Original Article

Screening of extracts of Japanese medicinal plants for HMG-CoA reductase inhibition

YOKOYAMA Satoru[1)], TAKENO Sayaka[1)], NAGATA Toshiro[1)], SHIMIZU Kuniyoshi[1)] and KONDO Ryuichiro[1)2)]

1) Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581 Japan
2) Corresponding Author: ryukondo@agr.kyushu-u.ac.jp

Received Oct 8, 2009 / Accepted Dec 22, 2009

Abstract

Aqueous, methanol, ethanol or dichloromethane extracts of 22 medicinal plants were examined for their inhibitory activity on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The samples which showed more than 80 % inhibition at the concentration of 1,000 μg/ml were selected as the potential HMG-CoA reductase inhibitory ingredients. The aqueous extract of herbal part of *Artemisia princeps* and the ethanol extract of fruit/seeds of *Trachycarpus wagnerianus* were selected and showed HMG-CoA reductase inhibitory activity with 88.1 ± 3.2 % and 83.7 ± 0.3 % at 1,000 μg/ml, respectively.

Key Words: *Artemisia princeps*, *Trachycarpus wagnerianus*, Hyperlipidemia, 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, Antihyperlipidemia

Introduction

Hyperlipidemia is one of important risk factors involved in the development of cardiovascular disease (Frishman, 1998). This disease is caused by increased blood cholesterol levels. Treatment of hyperlipidemia involves diet control, exercise, and the use of lipid-lowering diets and drugs (Stone, 1996). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1. 1. 1. 34), a rate-limiting enzyme in endogenous cholesterol synthesis, catalyzes the reductive deacylation of HMG-CoA to mevalonate in a two-step reaction (Bochar, Stauffacher, and Rodwell, 1999; Frimpong, and Rodwell, 1994). Therefore, lowering total cholesterol through the action of a HMG-CoA reductase inhibitor is very important for the remedy or prevention of hyperlipidemia.

HMG-CoA reductase inhibitors, statins, are the most effective drugs in lowering high cholesterol levels and are widely used for prevention of major coronary events (LaRosa, He, and Vupputuri, 1999). These drugs have been marketed in many countries and many new statins have been synthesized. Although their safety profile is excellent, they have been still linked to undesirable side effects, including myopathies (Pasternak, *et al*., 2002; Hamilton-Craig, 2001). Recently it has been reported that some statins, simvastatin and fluvastatin, induce nightmares in a clinical test (Wood, and Cummins, 2009). As these consequences, there continues to be a high demand for new oral antihyperlipidemia drugs.

Management of hyperlipidemia without any side effects is still a challenge to the medical system. Plant products are frequently considered to be less toxic and freer from side effects than synthetic ones. Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic, and hypolipidemics etc.

To search for new HMG-CoA reductase inhibitors with less side effects from natural resources, we conducted a screening with Japanese herbal medicines which have been used for remedy or prevention of hyperlipidemia or hypertension.

Material and Method

Materials

Male Wistar strain rats (7 weeks old) were purchased from Kyudo Co.,Ltd. (Saga, Japan). [3-^{14}C] HMG-CoA was obtained from GE Healthcare UK Ltd. (Buckinghamshire, United Kingdom) and [4-^{14}C] testosterone was purchased from PerkinElmer Life and Analytical Sciences Inc. (Boston, Massachusetts). The other reagents used in this study were provided from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant Extracts

Different plants/parts were obtained from the local market in Kashima (Saga, Japan). Plant materials (25 g) (aerial parts, root, leaf) shown in Table were crushed and extracted with distilled water, methanol or dichloromethane (1:15, w/v) for 24 h at a room temperature (25°C). Aqueous extracts were stored at -20°C after filtered through a filter paper and freeze-dried with a lyophilizer. The methanol or dichloromethane extracts were filtered through a filter paper and the filtrate was then concentrated using a rotary evaporator, respectively.

Preparation of Microsomes from Rat Liver

Microsomes were prepared according to the method previously described with a slight modification (Ong, Khor, and Tan, 1991). A male Wister strain rat (7 weeks old) was sacrificed by carbon dioxide. The liver was removed and minced with a pair of scissors. And then homogenized in 50 mM sodium phosphate buffer (pH 7.0) contained 75mM nicotinamide, 20 mM 2-mercaptoethanol, 2.5 mM EDTA and 250 mM sucrose (1:6, w/v) using a glass homogenizer fitted with a Teflon-coated plunger. The homogenate was centrifuged at 2,000g for 10 min and the supernatant was further ultracentrifuged twice at 100,000g for 60 min. The pellet was suspended in a small amount of 100 mM sodium phosphate buffer (pH 7.2) containing 20 mM 2-mercaptoethanol and 20 mM EDTA and used as the source of HMG-CoA reductase.

