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Rhei Rhizoma and Chunghyuldan Inhibit Pancreatic Lipase

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Abstract – Pancreatic lipase-inhibitory activity of the rhizome of Rhei Rhizoma and its antihyperlipidemic activity were measured. Rhei Rhizoma inhibited pancreatic lipase with IC_{50} value of 6.5 mg/ml (triolein as a substrate). Rhei Rhizoma significantly inhibited serum TG level in corn oil feeding-induced mice, and serum TG and cholesterol in Triton WR-1339-induced hyperlipidemic mice. However, Rhei Rhizoma did not show the hypolipidemic activity in high cholesterol diet-induced hyperlipidemic mice. When in vitro pancreatic lipase-inhibitory and in vivo antihyperlipidemic activities of Whangryunhaedoktang (WT) and Chunghyuldan (CD), which is consisted of ingredients of WT and Rhei Rhizoma, were measured, CD exhibited more potent inhibitory activities than WT. Therefore these results suggest that antihyperlipidemic activity of Rhei Rhizoma and CD may be more or less originated from the inhibition of pancreatic lipase.

Key words – *Rhei Rhizoma*, Chunghyuldan, Whangryunhaedoktang, pancreatic lipase, antihyperlipidemic activity

Introduction

Lipid metabolism normally maintains an elegant balance between synthesis and degradation. When the balance is lost, hypertriglyceride and hypercholesterolemia may develop. This can cause variety of serious diseases, such as arteriosclerosis, hypertension, obesity, diabetes, functional depression of some organs, etc. (Goldstein *et al.*, 1973). Pancreatic lipase is a key enzyme for lipid breakdown to absorb fatty acids (Rodwelll *et al.*, 1973). Pancreatic lipase (PL), one of the exocrine enzymes of pancreatic juice, catalyzes the hydrolysis of emulsified esters of glycerol and long chain fatty acids. Short chain fatty acids can be directly absorbed into the blood whilst long chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles (Cary *et al.*, 1983). The micelles are absorbed in to the mucosal cells of the intestine and the fatty acids and monoglycerides resynthesised into triglycerides. These triglycerides are formed into small particles known as chylomicrons, which consist of triglycerides, the sterol lipid cholesterol, and apoproteins. These chylomicrons are transported to the muscle and adipose tissue. Dietary triglyceride is usually stored in the adipose tissue. The pharmacological agents, which reduce the absorption of dietary triglyceride thereby reducing the probability of formation of arterosclerotic plaques, have been developed such as orlistat and clofibrate (Ballinger *et al.*, 2002). Many researchers reported the pancreatic lipase-inhibitory effect of natural products (Raghavendra and Prakash, 2002; Yamamoto *et al.*, 2000; Han *et al.*, 2001). However, the pancreatic lipase-inhibitory activities of Rhei Rhizoma have not been studied. Therefore we measured pancreatic lipase-inhibitory activity of the Rhizome of Rhei Rhizoma and its antihyperlipidemic activity, as part of our continuing search for biological active antiarteriosclerosis agents from the natural herbal resources. Furthermore, we investigated antihyperlipidemic activity of Chunghyuldan (CD), which is consisted of Whangryunhaedoktang (WT) and Rhei Rhizoma.

Materials and Methods Materials – Triton WR-1339, triolein, tributyrin, and pancreatic lipase were purchased from Sigma Chemical (USA). Total cholesteol and triglyceride assay kits were from Asan. Pharm. (Korea). Low-density lipoprotein (LDL) cholesterol assay kits were from BioMerieux (France). Orlistat (xenical) was kindly donated by Dr. I.K. Kim of the Korea Food and Drug Administration.The Rhizome of *Rheum palmatum* Linne (Family Polygonaceae) was purchased from Kyung Dong Market (Seoul, Korea) and identified by Dr. Nam-Je Kim, East-West Medical Research Institute, Kyung Hee Medical Center, Kyung Hee University. Voucher specimen (KHUVP01011) was deposited at the Herbarium of College of Pharmacy, Kyung Hee University. CD A (or CD B) was consisted of 80% EtOH extract of 1 g

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Rhei Rhizoma (or 4 g Rhei Rhizoma for CD B) and WT, of which the ingredients were 80% EtOH extracts of 4 g Coptidis Rhizoma, 4 g Phellodendri Cortex, 4 g Scutellariae Radix, and 4 g Gardeniae Fructus (Kim *et al.*, 2002; Hong *et al.*, 1982).

