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Hepatoprotective Activity of Chungpesagan-tang is Related to the Inhibition of β-Glucuronidase

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Abstract – β-Glucuronidase-inhibitory and hepatoprotective effects of Chungpesagan-tang, which has been used for liver diseases and stroke, on CCl_4-induced hepatotoxicity of rats was investigated. Chungpesagan-tang potently inhibited β-glucuronidases. Serum AST, ALT and LDH levels of the CCl_4 group orally treated with Chungpesagan-tang (100 mg/kg) were lowered to 47, 28 and 58% of the CCl_4-treated group, respectively. Among the ingredients of Chungpesagan-tang, Puerariae Radix, Scutellariae Radix and Rhei Rhizoma potently inhibited the β-glucuronidases and protected CCl_4-induced liver injury. The hepatoprotective activity of Puerariae Radix was affected by ingredients of Chungpesagan-tang: Scutellariae Radix had the synergistic activity, but Angelicae Tenussimae Radix exhibited the antagonistic activity. These results suggest that the β-glucuronidase inhibitor of herbal medicines may protect CCl_4-induced liver injury and puerarin should be a natural prodrug for the hepatoprotective effect.

Key words – Chungpesagan-tang, hepatoprotective activity, β-glucuronidase inhibitor.

Introduction

β-Glucuronidase has been discovered in animals, plants and bacteria (Stahl and Fishman, 1983). This enzyme catalyzes the hydrolysis of β-glucuronide conjugates of endogenous and exogenous compounds in the body, such as benzo[a]pyrene glucuronides, and of natural plant glucuronides such as glycyrrhizin. In the tissue of mammals, β-glucuronidase has been measured as a typical lysosomal enzyme through studies of subcellular fractionation. Pineda *et al.* (1959) demonstrated that liver damage caused an increase of the enzyme in blood, and liver cancer could be related to the enzyme (Mills and Smith, 1951; Mill *et al.*, 1953; Levy *et al.*, 1948). We found that silymarin, a commercial crude drug used as a hepatoprotective, inhibit β-glucuronidase *in vitro* as well as *in vivo* (Kim *et al.*, 1994). Recently we isolated glycyrrhizin from the rhizome of *Glycyrrhiza uralensis* as a β-glucuronidase inhibitor, and found that it had a potent hepatoprotective activity on CCl_4-induced liver injury of rats (Shim *et al.*, 2000).

Chungpesagan-tang has been frequently used for patients who suffer from stroke as well as liver diseases. This Chungpesagan-tang was developed from traditional Chinese medicines (Lee, 1996). Therefore, to evaluate the relationship between β-glucuronidase inhibitory and hepatoprotective activities, we investigated β-glucuronidase-inhibitory activity of Chungpesagan-tang and its hepatoprotective effect on CCl_4-induced liver injury of rats.

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Experimental

Reagents – *p*-Nitrophenyl-β-D-glucuronide and carbon tetrachloride were purchased from Sigma Co. (U.S.A). Diagnostic kits of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactic acid dehydrogenase (LDH) were purchased from Asan Pharmaceutical Co., Ltd. (Korea). Puerarin was isolated from the rhizome of *P. thunbergiana* according to the method of Hayakawa *et al.* (1984). The other chemicals were of analytical reagent grade.

Plant materials, extraction and isolation – The rhizome of *Scutellaria baicalensis*, the rhizome of *Angelica tenussima*, the rhizome of *Cimicifuga heracleifolia*, the rhizome of *Rheum palmatum* and

Table 1. The Constituent Herbs of Chungpesagan-tang

	Weight (g)	Voucher Number
Puerariae Radix (Root of *Pueraria thunbergiana* Bentham)	16	KHP990702-1
Angelicae Tenuissimae Radix (Root of *Angelica tenuissima* Nakai)	8	KHP990702-2
Scutellariae Radix (Root of *Scutellaria baicalensis* Georgi)	8	KHP990702-3
Platycodi Radix (Root of *Platycodon grandiflorum* A. De Candolle)	4	KHP990702-4
Raphani Semen (Seed of *Raphanus sativus* Linne)	4	KHP990702-5
Cimicifugae Rhizoma (Rhizome of *Cimicifuga heracleifolia* Komarov)	4	KHP990702-6
Angelicae Dahuricae Radix (Root of *Angelica dahurica* Bentham et Hook)	4	KHP990702-7
Rhei Rhizoma (Rhizome of *Rheum palmatum* Linne)	4	KHP990702-8
Total amount (Chungpesagan-tang)	52	

