as the principal flavonoids (13). Non-enzymatic lipid peroxidation of microsomes was markedly inhibited by baicalein and to a lesser extent by baicalin: the concentrations required for 50% inhibition of the lipid peroxidation were 0.5 and 6 μM for baicalein and baicalin, respectively (Fig. 2A). Baicalein and baicalin also prevented microsomes from the enzymatic lipid peroxidation (Fig. 2B). Flavonoids showed more effective protection against the non-enzymatic lipid peroxidation than the enzymatic peroxidation of microsomal lipids.

DISCUSSION

Reactive oxygen species including superoxide anion, hydrogen peroxide and hydroxyl radical are related to the pathogenesis of clinical and experimental diseases, and can be produced as by-products through the electron transport system of mitochondria and microsomes (3). One important

Fig. 1 Effect of Sho-saiko-to (TJ-9) and wogon (*Scutellaria* root extract) on the iron-induced lipid peroxidation of rat liver microsomes. Lipid peroxidation was expressed as the production of thiobarbituric acid-reactive substances (4), which was induced by (A) 10 μ M FeCl_3 and 0.5 mM ascorbic acid, and (B) 0.24 mM FeCl_3 , 2.4 mM ADP and 0.12 mM NADPH as described in Materials and Methods. TJ-9 and wogon was added at the indicated concentrations. ●, TJ-9; ○, wogon extract

mechanism of the cellular toxicity of oxygen may be due to membrane injury by lipid peroxidation (3). This report showed that TJ-9, the traditional herbal medicine, protected liver microsomes from iron-mediated lipid peroxidation. TJ-9, a mixture of several plant extracts, has been used for the treatment of human chronic hepatitis, and exerts a protective effect on the rat liver against galactosamine-induced hepatitis (1), which is caused by the production of reactive oxygen species (7, 8, 11). Inhibition by TJ-9 of microsomal lipid peroxidation demonstrated in this paper can give a biochemical basis for protection by this crude drug of the hepatotoxin-mediated liver injury.

Among the components of TJ-9, wogon the *Scutellaria* root extract markedly inhibited the lipid peroxidation of microsomes, whereas the extracts of licorice root and ginger showed only a little pro-

Fig. 2 Effect of baicalein and baicalin on the iron-induced lipid peroxidation of rat liver microsomes. Reaction conditions were similar to those described in the legend to Fig. 1, except that baicalein and baicalin were substituted for TJ-9 and wogon. A, Fe/ascorbic acid system; B, Fe/ADP/NADPH system. ●, baicalin; ○, baicalein

tection against microsomal lipid peroxidation. These results suggest that antioxidative effect of TJ-9 is due to the action of wogon, which contains large amounts of flavonoids with antioxidant properties. Baicalein and baicalin as principal flavonoids in wogon showed an inhibitory effect on the non-enzymatic and enzymatic lipid peroxidation of microsomes, but the protective action of baicalein was much more potent than that of baicalin, the glycoside of baicalein. These findings well agreed with the previous report indicating a potent inhibition of platelet lipoxygenase by baicalein, but only a slight inhibition by baicalin (12). Generally, flavonoid aglycones including naringenin, hesperetin and quercetin show more powerful antioxidative effect than their corresponding glycosides (10). Wogon the *Scutellaria* root extract, however, contains 1% baicalein and 14% baicalin on the basis of dry weight (13), and thus, the antioxidant action of the crude extract may be

largely dependent on the action of baicalin.

Recently, baicalein was shown to inhibit the iron-mediated lipid peroxidation by the formation of an inert iron-baicalein complex bound to microsomes (5). Baicalin the flavonoid glycoside may also exhibit an antioxidant effect on lipid peroxidation possibly by chelating iron, but the iron-baicalin complex may be less inert compared with iron-baicalein complex. Antioxidant activity of baicalin and baicalein may participate in the hepatoprotective action of Sho-saiko-to and its active ingredient, wogon.

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