Assay of HMG-CoA reductase Activity by Thin-Layer Chromatography

HMG-CoA reductase activity was detected according to the method previously described with a slight modification (Ong, Khor, and Tan, 1991). The reaction in a total volume of 100μl contained 100 mM sodium phosphate buffer (pH 7.2), 10 mM imidazole, 5 mM dithiothreitol, 10 mM EDTA, 20 mM NADPH, 30 mM [3-^{14}C] HMG-CoA (0.05μCi), 1μl of 1,000μg /ml plant extracts dissolved in dimethyl sulfoxide and 5μl liver microsomal preparation (7.7μg protein). The reaction was carried out in disposable test tubes, initiated by the addition of microsomal preparation, and incubated at 37°C for 15 min. The reaction was terminated by the addition of 25μl of 1 M HCl and further incubated at 37°C for 60 min to allow lactonization of mevalonate to mevalonolactone. After the incubation, 1.6 pmol [4-^{14}C] testosterone (0.08 nCi) was added as an internal standard and 130μl of ethyl acetate was added to the samples to extract mevalonolactone and testosterone. An aliquot (100μl) of the supernatant was spotted on an activated silica 60G thin-layer plate (0.25-mm thickness) and developed in benzene-acetone (1:1, v/v). The plate was exposed to imaging plate for 15 h and detected radioactivity by imaging analyzer. All values were expressed as the mean ± S.D.

Results and Discussion

To confirm the enzymatic method using rat liver microsome as HMG-CoA reductase, the concentration required for 50% inhibition (IC_{50}) was measured with lovastatin. IC_{50} for lovastatin was calculated to be 0.025μM, whereas the reported value is 0.023μM (Endo, Kuroda, and Tanzawa, 1976). Hence we concluded that the method we modified is acceptable for further experiment.

To search for new HMG-CoA reductase inhibitors from natural resources, we conducted a screening with Japanese herbal medicines which have been used for remedy or prevention of hyperlipidemia or hypertension. Also, to pick out more desirable ingredients, the extract that showed the percentage of HMG-CoA reductase inhibition is more than 80 % at the concentration of 1,000μg/ml, was judged as possible active ingredients. The result showed that aqueous extract prepared from *Artemisia princeps* and ethanol extract prepared from fruits and seeds of *Trachycarpus wagnerianus* had potent inhibitory activity on HMG-CoA reductase with 88.1 ± 3.2 % inhibition and 83.7 ± 0.3 % inhibition at 1,000μg/ml, respectively (Table).

Both aqueous and ethanol extract from *A. princeps* had comparably high inhibitory activity with 88.1 ± 3.2 % (aqueous) and 60.7 ± 6.1 % (ethanol) at 1,000μg/ml. Essential oil from *A. princeps* has inhibitory activity of low density lipoprotein (LDL) oxidation and upregulates the expression of LDL receptor (Han, *et al*., 2009; Chung, *et al*., 2007). HMG-CoA reductase is a key factor in the expression of LDL receptor (Wang, *et al*., 1993), so

Table Plants, plant parts, extract solvents and HMG-CoA reductase inhibitory activity