the rhizome of *Angelcia dahurica* were produced in China and the rhizomes of *Pueraria thunbergiana*, the rhizome of *Planticodon gradiflorum*, the semen of *Raphanus satius* were in Korea. They were purchased from Kyungdong Crude Drug Market, Seoul, Korea, 1999. It was identified by N.-J. Kim (East-West Medical Research Institute, Kyung Hee University) and voucher specimens (KHP 990702-1~990702-8) were kept at College of Pharmacy, Kyung Hee University. Herbal medicine (100 g) and Chungpesagan-tang (Table 1) were extracted twice with 500 ml of boiling water. After evaporation, each extract were used.

Animals – Male mice (Spray Dawley, male, 180-200 g) were maintained on pellet food (Samyang Co., Korea) and tap water. Five rats in each group were used. Samples were administered orally 30 min after 20% CCl_4 diluted with olive oil (1 ml/100 g) on each group (except normal control group). These rats were anaesthetized with 20% urethane (0.1 ml/100 g body weight) 24 h after the administration of sample, and then 1 ml of blood was taken out by cardiac puncture. The serum was obtained by centrifugation (3000 rpm, 10 min) and used for measuring the activities of AST, ALT and LDH (Reitman and Frankel, 1957; Wroblewski and LaDue, 1955).

Preparation and assay of β-glucuronidase – A β-glucuronidase-producing bacterium, *E. coli* HGU-3, previously isolated by us was cultured in 5 liter of the broth at 37°C for 18 h (Kim *et al.*, 1995). The cultured bacteria were collected at 6000 rpm for 30 min and β-glucuronidase was purified by ammonium sulfate fractionation (saturated 40-50%), DEAE-cellulose column chromatography and Sephacryl S-300 column chromatography according to our previous method (Kim *et al.*, 1995). Its specific activity was 0.3 μmol/min/mg protein. In addition, β-glucuronidase of rat liver was partially purified according to Akao *et al.* (1991). Its specific activity was 0.7 μmol/min/mg protein.

The β-glucuronidase assay mixture (total volume of 0.5 ml) contained 20 μl of 10 mM p-nitrophenyl-β-D-glucuronide, 0.38 ml of 0.1 M phosphate buffer (pH 7.0), 40 μl of water (or inhibitor) and 60 μl of the enzyme purified above (Kim *et al.*, 1995). The assay mixture was incubated at 37°C for 50 min. The reaction was stopped by the addition of 0.5 ml of 0.25 N NaOH, and the absorbance was measured at 405 nm.

Results

Effect of Chungpesagan-tang on CCl_4-induced liver injury – The hepatoprotective effects of Chungpesagan-tang on CCl_4-induced liver injury in rats are shown in Table 2. In normal rats, serum GOT, GPT and LDH levels were 305±34.8 and 345±34.2 Karmen units, and 258±58.7 Wroblewski units, respectively. In CCl_4-treated animals, serum GOT and GPT levels were elevated to 2289±165.9 and 2791±332.1 Karmen units, respectively. LDH was also elevated to 1478± 224.4 Wroblewski units 20 h after CCl_4 administration. In contrast, serum

GOT, GPT and LDH levels of the CCl_4 group orally treated with 100 mg/kg of Chungpesagan-tang were lowered to 58.8, 75.7 and 51.7% of the CCl_4-treated group, respectively. However, in the group treated with 500 mg/kg of Chungpesagan-tang GOT, GPT and LDH levels were inferior to those of the group treated with 100 mg/kg Chungpesagan-tang.