Extraction of herbal medicines – Each herbal medicine (1 kg) was extracted twice with water or 80% ethanol in boiling water for 2 h. These extracts were filtered and evaporated in a rotary vacuum evaporator and then finally lyophilized with a freezing dryer. Dry weight yields (%) of extracts are shown in Table 1. To standardize the quality of these herbal medicines used in experiments, berberine, baicalin, geniposide, and sennoside A in WT, CD and Rhei Rhizoma were quantitatively assayed according to the previous methods (Oshima *et al.*, 1983; The Korean Pharmacopia, 1997). The main component contents of herbal medicine and formulae were shown in Table 2.

Activity assay of pancreatic lipase – The enzyme activity assay was performed according to the previous method (Junge, 1984). The reaction mixture (3.06 ml) containing 135 mM triolein (or tributyrin) emulsified in gum acasia, 2 mM sodium thioglycolate, and pancreatic lipase (0.6 unit using triacetin as a substrate) and the sample was adjusted to pH 8.8 with 0.1 M NaOH, incubated at 25°C and titrated with 10 mM NaOH to adjust at the pH 8.8. The inhibitory activity of the sample was calculated from the titrant volume.

Animals – Male ICR mice (20-25 g) were purchased from Daehan Experimental Animal (Korea) and fed a commercial diet (Samyang, Korea). These animals were kept for at least 7 days prior to the experiments. To evaluate the hypolipidemic effect, three kinds of hyperlipidemic animal models were prepared.

First, hyperlipidemic mouse model by corn oil was prepared according to the method of Duhault *et al.* (1976). Six mice were used per group. Corn oil (1g/kg) was orally administered 2 h after each sample were orally administered. Each samples was orally administered once per experiment. On 2 h after the administration of corn oil, blood samples of mice were drawn by cardiac puncture under ether anesthesia.

Second, hyperlipidemic mouse model by Trition WR-1339 was prepared according to the method of Kusama *et al.* (1988). Triton WR-1339 was injected at the end of the regular 16-h fasting period as a 10% solution in saline at a dose of 200 mg/kg body weight into the tail veins of mice under light ether anesthesia. Six mice were used per group. These mice were anesthetized with ether 18 h after Triton WR-1339 injection and 1-1.5 ml of blood was withdrawn by cardiac puncture. Sera were obtained by centrifugation (1500×g, 10 min). Tested samples and orlistat were administered orally once a day for 3 days. The final administrations of these samples were performed 1 h before Triton

Table 1. The extracted yields and component contents from ingredients of Chunghyuldan and Whangryunhaedoktang

Herb	Scientific name	CD A (g)	CD B (g)	WT (g)	Yield (%)	
					80% EtOH	Water
Coptidis Rhizoma	*Coptis japonica* MAKINO from Japan	4	4	4	15.8	11.8
Phenodendri Cortex	*Phellodendron amurense* RUPRECHT from China	4	4	4	10.9	9.3
Scutellariae Radix	*Scutellaria baicalensis* GEORGI from Young-am, Korea	4	4	4	32.0	34.3
Gardeniae Fructus	*Gardenia jasminoides* ELLIS from Young-am, Korea	4	4	4	28.3	18.3
Rhei Rhizoma	*Rheum palmatum* LINNE from China	1	4	0	35.3	18.3

WT, Whangryunhaedoktang; CD A, Chyunghyundan A; CDB, Chyunghyuldan B.
Values indicate the major component content.

Table 2. The content of major components in 80% alcoholic and water extracts of Chunghyuldan and its ingredients

	Content (%)			
	Berberine	Baicalin	Geniposide	Sennoside A
Rhei Rhizoma.	–	–	–	0.76 (0.09)
Chunghyuldan A	5.07	5.74	4.99	0.06
Chunghyuldan B	3.97	4.50	3.91	0.22
Whngryunhaedoktang	5.59	6.32	5.49	0

Values are contents of major components in 80% alcoholic extracts.
Values in Parenthesis are contents of major components in water extracts.