1	*Acer nikoense*	Aceraceae	Nikko maple	Megusurinoki	branch	M	0.6 ± 5.3
2	*Ajuga decumbens*	Lamiaceae	Creeping bugleweed	Kiransou	herb	W	24.1 ± 7.6
						M	5.8 ± 9.2
3	*Angelica keiskei*	Apiaceae	Ashitaba	Ashitaba	leaf	E	70.0 ± 0.2
4	*Aralia cordata*	Araliaceae	Japanese spikenard	Udo	rhizome	W	40.6 ± 13.0
5	*Artemisia princeps*	Asteraceae	Japanese mugwort	Yomogi	herb	W	88.1 ± 3.2
						M	16.0 ± 1.0
						E	60.7 ± 6.1
6	*Carthamus tinctorius*	Asteraceae	Safflower	Benibana	flower	W	1.4 ± 5.3
7	*Cassia obtusifolia*	Caesalpiniaceae	Senna obtusifolia	Ebisugusa	seeds	W	-1.9 ± 5.8
						M	1.5 ± 1.8
8	*Cirsium japonicum*	Asteraceae	Japanese thistle	Noazami	leaf	W	34.5 ± 11.4
9	*Crataegus cuneata*	Rosaceae	Japanese hawthorn	Sanzashi	fruit/ seeds	W	22.1 ± 1.3
						M	-12.0 ± 2.3
10	*Equisetum arvense*	Equisetaceae	Field horsetail	Sugina	herb	M	5.2 ± 9.7
11	*Glechoma hederacea* subsp. *grandis*	Lamiaceae	Field balm	Kakidooshi	herb	W	61.4 ± 2.3
						M	2.3 ± 0.7
12	*Glehnia littoralis*	Apiaceae	Beach silvertop	Hamabouhuu	root	W	34.2 ± 3.8
13	*Glycine max*	Fabaceae	Soybean	Daizu	seeds	W	0.5 ± 11.0
14	*Gynostemma pentaphyllum*	Cucurbitaceae	Five-leaf ginseng	Amachazuru	above ground part	W	-34.4 ± 0.9
						M	3.9 ± 11.0
						E	62.0 ± 8.2
15	*Houttuynia cordata*	Saururaceae	Heart leaf	Dokudami	herb	M	13.2 ± 2.0
16	*Hypericum erectum*	Guttiferae	Upright St. John's wort	Otogirisou	herb	M	2.6 ± 13.9
17	*Lycium rhombifolium*	Solanaceae	Chinese wolfberry	Kuko	leaf	M	-5.3 ± 4.2
18	*Morus lhou*	Moraceae	Roguwa	Roguwa	root bark	W	42.9 ± 8.3
19	*Plantago asiatica*	Plantaginaceae	Chinese plantain	Oobako	seeds	W	12.1 ± 5.9
						M	-13.5 ± 5.2
20	*Plantago asiatica*	Plantaginaceae	Chinese plantain	Oobako	herb	W	16.6 ± 1.1
						M	-10.2 ± 5.4
21	*Platycodon grandiflorus*	Campanulaceae	Balloon flower	Kikyou	root	W	54.8 ± 5.5
						M	2.5 ± 11.5
						E	53.9 ± 0.1
22	*Polygonatum falcatum*	Liliaceae	Solomon's seal	Narukoyuri	rhizome	W	60.2 ± 0.1
						M	10.0 ± 2.4
						E	59.0 ± 6.6
23	*Trachycarpus wagnerianus*	Arecaceae	Chusan palm	Toujuro	fruit/seeds	M	76.6 ± 1.7
						E	83.7 ± 0.3
24	*Trachycarpus wagnerianus*	Arecaceae	Chusan palm	Toujuro	leaf	M	2.9 ± 1.6

*W: Water extract, M: Methanol extract, E: Ethanol extract, D: Dichloromethane extract
Inhibitory activities were assayed at the concentration of 1000μg/ ml
Data given as mean ± standard deviation, n = 3

the upregulation of LDL receptor of an essential oil prepared from *A. princeps* might be related with HMG-CoA reductase inhibitory activity, but still remained unclear. Also, there is no report about HMG-CoA reductase inhibitory activity of an aqueous extract of *A. princeps*, so it is predicted that various inhibitors contained in *A. princeps*. In addition to this possibility, many beneficial functions to remedy for hyperlipidemia were found from *A. princeps* (Min, *et al*., 2009; Choi, *et al*., 2007; Kim, *et al*., 2008; Han, *et al*., 2009). To evaluate the functions of the extract of *A. princeps* is benefit for finding out of a new agent for hyperlipidemia.

In this study, HMG-CoA inhibitory activity of *T. wagnerianus* was revealed. The ethanol extract of fruit/seeds of *T. wagnerianus* showed the potent HMG-CoA reductase inhibitory activity with 83.7 ± 0.3 % at 1,000μg/ml. Antihypertension activity of *T. wagnerianus* has been known in folk remedies. However, researches about *T. wagnerianus* are very few, so it's necessary to accumulate the information about bioactive

substances contained in *T. wagnerianus*. This report will be clue to know how to exploit of *T. wagnerianus* in healthcare.

Intriguingly, ethanol extract from ground part of *Gynostemma pentaphyllum* inhibited HMG-CoA reductase activity comparably with 62.0 ± 8.2 % at 1,000μg/ml, but its aqueous extract enhanced inversely with -34.4 ± 0.9 % at 1,000μg/ml. Preventive effect for atherosclerosis of *G. pentaphyllum* has been already reported but HMG-CoA reductase inhibitory or enhancive activities are not known (Tan, and He, 2007). Not only antihyperlipidemia but antihypocholesterolemia agents might be proposed from compounds contained in *G. pentaphyllum.*

In conclusion, two of the plants tested here (*A. princeps* and *T. wagnerianus*) exhibited strong inhibitory activity on HMG-CoA reductase. These plants have been known as herbal medicines for hemostasis or antihypertension primarily and in use for many years as packs or tea. Particularly, some studies show that *A. princeps* has many beneficial functions to remedy for hyperlipidemia. Because of enormous clinical data from folk remedies and these multifunctional roles, *A. princeps* has been featured their possible in home-made therapies for hyperlipidemia. Whereas *T. wagnerianus* is not investigated well yet, so other beneficial indications may be found in future. Further studies may lead to their use as safe alternatives to synthetic antihyperlipidemia drugs.