β-Glucuronidase-inhibitory activity of some polyprescriptions – To understand the relationship between β-glucuronidase-inhibitory and hepatoprotective activities of herbal medicinal polyprescriptions, a polyprescription for liver diseases and a polyprescription for psychological diseases were selected and their inhibitory activity on β-glucuronidases of *E. coli* HGU-3 and of rat liver was investigated (Table 3). Chungpesagan-tang for the therapy of liver diseases potently inhibited β-glucuronidases. However, Kwiinahnsim-tang, which is a polyprescription for the therapy of psychological diseases, did not inhibit the β-glucuronidase. Therefore, we also measured the β-glucuronidase-inhibitory activity of the ingredients of Chungpesagan-tang. Among the ingredients, Puerariae Radix, Scutellariae Radix and Rhei Rhizoma potently inhibited the β-glucuronidases. These IC_{50} values were 1.21, 0.9 and 0.4 mg/ml for β-glucuronidase of *E. coli* HGU-3, and 0.83, 0.35 and 0.11 mg/ml for that of rat liver, respectively.

To understand the drug interaction of ingredients on Chungpesagan-tang, we also measured the inhibitory effect of herbal medicines combined with Puerariae Radix on β-glucuronidase activity. The inhibitory activity of Puerariae Radix on β-glucuronidase

Table 2. Protective Effects of Chungpesagan-tang on Serum GPT Activities of Rats Treated with CCl_4

	Dose (mg/kg)	GPT (Karman Unit)	GOT (Karmen Unit)	LDH (Wroblewski Unit)
Control		345± 34.2	305± 34.8	258± 58.7
CCl_4		2791±332.1[a]	2289±165.9[a]	1478±224.4[a]
Chungpesagan-tang	100	2112±220.9[b]	1346±171.1[b]	764±46.9[b]
Chungpesagan-tang	500	2506±262.2	1823±143.8	895±35.0[b]
Silymarin	100	1515±181.4[b]	1128±170.7[b]	781±151.2[b]

[a]Significantly different from control group ($p<0.05$).
[b]Significantly different from CCl_4-treated control group ($p<0.05$).

Table 3. Inhibitory Effect of Ingredients of Chungpesagan-tang on β-glucuronidases

Herbal medicine	Extract Yield (%)	Puerarin / Puerariae Radix (%)	IC_{50} (mg/ml)	
			E. coli	Rat liver
Puerariae Radix	29.1	1.06	1.21	0.83
Scutellariae Radix	40.0		0.90	0.35
Angelicae Tenuissimae Radix	25.0		>1.5	>1.5
Raphani Semen	13.2		>1.5	>1.5
Platycodi Radix	38.2		>1.5	>1.5
Cimicifugae Rhizoma	11.8		>1.5	>1.5
Angelicae Dahuricae Radix	31.6		>1.5	>1.5
Rhei Rhizoma	19.4		0.4	0.11
Puerariae Radix:Scutellariae Radix (=2:1, PS)	29.9	1.15	1.41	0.09
Puerariae Radix:Angelicae Tenuissimae Radix (= 2:1)	22.5	1.85	>1.5	>1.5
Puerariae Radix:Raphani Semen (=4:1)	26.0	1.45	>1.5	0.89
Puerariae Radix:Platycodi Radix (=4:1)	32.7	1.90	>1.5	>1.5
Puerariae Radix:Cimicifugae Rhizoma (=4:1)	23.9	1.03	1.50	0.30
Puerariae Radix:Angelicae Dahuricae Radix (=4:1)	27.7	1.54	>1.5	0.90
Puerariae Radix:Rhei Rhizoma (=4:1)	32.5	1.75	1.09	0.05
Puerariae Radix:Scutellariae Radix:Angelicae Tenuissimae Radix (=2:1:1, PSA)	22.0	0.94	>1.5	>1.5
Kwiiahnshim-tang			>1.5	>1.5
Chungpesagan-tang	36.0	1.30	0.82	0.92

activity was synergistically effectively by Scutellariae Radix or Rhei Rhizoma. However, the other ingredients did not affect, but also interfered the inhibitory activity of Puerariae Radix on β-glucuronidase activity.

