

Effect of Green Tea and *Pueraria radix* Tea on Apolipoprotein B₁₀₀ Production and Low Density Lipoprotein Activity

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Abstract

In this study, we investigated the effects of green tea and *Pueraria radix* tea on the production of Apo B₁₀₀ in Hep G₂ liver cells and on the expression of the low density lipoprotein (LDL) receptor. Treatment with green tea resulted in a 60.7% decrease on the Apo B₁₀₀ concentration in Hep G₂ cells. *Pueraria radix* tea decreased Apo B₁₀₀ concentration by 63.5% in Hep G₂ cells. Green tea and *Pueraria radix* tea significantly decreased Apo B₁₀₀ concentration by 64.8% and 61.8%, respectively, in the media. Treatment of the cells with green tea and *Pueraria radix* tea also significantly decreased the intracellular total cholesterol, but total cholesterol concentrations in the media increased by 26.4% (green tea) and 23.6% (*Pueraria radix* tea) above that measured in the media of control cells. The addition of green tea and *Pueraria radix* to the media of the Hep G₂ cells increased the LDL receptor binding activities by 84.1% and 79.4%, respectively.

Key words: *Pueraria radix*, green tea, Apo B₁₀₀, Hep G₂ cells, cholesterol, LDL receptor

INTRODUCTION

Atherosclerosis is a complex disease that takes many years to develop. It is believed to involve an inflammatory response that results in the accumulation of lipids in the arterial wall (1). The primary therapeutic strategy aimed at reducing the frequency and progression of atherosclerosis is to decrease arterial exposure to pro-atherogenic lipoproteins (2). Hyperlipoproteinemia is one of several risk factors known to promote atherogenesis, both in human subjects and in experimental animals (3). High plasma cholesterol has been ranked as one of the greatest risk factors, contributing to the prevalence and severity of coronary heart disease. Current strategies include improving dietary habits, lifestyle changes, and early diagnosis and treatment of dyslipidemias (2).

Apo B₁₀₀ is exclusively synthesized by the liver and is the principal constituent of very low density lipoprotein (VLDL), the precursor of low LDL. Apo B containing lipoproteins are associated with increased atherosclerotic risk. Current therapeutic interventions focus on reducing the plasma concentration of LDL and chylomicron remnants by decreasing their production and also by promoting clearance from plasma by the hepatic LDL receptor (4). The rate of VLDL production is dependent on Apo B₁₀₀, so regulation of Apo B₁₀₀ secretion can be important factor of lipid metabolism (5)

Tea is one of the most popular beverages in Oriental

society. There is an increasing interest in green tea as a protective agent against cardiovascular disease (6,7). In this regard, several epidemiologic studies have shown an inverse association between green tea consumption and coronary heart diseases (8,9). Numerous intervention studies in animal models have found that green tea exhibits a hypocholesterolemic effect (10-12). In fact, increased consumption of green tea has been associated with decreased serum triacylglycerols and cholesterol. The effect of drinking green tea on plasma lipoproteins appears to be characterized by decreasing LDL cholesterol and increasing HDL cholesterol (13).

Pueraria lobata is a perennial leguminous vine of the genus *Pueraria* native to eastern Asia and is a traditional oriental medicinal plant (14). Crude extract of the root (*Pueraria radix*) has been used for many disorders such as fevers, gastrointestinal disorders, muscle aches, allergies, respiratory problems, skin problems, high blood pressure, migraine headaches and lowering cholesterol (15). Several studies have shown its benefits in treating chronic alcoholism, reducing the desire for alcohol and protecting organs from damage by alcohol (15-17). However, the efficacy, active constituents and mechanisms of action of this root have been little examined. We have recently demonstrated that *Pueraria radix* has lipid lowering properties in animals. Significant lipid lowering effects were observed in rats when they were supplemented with *Pueraria radix* in association with a high

fat-high cholesterol diet compared to control rats (18).

Hep G₂ cells synthesize and secrete lipoproteins and cholesterol in the same way as human hepatocytes (19). We examined the effects of green tea and *Pueraria radix* tea on VLDL from liver cells. The aim of this study was to compare the effects of green tea and *Pueraria radix* tea on production of Apo B₁₀₀ in Hep G₂ liver cells and on the expression of the LDL receptor, the major mechanism by which cholesterol is removed from circulation. In this study, cells were incubated with the green tea and *Pueraria radix* tea.