WR-1339 injection.

Third, hypercholesterolemic mouse model by high cholesterol diets was prepared according to the method of Niiho *et al.* (1990). Mice were classified into 4 groups (HC, water extract of Rhei Rhizoma, orlistat and normal control groups). Each group contained 5 mice. The HC group was fed on normal diet (95.75~96.5%) supplemented with 1% cholesterol, 0.25% cholic acid and 2.5% olive oil (CCO) for 7 consecutive days. The normal control group received a solid normal diet (rodent chow: 32% protein, 5% fat, 2% fiber, and 60% nitrogen free extract) alone. Rhei Rhizoma water extract group was fed on normal diet (95.75~96.5%) supplemented with CCO and orally administered water extract of Rhei Rhizoma (1 g/kg/day). Rhei Rhizoma water extract group was fed on normal diet (95.75~96.5%) supplemented with CCO and orally administered oristat (1 g/kg/day). After 16-h fasting period from final administration of sample, blood samples of mice were drawn by cardiac puncture under ether anesthesia.

Determination of total serum cholesterol, triglyceride, and LDL cholesterol – Total cholesterol was measured by the enzyme method designed by Allain *et al.* (1974). Serum triglyceride was measured by the method designed by Sardsai *et al.* (1968). LDL Cholesterol was measured by the enzyme method designed by Mainard *et al.* (1986).

Statistical analysis – All the data from the *in vivo* experiments were expressed as mean ± standard deviation and statistical significance was determined using Students *t*-test.

Table 3. The pancreatic lipase-inhibitory activity of Rhei Rhizoma

Substrate	IC_{50} (mg/ml)	
	Triolein	Tributyrin
Rhei Rhizoma	6.5	10.5
Xenical	0.8	2.1

IC_{50} indicates 50% inhibitory concentration.

Results and Discussion

***In vitro* pancreatic lipase-inhibitory and *in vivo* antihyperlipidemic effects of Rhei Rhizoma** – As part of our continuing search for biological active anti-arteriosclerosis agents from the natural herbal resources, pancreatic lipase-inhibitory activity of two hundred herbal medicines was measured. Among tested herbal medicines, water extract of Rhei Rhizoma potently inhibited it (Table 3).

Therefore, to evaluate the hypolipidemic effects of Rhei Rhizoma, we also measured their inhibitory effects in corn oil feeding-induced hyperlipidemic mice (Table 4). Triglyceride level, not cholesterol, in serum was increased by treatment with corn oil. In the Rhei Rhizoma (1 g/kg) group, serum triglyceride level was significantly decreased to less than that of corn oil alone group. We measured their hypolipidemic activities in Triton WR-1339-induced hyperlipidemic mice

Table 4. The effects of Rhei Rhizoma on serum triglyceride, total cholesterol, and low density lipoprotein cholesterol level in corn oil feeding induced hyperlipidemic mice

Group	Dose (g/kg/day)	TG level (mg/dl)	TC level (mg/dl)	LDL level (mg/dl)
Normal	–	94.9±12.65	156.7±21.30	36.2±7.13
Control	–	155.2±5.12#	155.5±10.10	58.5±13.6
Rhei Rhizoma	1.0	87.61±8.54*	155.9± 6.82	42.0±5.27
Xenical	1.0	98.2±4.07*	143.8± 7.61	52.7±5.32

TC indicates total cholesterol, TG indicates triglyceride, and LDL indicates low density lipoprotein cholesterol.
Each value is mean±S.D. (n=10).
#Significantly different from the normal group ($p<0.05$).
*Significantly different from the control group ($p<0.05$).

Table 5. The effects of Rhei Rhizoma on serum triglyceride, total cholesterol, and low density lipoprotein cholesterol level in Triton WR-1339 induced hyperlipidemic mice

Group	Dose (g/kg/day)	TG level (mg/dl)	TC level (mg/dl)	LDL level (mg/dl)
Normal		79.6±3.9	212.1±9.0	83.0±1.5
Control		1040.0±11.2#	337.5±17.7#	>100
Rhei Rhizoma	1.0	619.5±7.5*	247.2±4.2*	72.4±6.9
Xenical	1.0	892.4±9.8	242.0±5.6*	21.0±3.1

TC indicates total cholesterol, TG indicates triglyceride, and LDL indicates low density lipoprotein cholesterol.
Each value is meanS.D. (n=10).
Significantly different from the normal group ($p<0.05$).
* Significantly different from the control group ($p<0.05$).