Effect of ingredients of Chungpesagan-tang on CCl_4-induced liver injury – The hepatoprotective effects of Chungpesagan-tang, Puerariae Radix, Scutellariae Radix and Rhei Rhizoma, which were the potent inhibitor of β-glucuronidase, on CCl_4-induced liver injury in rats are shown in Table 4. In CCl_4-treated animals, serum GOT, GPT and LDH levels were dramatically elevated, comparing with normal rats. However, serum GOT, GPT and LDH levels of the group orally treated with Puerariae Radix (100 mg/kg) on CCl_4-induced liver injury were significantly lowered to 83.9, 58.9 and 55.1% of the CCl_4-treated group, respectively. Serum GOT, GPT and LDH levels of the group orally treated with Scutellariae Radix (100 mg/kg) on CCl_4-induced liver injury in mice were also significantly lowered. These hepatoprotective activities of Puerariae Radix or Scutellariae Radix were dose-dependent. Rhei Rhizoma had a potent hepatoprotective activity at a dose of 100 mg/kg. However, Rhei Rhizome was not effective at a dose of 500 mg. Serum GOT, GPT and LDH levels of the group orally treated with Puerariae Radix combined with Scutellariae Radix (PS, 100 mg/kg) on CCl_4-induced liver injury in mice were similar to those of the group treated with Puerariae Radix only. However, when this PS polyprescription combined with Angelicae Tenuissimae Radix (PSA) was treated on mice with CCl_4-induced liver injury, the hepatoprotective activity of the PSA polyprescription was inferior to that of PS polyprescription.

Discussion

There have been some efforts to prove the relation between the fluctuation of serum β-glucuronidase level and hepatic diseases: Liver damage caused an increase of the enzyme in blood (Pineda *et al.*, 1959; Mills and Smith, 1951; Kim *et al.*, 1994). Silymarin inhibited rat liver microsomal β-glucuronidase and had the hepatoprotective effect *in vivo* as well as *in vitro* (Kim *et al.*, 1994). We suggested that the hepatoprotective activity of glycyrrhizin as well as silymarin might be caused by the β-glucuronidase inhibition. Here, we measured inhibitory effects of Chungpesagan-tang and its ingredients. Chungpesagan-tang had a good β-glucuronidase-inhibitory activity. It also had a good hepatoprotective activity on CCl_4-induced liver injury of mice. Among its ingredients, Puerariae Radix, Scutellariae Radix and Rhei Rhizoma had a good β-glucuronidase-inhibitory activity as well as a potent hepatoprotective activity on CCl_4-induced liver injury of mice. However, Rhei Rhizoma was not effective at a dose of 500 mg (not 100 mg/kg). This result coincided that Chungpesagan-tang had a hepatoprotective at a low dose, but was not effective at a high dose. Therefore, we thought that, to cure liver injury with herbal medicines, the selection of their dosages should be important.

To understand drug interaction on Chungpesagan-tang, we also measured the inhibitory effect of ingredients combined with Puerariae Radix on β-glucuronidase and hepatoprotective activities. The inhibitory activity of Puerariae Radix on β-glucuronidase

Table 4. Protective Effects of Ingredients of Chungpesagan-tang on Serum GPT Activities of Rats Treated with CCl_4

	Dose (mg/kg)	GPT (Karman Unit)	GOT (Karmen Unit)	LDH (Wroblewski Unit)
Control		334± 38.5	309± 65.2	245± 88.1
CCl_4		2772±232.1[a]	2019±165.5[a]	1448±211.2[a]
Puerariae Radix	100	1635± 63.6[b]	1693±201.1[b]	798±185.8[b]
Puerariae Radix	500	1527± 75.3[b]	1475±100.8[b]	729±289.3[b]
Scutellariae Radix	100	2055±222.9[b]	1544± 76.1[b]	1277±289.4
Scutellariae Radix	500	1978±139.9[b]	1723±163.9[b]	957± 13.7[b]
Rhei Rhizoma	100	1664±143.2[b]	1413±122.5[b]	869± 48.6[b]
Rhei Rhizoma	500	3049±257.6	2221±145.2	1593± 52.7
P+S	100	1687±115.8[b]	1558±110.3[b]	811± 30.0[b]
P+S+A	100	2151±217.8[b]	2042±130.1	857± 53.2[b]
Silymarin	100	1335±120.1[b]	1168±131.7[b]	821± 72.5[b]