MATERIALS AND METHODS

Preparation of green tea and *Pueraria radix* tea

The green tea was prepared fresh for every experiment by brewing 10 g of green tea leaves for 2 min in 100 mL of hot water (80°C) followed by paper filtration.

The roots of *Pueraria lobata* (*Pueraria radix*, chick in Korean) collected in Korea were dried at room temperature in the shade. They were chopped and extracted with distilled boiling water, filtered, and then diluted to a 10% (w/v) solution.

Hep G₂ cell culture

Human cell line Hep G₂ cells were grown under 5% CO₂ at 37°C in Dulbecco's modified Eagles media (DMEM) containing 10% (v/v) fetal calf serum (v/v) and 2% penicillin-streptomycin-glutamine. Cells were grown in 175 cm² flasks containing DMEM supplemented with 10% fetal calf serum until confluent. Cells were then subcultured at 5 × 10⁵ cells into 25 cm² flasks containing the same media. Cells were preincubated in serum free medium supplemented with 1% bovine serum albumin (BSA) then incubated for 24 hours with 10 mL of the media containing 200 µL of the 10% (w/v) freshly brewed green tea or 200 µL of 10% (w/v) *Pueraria radix* tea.

The media from each flask was measured and kept on ice. 3 mL of cold PBS was added to each flask and the Hep G₂ cells were scraped off the flasks into the PBS buffer and were pelleted by centrifugation (1,400 g at 4°C for 5 minutes). The pelleted cells were resuspended in 800 µL of solubilisation buffer, prepared by mixing with 1% Triton in PBS and 1% protease in PBS (5 : 1 molar ratio); unsolubilised cell matter was removed by pelleting with centrifugation at low speed for 5 minutes. The protein content of the solubilised cell supernatant was measured by BSA method (20).

Cholesterol analysis

An aliquot of cells was used to determine total cholesterol concentrations by gas chromatography/mass spectrometry (Hewlett-Packard HP 5890) and another small

aliquot was used to measure cellular protein.

Apo B₁₀₀ quantitation

Apo B₁₀₀ was quantitated in solubilised cells and in the media by Western Blotting using an enhanced chemiluminescence (ECL) detection system.

The solubilised cell samples and media samples were separated by electrophoresis on 3~8% SDS polyacrylamide gradient gel at 150 volts for 60 minutes and electrotransferred onto polyvinylidene di-fluoride (PVDF) membrane at 30 volts for 90 minutes. The membranes were then blocked for 1 hour in 10% skim milk powder solution at room temperature. After washing with TBST, the membranes were incubated in a 1 : 5000 dilution of a primary Apo B antibody in TBST for 1 hour. Rising again with TBST, the membranes were incubated for an hour in 1 : 30000 dilution of secondary antibody (anti-rabbit horse radish peroxidase conjugated) in TBST. Washing with TBST, the PVDF membranes were incubated in the ECL solution for one minute, exposed to the film in a Kodak X-Omatic cassette and developed in an Agfa-Gaevent Radioprint X-ray developer. Apo B₁₀₀ on the film was quantitated by densitometric analysis of the scanned film.

LDL receptor binding activity

Following incubation, the cells from each flask were harvested, resuspended in phosphate buffered saline (PBS) and the protein content was measured (20). Determination of the specific LDL receptor binding activity was measured by the method of Roach et al. (21). The intact Hep G₂ cells (100 µg protein) were incubated for 1 hour at room temperature with LDL-gold conjugates (20 µg protein/mL) in the absence or presence of 20 mM EDTA to determine total and non-specific binding, respectively. After 1 hour, the cells were pelleted by centrifugation, washed and resuspended in 4% (w/v) gum arabic and a silver enhancement IntenSE BL kit solution (Amersham, Australia) was then added. The silver enhancement reaction and absorbance measurements (500 nm) were carried out using the Bio autoanalyser (Roche Diagnostica, New Zealand). The amount of LDL bound to the cells was expressed as ng LDL protein bound per mg cell protein.

Determination of the LDL receptor protein was done by Western blotting with a polyclonal anti-LDL receptor antibody (22).

Statistical analysis

Statistical analysis was carried out using SPSS (SPSS 10.0, SPSS Institute, USA). Means were compared using one-way analysis of variance followed by Duncans multiple range test. Differences were considered significant at $p < 0.05$.