(Table 5). Triglyceride, total cholesterol, and LDL cholesterol levels in serum were increased by treatment with Triton WR-1339. This result was similar to those of the previous report.[12] However, compared with triglyceride, total cholesterol and LDL cholesterol levels in Triton WR-1339-alone group, those in Rhei Rhizoma-treated groups were significantly decreased. However, Rhei Rhizoma water extract did not decrease serum cholesterol levels on hypercholesterolemic mice induced by feeding high cholesterol diet (Table 6).

Accordingly, we believe that hypolipidemic activity of Rhei Rhizoma should be due to the inhibition of pancreatic lipase.

***In vitro* pancreatic lipase-inhibitory and *in vivo* antihyperlipidemic effects of WT and CD** – Rhei Rhizoma exhibited *in vitro* pancreatic lipase-inhibitory and *in vivo* hypolipidemic activities. Therefore, Rhei Rhizoma was added in herbal formulae and we evaluated whether the hypolipidemic activity of Rhei Rhizoma could explain CD inhibited pancreatic lipase (Table 7). However, WT did not inhibit it. Between CDs, CD B, which contained higher concentration of Rhei Rhizoma than CD A, exhibited more potent inhibitory activity.

We also measured hypolipidemic activity of WT and CD in hyperlipidemic mice induced by corn oil feeding (Table 8). Triglyceride level in serum was increased by treatment with corn oil. However, compared with triglyceride level in corn oil alone group, that in CD-treated group was significantly decreased. CD showed more potent inhibition than WT. Among tested herbal formulae, CD B contained high concentration of Rhei Rhizoma exhibited the most potent inhibition (Table 9). CDs dose-dependently lowered serum

Table 6. The effects of Rhei Rhizoma on serum triglyceride, total cholesterol, and low density lipoprotein cholesterol level in high cholesterol diet induced hyperlipidemic mice

Group	Dose (g/kg/day)	TG level (mg/dl)	TC level (mg/dl)	LDL level (mg/dl)
Normal	–	31.7±12.10	118.6±15.75	36.8±13.21
Control	–	33.8±10.43	184.8±20.69#	101.1±2.82#
Rhei Rhizoma	1.0	28.7±8.73	206.0±48.33	90.8±12.54
Xenical	1.0	11.2±4.37*	207.2±1.92	92.8±7.34

TC indicates total cholesterol, TG indicates triglyceride, and LDL indicates low density lipoprotein cholesterol.
Each value is mean±S.D. (n=10).
#Significantly different from the normal group ($p<0.05$).
*Significantly different from the control group ($p<0.05$).

Table 7. The pancreatic lipase-inhibiory activity of Chunghyuldan and Whanryunhaedoktang

	IC_{50} (mg/ml)	
	Triolein	Tributyrin
Whangryunhaedoktang	>20	>20
CD A	10.3	14.7
CD B	6.5	9.0
Xenical	0.8	2.1

IC_{50} indicates 50% inhibitory concentration.

Table 8. The effects of Chunghyuldan and Whangryunhaedoktang on serum triglyceride, total cholesterol, low and high density lipoprotein cholesterol level in corn oil feeding induced hyperlipidemic mice

Group	Dose (g/kg/day)	TG level (mg/dl)	TC level (mg/dl)	HDL level (mg/dl)	LDL level (mg/dl)
Normal	–	129.9±9.9	144.5±15.2	59.7±10.2	60.2±7.7
Control	–	234.3±22.5#	164.0±23.2	60.1±4.6	55.8±7.3
CD A	1	170.4±39.0*	158.1±15.8	68.3±6.1	55.31±6.7
CD B	1	158.6±47.9*	139.2±26.3	59.7±8.5	38.9±4.1*
WT	1	172.7±15.5*	154.7±10.8	61.4±6.6	49.1±6.6
Xenical	0.1	90.7±29.8*	146.9±9.9	61.4±9.0	60.6±15.7

TC indicates total cholesterol, TG indicates triglyceride, LDL indicates low density lipoprotein cholesterol, and HDL indicates high density lipoprotein cholesterol.
Each value is mean ± S.D. (n=10).
#Significantly different from the normal group ($p<0.05$).
*Significantly different from the control group ($p<0.05$).