[a]Significantly different from control group ($p<0.05$).
[b]Significantly different from CCl_4-treated control group ($p<0.05$).
P: Puerariae Radix, S: Scutellariae Radix, A: Angelicae Tenuissimae Radix.

activity was synergistically effective by Scutellariae Radix or Rhei Rhizoma. However, the other ingredients did not affect, but also interfered the inhibitory activity of Puerariae Radix on β-glucuronidase activity. Scutellariae Radix activated synergistically hepatoprotective activity of Puerariae Radix. However, Angelicae Renuissimae Radix interfered antagonistically the hepatoprotective activity of PS polyprescription.

We also measured β-glucuronidase-inhibitory activity of some herbal medicinal polyprescriptions (Data not shown). Chungpesagan-tang, which is used for liver diseases, had a potent activity. However, Kwiinahnsim-tang, which is used for the psychological diseases (not liver diseases), did not inhibit β-glucuronidases. These results suggest that the β-glucuronidase should be closely related to the liver injury, which could be protected by Chungpesagan-tang, an inhibitor of β-glucuronidase.

References

Akao, T. A., Akao, T. I., Hattori, M., Kanaoka, M., Yamamoto, K., Namba, T. and. Kobashi, K., Hydrolysis of glycyrrhizin to 18β-glycyrrhetyl monoglucuronide by lysosomal β-D-glucuronidase of animal livers. *Biochem. Pharmacol.* **41**, 1025-1029 (1991).

Hayakawa, J., Noda, N, Yamada, S. and Uno, K., Studies on physical and chemical quality evaluation of crude drugs preparations. I. Analysis of Puerariae Radix and species Pueraria. *Yakugaku Zasshi* **104**, 50-56 (1984).

Kim, D.-H., Jin, J.-H., Park, J.-B. and Kobashi, K., Silymarin and its components are inhibitors of β-glucuronidase. *Biol Pharm. Bull.* **17**, 443-445 (1994).

Kim, D.-H., Jin, Y.-H., Jung, E.-A., Han, M.J. and Kobashi, K., Purification and characterization of βglucuronidase from *Escherichia coli* HGU-3, a human intestinal bacterium. *Biol. Pharm. Bull.* **18**, 1184-1188 (1995).

Lee, J. M., Reduohanxiao-tang. *In:* Longevity and life Preservation in Oriental Medicine. S.H. Choi (translated), Kyung Hee Univ. Press, Seoul, 1996, pp. 153-175.

Levy, G. A., Kerr, L. M. H. and Campbell, J. C., β-Glucuronidase and cell proliferation. *Biochem. J.* **42**, 462-468 (1948).

Mills, G. T., Paul, J. and Smith, E. E. B., Studies in β-glucuronidase, the influence of age, partial hepatectomy and other factors in the β-glucuronidase. *Biochem. J.* **53**, 232-245 (1953).

Mills, G. T. and Smith, E. E. B., The β-glucuronidase activity of chemically induced rat hepatoma. *Science* **114**, 690-692 (1951).

Pineda, E. P., Goldberg, J. A., Banks, B. M and Rutenburg, A. M., The significance of serum β-glucuronidase activity in patients with liver disease. *Gastroenterol.* **36**, 202-213 (1959).

Shim, S.-B., Kim, N.-J. and Kim, D.-H., β-Glucuronidase inhibitory activity and hepatoprotective effect of 18-glycyrrhetinic acid from the Rhizomes of Glycyrrhiza uralensis. *Planta Med.* **66**, 40-43 (2000).

Stahl, P. D. and Fishman, W. H., β-Glucuronidase. *In:* Method of Enzymatic Analysis. Bergmeyer, H.U. (ed), 3rd edition, Weinheim: Verlag Chemie, GmbH., 1983, Vol. 4, pp. 246-256.

Reitman, S. and Frankel, S., A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Path.* **28**, 56-63 (1957).

Wroblewski, F. and LaDue, J. S., Lactic dehydrogenase activity in blood. *Proc. Soc. Exp. Biol. Med.* **90**, 210-213 (1955).

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