RESULTS

The effects of green tea and *Pueraria radix* tea on Apo B₁₀₀ production and secretion in Hep G₂ cells

The effects of green tea and *Pueraria radix* tea on Apo B₁₀₀ production in Hep G₂ cells were determined. Cells were incubated with green tea and *Pueraria radix* tea for 24 hours, and the Apo B₁₀₀ content in these cells as well as the amount of Apo B₁₀₀ secreted into culture medium were determined. Fig. 1 shows that treatment with green tea resulted in a 60.7% decrease in Apo B₁₀₀ concentrations in Hep G₂ cells. *Pueraria radix* tea decreased Apo B₁₀₀ concentrations by 63.5% in Hep G₂ cells.

The green tea and *Pueraria radix* tea also significantly decreased Apo B₁₀₀ concentration by 64.8% and 61.8%, respectively in the media (Fig. 2). The pattern of decrease in cellular Apo B₁₀₀ levels is very similar to that of the

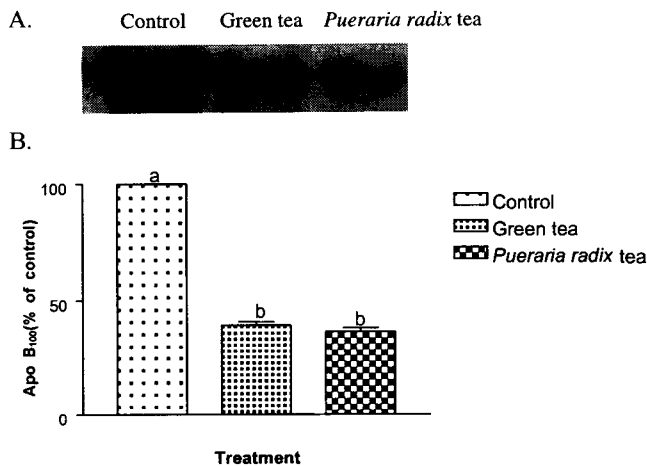


Fig. 1. The effects of green tea and *Pueraria radix* tea on Apo B₁₀₀ production in Hep G₂ cells.

A, Photographs of chemiluminescent detection of the blots are shown. B, Apo B₁₀₀ in the cells is expressed as a percentage of control SEM (n=4). Different letters above bar graphs indicate significance at p < 0.05.

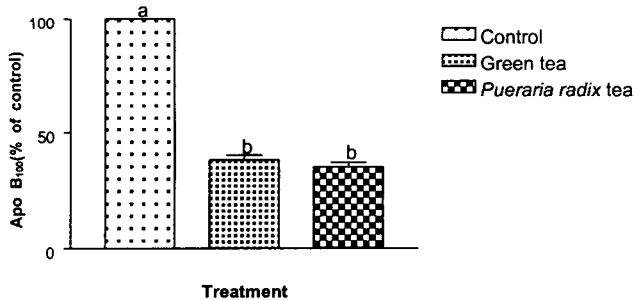


Fig. 2. The effects of green tea and *Pueraria radix* tea on Apo B₁₀₀ concentration secreted into the media by Hep G₂ cells. Apo B₁₀₀ in the cells is expressed as a percentage of control SEM (n=4). Different letters above bar graphs indicate significance at p < 0.05.

secreted Apo B₁₀₀. There was no significant difference between the treatment with green tea and that with *Pueraria radix* tea.

The effects of green tea and *Pueraria radix* tea on cell cholesterol

The effects of green tea and *Pueraria radix* tea on cholesterol content in Hep G₂ cells were studied by measuring the cholesterol content in the cells. As shown in Fig. 3, treatment of the cells with green tea and *Pueraria radix* tea significantly decreased the intracellular total cholesterol. The intracellular cholesterol concentration of the green tea group was lower than that of *Pueraria radix* tea group. However, media cholesterol concentrations were 26.4% (green tea) and 23.6% (*Pueraria radix* tea) higher than in the media of control cells (Fig. 4). There appeared to be an increase in the export of cholesterol from the cells into the media when the cells were exposed to the green tea and *Pueraria radix* tea.

The effects of green tea and *Pueraria radix* tea on LDL receptor

As shown in Table 1, the addition of green tea to the media of the Hep G₂ cells increased the LDL receptor

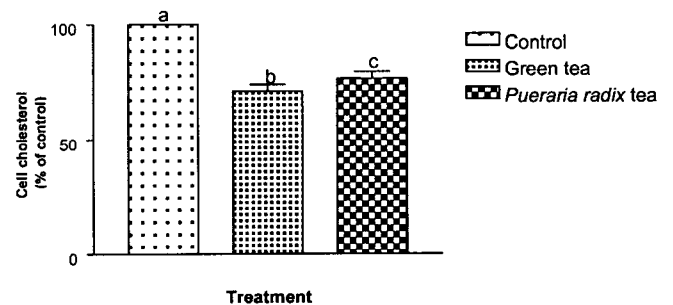


Fig. 3. The effects of green tea and *Pueraria radix* tea on intracellular total cholesterol concentrations.