Table 9. The effects of Chungyuldans on serum triglyceride, total cholesterol, and low density lipoprotein cholesterol level in corn oil feeding induced hyperlipidemic mice

Group	Dose (g/kg/day)	TG level (mg/dl)	TC level (mg/dl)	LDL level (mg/dl)
Normal	–	94.9±12.6	156.7±1.3	36.2±7.1
Control	–	155.2±5.1#	155.5±10.1	58.5±13.6
CD A	0.1	132.0±16.2*	155.5±10.1	55.3±1.8
	0.2	99.1±12.1*	156.2±1.9	53.4±1.3
CD B	0.1	126.5±12.2*	148.2±3.0	45.6±1.2
	0.2	79.6±11.8*	146.4±4.7	38.7±0.5
Xenical	10	98.0±4.0*	143.7±7.6	52.7±5.3

TC indicates total cholesterol, TG indicates triglyceride, and LDL indicates low density lipoprotein cholesterol.
Each value is mean±S.D. (n=10).
#Significantly different from the normal group ($p<0.05$).
*Significantly different from the control group ($p<0.05$).

Table 10. The effects of Chungyuldans on serum triglyceride, total cholesterol, and low density lipoprotein cholesterol level in Triton WR-1339 induced hyperlipidemic mice

Group	Dose (g/kg/day)	TG level (mg/dl)	TC level (mg/dl)	LDL level (mg/dl)
Normal	–	79.6±3.9	212.1±9.0	83.0±1.5
Control	–	1040.0±11.2#	337.5±17.7#	>100
CD A	0.1	991.9±41.8	316.1±15.0	51.3±2.5*
	0.2	895.6±66.7*	277.7±13.8*	38.3±2.0*
CD B	0.1	791.6±30.5*	286.4±22.1*	48.3±2.5*
	0.2	614.9±26.5*	256.4±8.5*	45.1±1.6*
Xenical	10	540.5±10.6*	332.7±20.3	77.9±9.7*

TC indicates total cholesterol, TG indicates triglyceride, and LDL indicates low density lipoprotein cholesterol.
Each value is mean±S.D. (n=10).
#Significantly different from the normal group ($p<0.05$).
*Significantly different from the control group ($p<0.05$).

TG levels.

We also measured hypolipidemic activity in Triton WR-1339-induced hyperlipidemic mice (Table 10). Triglyceride, total cholesterol, and LDL cholesterol levels in serum were increased by treatment with Triton WR-1339. However, compared with triglyceride, total cholesterol and LDL cholesterol levels in Triton WR-1339-alone group, those in CD or WT-treated groups were significantly decreased. CD showed more potent inhibition than WT. Among tested herbal formulae, CD B containing high concentration of Rhei Rhizoma exhibited the most potent inhibition. These results suggest that hypolipidemic activity of Rhei Rhizoma should be due to the inhibition of pancreatic lipase.

Judging from these results, we propose that pancreatic lipase inhibitor may be effective as a hypolipidemic agent.

References

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond W., Fu P. C. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**, 470-5 (1974).

Ballinger, A., Peikin, S. R., Orlistat: its current status as an antiobesity drug. *Eur. J. Pharmacol.*, **440**, 109-117 (2002).

Carey, M. C., Small, D. M., Bliss, C. M., Lipid digestion and absorption. *Annu. Rev. Physiol.*, **45**, 651-677 (1983).

Duhault, J., Boulanger, M., Beregi, L., Sicot, N., Bouvier, F. A new type of hyperlipidemic agent comparative assay in rats. *Atherosclerosis*. **23**, 63-72 (1976).

Han, L. K., Kimura, Y., Kawashima, M., Takaku, T., Taniyama, T., Hayashi, T., Zheng, Y. N., Okuda, H. Antiobesity effects in rodents of dietary teasaponin, a lipase inhibitor. *Int. J. Obes.* **25**, 1459-1464 (2001).

