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Original Article

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

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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). [$3\text{-}^{14}\text{C}$] HMG-CoA was obtained from GE Healthcare UK Ltd. (Buckinghamshire, United Kingdom) and [$4\text{-}^{14}\text{C}$] 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 Wistar 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 100 μ l contained 100 mM sodium phosphate buffer (pH 7.2), 10 mM imidazole, 5 mM dithiothreitol, 10 mM EDTA, 20 mM

NADPH, 30 mM [$3\text{-}^{14}\text{C}$] HMG-CoA (0.05 μ Ci), 1 μ l of 1,000 μ g/ml plant extracts dissolved in dimethyl sulfoxide and 5 μ l liver microsomal preparation (7.7 μ g 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 25 μ l 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 [$4\text{-}^{14}\text{C}$] testosterone (0.08 nCi) was added as an internal standard and 130 μ l of ethyl acetate was added to the samples to extract mevalonolactone and testosterone. An aliquot (100 μ l) 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 \pm 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 0.025 μ M, whereas the reported value is 0.023 μ M (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 1,000 μ g/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 \pm 3.2 % inhibition and 83.7 \pm 0.3 % inhibition at 1,000 μ g/ml, respectively (Table).

Both aqueous and ethanol extract from *A. princeps* had comparably high inhibitory activity with 88.1 \pm 3.2 % (aqueous) and 60.7 \pm 6.1 % (ethanol) at 1,000 μ g/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	<i>Acer nikoense</i>	Aceraceae	Nikko maple	Megusurinoki	branch	M	0.6 ± 5.3
2	<i>Ajuga decumbens</i>	Lamiaceae	Creeping bugleweed	Kiransou	herb	W	24.1 ± 7.6
						M	5.8 ± 9.2
3	<i>Angelica keiskei</i>	Apiaceae	Ashitaba	Ashitaba	leaf	E	70.0 ± 0.2
4	<i>Aralia cordata</i>	Araliaceae	Japanese spikenard	Udo	rhizome	W	40.6 ± 13.0
5	<i>Artemisia princeps</i>	Asteraceae	Japanese mugwort	Yomogi	herb	W	88.1 ± 3.2
						M	16.0 ± 1.0
						E	60.7 ± 6.1
6	<i>Carthamus tinctorius</i>	Asteraceae	Safflower	Benibana	flower	W	1.4 ± 5.3
7	<i>Cassia obtusifolia</i>	Caesalpiniaceae	Senna obtusifolia	Ebisugusa	seeds	W	-1.9 ± 5.8
						M	1.5 ± 1.8
8	<i>Cirsium japonicum</i>	Asteraceae	Japanese thistle	Noazami	leaf	W	34.5 ± 11.4
9	<i>Crataegus cuneata</i>	Rosaceae	Japanese hawthorn	Sanzashi	fruit/ seeds	W	22.1 ± 1.3
						M	-12.0 ± 2.3
10	<i>Equisetum arvense</i>	Equisetaceae	Field horsetail	Sugina	herb	M	5.2 ± 9.7
11	<i>Glechoma hederacea</i> subsp. <i>grandis</i>	Lamiaceae	Field balm	Kakidooshi	herb	W	61.4 ± 2.3
						M	2.3 ± 0.7
12	<i>Glehnia littoralis</i>	Apiaceae	Beach silvertop	Hamabouhuu	root	W	34.2 ± 3.8
13	<i>Glycine max</i>	Fabaceae	Soybean	Daizu	seeds	W	0.5 ± 11.0
14	<i>Gynostemma pentaphyllum</i>	Cucurbitaceae	Five-leaf ginseng	Amachazuru	above ground part	W	-34.4 ± 0.9
						M	3.9 ± 11.0
						E	62.0 ± 8.2
15	<i>Houttuynia cordata</i>	Saururaceae	Heart leaf	Dokudami	herb	M	13.2 ± 2.0
16	<i>Hypericum erectum</i>	Guttiferae	Upright St. John's wort	Otogirisou	herb	M	2.6 ± 13.9
17	<i>Lycium rhombifolium</i>	Solanaceae	Chinese wolfberry	Kuko	leaf	M	-5.3 ± 4.2
18	<i>Morus lhou</i>	Moraceae	Roguwa	Roguwa	root bark	W	42.9 ± 8.3
19	<i>Plantago asiatica</i>	Plantaginaceae	Chinese plantain	Oobako	seeds	W	12.1 ± 5.9
						M	-13.5 ± 5.2
20	<i>Plantago asiatica</i>	Plantaginaceae	Chinese plantain	Oobako	herb	W	16.6 ± 1.1
						M	-10.2 ± 5.4
21	<i>Platycodon grandiflorus</i>	Campanulaceae	Balloon flower	Kikyuu	root	W	54.8 ± 5.5
						M	2.5 ± 11.5
						E	53.9 ± 0.1
22	<i>Polygonatum falcatum</i>	Liliaceae	Solomon's seal	Narukoyuri	rhizome	W	60.2 ± 0.1
						M	10.0 ± 2.4
						E	59.0 ± 6.6
23	<i>Trachycarpus wagnerianus</i>	Arecaceae	Chusan palm	Toujuro	fruit/seeds	M	76.6 ± 1.7
						E	83.7 ± 0.3
24	<i>Trachycarpus wagnerianus</i>	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 1,000 μ g/ml, but its aqueous extract enhanced inversely with -34.4 ± 0.9 % at 1,000 μ g/ml. Preventive effect for atherosclerosis of *G. pentaphyllum* has been already reported but HMG-CoA reductase inhibitory or enhance 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.

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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

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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1,000 μ g/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1,000 μ g/mlの試験でそれぞれ $88.1 \pm 3.2\%$ と $83.7 \pm 0.3\%$ 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，トウジユロ，高脂血症，HMG-CoAレダクターゼ，抗高脂血症