Reference

Bochar DA, Stauffacher CV, Rodwell VW (1999) Investigation of the conserved lysines of Syrian hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase. Biochemistry 38: 15848-15852

Choi HJ, Kim EJ, Han MJ, Baek NI, Kim DH, Jung HG, Kim NJ (2007) Hepatoprotective effect of fermented *Artemisia princeps* Pampanini by lactic acid bacteria. Saengyak Hakhoechi 38: 245-253.

Chung MJ, Kang AY, Park SO, Park KW, Jun HJ, Lee SJ (2007) The effect of essential oils of dietary wormwood (*Artemisia princeps*), with and without added vitamin E, on oxidative stress and some genes involved in cholesterol metabolism. Food Chem Toxicol 45: 1400-1409.

Endo A, Kuroda M, Tanzawa K (1976) Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity. FEBS Lett 72: 323-326

Frimpong K, Rodwell VW (1994) Catalysis by Syrian hamster 3-hydroxy-3-methylglutaryl- CoA reductase. Proposed roles of histidine 865, glutamate 558, and aspartate 766. J Biol Chem 269: 11478-11483

Frishman WH (1998) Biologic markers as predictors of cardiovascular disease. Am J Med 104: 18S-27S

Hamilton-Craig I (2001) Statin-associated myopathy. Med J Aust 175: 486-489

Han JM, Kim MJ, Baek SH, An S, Jin YY, Chung HG, Baek NI, Choi MS, Lee KT, Jeong TS (2009) Antiatherosclerotic effects of *Artemisia princeps* Pampanini cv. Sajabal in LDL receptor deficient mice. J Agric Food Chem 57: 1267-1274.

Kim MJ, Han JM, Jin YY, Baek NI, Bang MH, Chung HG, Choi MS, Lee KT, Sok DE, Jeong TS (2008) In vitro antioxidant and anti- inflammatory activities of jaceosidin from *Artemisia princeps* Pampanini cv. Sajabal. Arch Pharm Res 31: 429-437

LaRosa JC, He J, Vupputuri S (1999) Effect of statins on risk of coronary disease: a meta-analysis of randomized controlled trials. JAMA 282: 2340-2346

Min SW, Kim NJ, Baek NI, Kim DH (2009) Inhibitory effect of eupatilin and jaceosidin isolated from *Artemisia princeps* on carrageenan-induced inflammation in mice. J Ethnopharmacol 125: 497-500.

Ong KK, Khor HT, Tan DTS (1991) Assay of 3-hydroxy-3-methylglutaryl CoA reductase activity using anionic-exchange column chromatography. Anal Biochem 196: 211-214

Pasternak RC, Smith SC Jr, Bairey-Merz CN, Grundy SM, Cleeman JI, Lenfant C (2002) ACC/AHA/NHLBI clinical advisory on the use and safety of statins. Circulation 106: 1024-1028.

Stone NJ (1996) Lipid management: current diet and drug treatment options. Am J Med 101: 40S-49S

Tan H, He Q (2007) Prevention effect of *Gynostemma pentaphyllum* on the rabbits suffered from atherosclerosis caused by high cholesterol feedstuff and testosterone level. Shanxi Yiyao Zazhi 36: 489-492.

Wang X, Briggs MR, Yokoyama C, Goldstein JL, Brown MS (1993) Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter II. J Biol Chem 268: 14497-14504.

Wood RL, Cummins DF (2009) Simvastatin- and fluvastatin-associated nightmares. J Pharm Pract 22: 421-425

HMG-CoAレダクターゼ阻害活性を有する生薬抽出物のスクリーニング

横山　聡[1)]・竹野　明香[1)]・永田　敏郎[1)]・清水　邦義[1)]・近藤　隆一郎[1)]
1）九州大学大学院農学研究院森林資源科学部門　福岡県福岡市東区箱崎6-10-1

要　旨

生薬として用いられている薬用植物22種類を水，メタノール，エタノール，ジクロロメタンで抽出し，各抽出物の3-hydroxy-3-methylglutaryl coenzyme A（HMG-CoA）レダクターゼ阻害活性を測定した。抽出物濃度1,000μg/mlで80%以上の阻害活性を有する試料を選抜した結果，*Artemisia princeps*と*Trachycarpus wagnerianus*がHMG-CoAレダクターゼ阻害成分を含む試料として見出された。*Artemisia princeps*の全草の水抽出物と*Trachycarpus wagnerianus*の果実と種子の混合物のエタノール抽出物は，抽出物濃度1,000μg/mlの試験でそれぞれ88.1±3.2%と83.7±0.3%のHMG-CoAレダクターゼ阻害活性を示した。

キーワード：ヨモギ，トウジュロ，高脂血症，HMG-CoAレダクターゼ，抗高脂血症