Isolation of Cholesteryl Ester Transfer Protein Inhibitors from *Panax ginseng* Roots

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We have isolated cholesteryl ester transfer protein (CETP) inhibitors from the extract of Korean *Panax ginseng* C. A. MEYER roots and identified them as polyacetylene analogs. These compounds inhibit human CETP with IC_{50} values of around 20—35 mg/ml.

Key words *Panax ginseng*; cholesteryl ester transfer protein (CETP); panaxynol; panaxydol; panaxytriol

Cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein with a molecular mass of 74 kDa, is a lipid transfer protein found in plasma which mediates the transfer of cholesteryl ester (CE) and triglyceride (TG) between high-density lipoprotein (HDL) and other low-density lipoproteins (VLDL, LDL).[1,2]

Recent studies on a Japanese family with homozygous genetic deficiency of CETP caused by a point mutation in the CETP gene have shown that the levels of HDL and apo A-I were profoundly increased, and the CETP deficiency was associated with a reduced rate of coronary artery disease. Therefore, specific inhibitors of plasma CETP might be good candidates as therapeutic agents for atherosclerotic cardiovascular diseases.[3-7] However, only a few CETP inhibitors are known.[8-10]

Panax ginseng C. A. MEYER has been used as a herbal medicine in Korea, China and Japan for hundreds of years. It was reported that the petroleum ether fraction extracted from ginseng roots inhibits β-hydroxy-β-methyl-glutaryl-CoA (HMG-CoA) reductase,[11,12] and antiplatelet effects of panaxynol isolated from *Panax ginseng* roots were also reported by Teng *et al.*[13] The most interesting results were reported by Schultz *et al.*[14] and Dixit *et al.*,[15] who performed feeding experiments in human and monkeys, respectively, with *Panax ginseng* roots. They found that the total serum cholesterol and LDL-cholesterol were decreased, whereas, the HDL-cholesterol level was increased. These results prompted us to search for CETP inhibitors from the extract of Korean ginseng roots.

We found that the petroleum ether fraction of ginseng roots strongly inhibits CETP. Finally, **1a**, **1b** and **1c** were isolated from the extract of Korean *Panax ginseng* C. A. MEYER roots as CETP inhibitors. Herein, we describe the isolation and biological activity of **1a**, **1b** and **1c**.

Experimental

Ginseng roots (5 years old) harvested in Keumsan, Korea in 1994, were purchased at Keumsan Ginseng Market. The ginseng roots (1 kg) were milled and extracted with 5 l of methanol. The extract was filtered and concentrated under reduced pressure to give a dark residue (43 g), which inhibited CETP by 55% at 100 μg/ml. The residue was suspended in water (1 l) and was partitioned twice between hexane (2 × 500 ml) and water. The hexane layers were combined and concentrated to give an oily residue (1.3 g), which strongly inhibited CETP by 90% at 100 μg/ml. This residue was subjected to flash chromatography (E. Merck, Silica gel 60, 230—400 mesh) with a gradient of hexane–ethyl acetate (10:0 to 8:2, v/v). For further purification, preparative TLC on silica gel with 5% EtOAc–hexane, followed by HPLC (Phenomenex, Ultracarb 10 ODS, 250 × 21.2 mm, 95% MeOH/H_2O at 250 nm) yielded **1a** (120 mg/kg), **1b** (50 mg/kg) and **1c** (10 mg/kg).

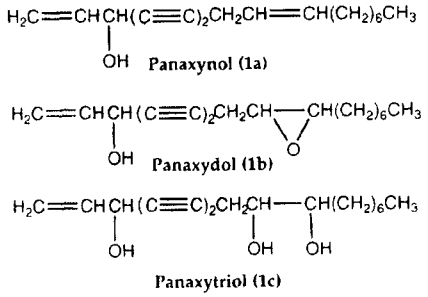

Fig. 1. Structures of the CETP Inhibitors

Results and Discussion

Compounds **1a**, **1b** and **1c** were assigned the molecular formulae $C_{17}H_{24}O$ (M^+: 224), $C_{17}H_{24}O_2$ (M^+: 260) and $C_{17}H_{26}O_3$ (M^+: 278) from electron impact-MS (EI-MS) and ^{13}C-NMR spectra, indicating 5 (for **1c**) and 6 (for **1a**, **1b**) degrees of unsaturation. From NMR experiments (^1H- and ^{13}C-NMR spectra, and ^1H–^1H correlation spectroscopy (COSY), HMQC), the purified CETP inhibitors were identified as panaxynol (**1a**), panaxydol (**1b**) and panaxytriol (**1c**), and this was confirmed by comparision of the spectral data with the reported values.[16-19]

The CETP assay was conducted using a Scintillation Proximity Assay (SPA) kit which was provided by Amersham (US patent 4 568 649). In this CETP-SPA assay, the transfer of [^3H]cholesteryl ester from [^3H]CE-HDL to biotinylated LDL was measured by scintillation counter with SPA bead after incubation of the donor ([^3H]CE-HDL) and the acceptor (biotinylated LDL) in the presence of human recombinant CETP or CETP isolated from human blood for 4 h.[20] The polyacetylene compounds inbihited CETP with IC_{50} values of 25 μg/ml (**1a**), 20 μg/ml (**1b**) and 35 μg/ml (**1c**).

This is the first report of the isolation of CETP inhibitors from an edible source and the first report that ginseng produces CETP inhibitors. Although the polyacetylene analogs **1a** and **1b** only mildly inhibit CETP, the results are of interest in connection with the pharmaceutical activities of ginseng. It also shows that many components of ginseng may contribute to decrease the total serum

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cholesterol and LDL-cholesterol through different mechanisms, such as inhibition of HMG-CoA reductase, CETP and other unknown factors.

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