Hayakawa J, Noda N, Yamada S, Mikami E, Uno K. Studies on physical and chemical quality evaluation of crude drugs preparations. III. Analysis of Gardenia fruits and its preparations. *Yakugaku Zasshi*. **105**, 996-1000 (1985).

Hong, N. D., Kim, J. W., Doo, H. K., Kim, N. J., Studies on the efficacy of combined preparation of crude drugs (V)-Effect of "Whangryonhaedok-Tang" on the antiinflammatory, ileum of mice, blood pressure, blood glucose, total cholesterol and antibacterial activity. *Kor. J. Pharmacog.* **13**, 26-32 (1982)

Junge, W., "Methods of Enzymatic Analysis," Vol. 4., ed. by Bergmeyer H. U., Verlag Chemie GmbH, Weinheim, 1984, pp. 15-25.

Kim, Y. S., Jung, E. A., Chang, J. C., Yang, H. K., Kim, N. J.,

Cho, C. H., Bae, H. S., Lee, K. S., Kim, D. H. Effect of Daio-Orengedokuto inhibits HMG-CoA reductase and pancreatic lipase. *Biol. Pharm. Bull.* **25**, 1442-1445 (2002).

Kusama, H., Nishiyama, M., Ikeda, S., Pharmacological investigation of bezafibrate, a hypolipidemic agent, (I). Effect of bezafibrate on normal and experimental hyperlipidemia in rats. *Folia pharmacol. Japon,* **92**, 175-180 (1988)

Mainard, F., Madec, Y., Cholesterol, phospholipid and apo B composition of LDL; Comparison of precipitation and ultracentrifugation methods. *Ann. Biol. Clin.* **44**, 618-623 (1986)

Niiho, H., Yamazaki, T., Nakajima, Y., Itoh, H., Takeshita, T., Kinjo, J., Nohara, T. Pharmacological studies on Puerariae Flos. II. The effects of Puerariae Flos on alcohol-induced unusual metabolism and experimental liver injury in mice. *Yakugaku Zasshi.* **110**, 604-11 (1990)

Ohshima, Y, Takahashi, K., Hiraga, Y., Shibata, S. Studies on the evaluation of Chinese drug preparations. Determination of sennosides in the drug preparation containing Rhubarb. *Shoyakugaku Zasshi.* **37**, 217-222 (1983).

Olsson, A. G., Lang, P. D., Vollmar, J., Effect of bezafibrate during 4,5 years of treatment of hyperlipidemia. *Atheroscleosis*, **55**, 195-199 (1985)

Raghavendra, M. P., Prakash, V. Phenylboronic acid - a potent inhibitor of lipase from Oryza stiva. *J. Agric. Food Chem.* **50**, 6037-6041 (2002)

Sardesai, V. M., Mannig, J. A., The determination of triglycerides in plasma and tissues. *Clin. Chem.* **14**, 156-158 (1968)

Suenaga, T., Shirakawa, T., Harada, N., Tao, C., Yamasaki, M., Komatsu, H., Matsumoto, N., Kida, M., Kawamoto, Y., Murakami, Y., Kakamura, M., Mieno, H., Inoue, M., Kajiyama, G., Mechanism of protective effects of Ouren-Gedoku-To and San'ou-Syanshin-To on the gastric mucosa. *Folia pharmacol japon.* **98**, 319-325 (1991)

The Korean Pharmacopia. 7th ed. pp. 721, 766, 778, 779, 781, 1997.

Yamamoto, M., Shimura, S., Itoh, Y., Ohsaka, T., Egawa, M., Inoue, S. Antiobesity effects of lipase inhibitor CT-II an extract from edible herbs, Nomame Herba, on rats fed a high-fat diet. *Int. J. Obes. Relat. Metab. Disord.* **24**, 758-764 (2000)

Yoshinaga, S., Kimura, M., Tanaka, A., Tomonaga, M., The effect of Kampo medicine "Oren-gedoku-to" on clinical manifestations and cerebral blood flow in chronic stage of cerebrovascular diseases. *J. Med. Pharm. Sco. WAKAN-YAKU,* **9**, 22-27 (1992)

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