Cholesterol in the cells is expressed as a percentage of control SEM (n=4). Different letters above bar graphs indicate significance at p < 0.05.

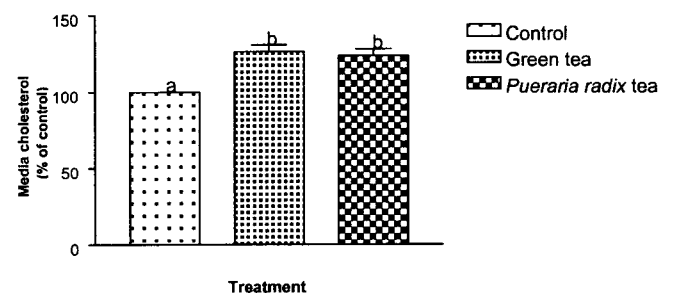


Fig. 4. The effects of green tea and *Pueraria radix* tea on media total cholesterol concentrations.

Cholesterol in the media is expressed as a percentage of control SEM (n=4). Different letters above bar graphs indicate significance at p < 0.05.

Table 1. Effect of green tea and *Pueraria radix* tea on the LDL receptor binding activity and protein in Hep G₂ cells

	LDL receptor binding activity (ng LDL/mg cell protein)	LDL receptor protein (arbitrary absorbance units)
Control	32.0 ± 3.2 ^{1)a2)}	0.31 ± 0.02 ^a
Green tea	58.9 ± 3.5 ^b	3.40 ± 0.21 ^b
<i>Pueraria radix</i> tea	57.4 ± 3.6 ^b	3.48 ± 0.18 ^b

¹⁾Mean ± SE.

²⁾Values with different superscripts are significantly different at = 0.05 by Duncans multiple range test.

binding activity (84.1%). *Pueraria radix* tea also increased the LDL receptor binding activity (79.4%).

Both green tea and *Pueraria radix* tea caused an increase in the amount of LDL receptor protein measured by blotting with the polyclonal LDL receptor antibody. There was no significant difference between the effect of green tea and that of *Pueraria radix* tea.

DISCUSSION

Many studies have found reduction in VLDL and LDL cholesterol levels in animals consuming green tea and *Pueraria radix*, suggesting that green tea and *Pueraria radix* attenuate the secretion of Apo B₁₀₀ in liver cells (10-12,15-18). Therefore, in this study, we examined the effects of green tea and *Pueraria radix* tea on production of Apo B₁₀₀ in Hep G₂ liver cells and on the expression of the LDL receptor, a cell surface protein which controls plasma cholesterol concentrations. For this purpose, human Hep G₂ liver cells, known to express LDL receptors amenable to regulation, were cultured in green tea and *Pueraria radix* tea.

Results demonstrated that when hepatocytes were incubated in the green tea, cellular cholesterol was 28.8% lower. In the *Pueraria radix* tea, cellular cholesterol was 23.5% lower. Cholesterol appears to play an important role in the regulation of Apo B secretion by hepatocytes. The rate of cholesterol synthesis may regulate Apo B secretion by determining the availability of cholesteryl esters (23,24). In cell cultures, Apo B secretion has been shown to be stimulated by cholesterol and inhibited by HMG-Co A reductase inhibitors (25,26). There is a very high correlation ($r=0.956$) between cell cholesterol and the concentration of cholesterol in the media (27). This suggests that the concentration of cholesterol in the media is linked to the amount of cholesterol synthesized by the cells. Our findings show that these teas significantly suppress intracellular cholesterol production. Apo B₁₀₀ is mainly synthesized and secreted by hepatocytes. This apolipoprotein is associated with VLDL and LDL in plasma. The rate of VLDL production is dependent on Apo B₁₀₀, so the reduced production and secretion of Apo B₁₀₀ might decrease the production of VLDL in the liver.

Extra cholesterol was found in the media where its concentration was increased by the teas 26.4% (green tea) and 23.6% (*Pueraria radix* tea) over control. Therefore, green tea and *Pueraria radix* tea appear to have increased the export of cholesterol from the cells into the media. Cholesterol could move from the cells into the media by normal diffusion down the concentration gradient (22). This could explain why the concentration of cholesterol is seen to increase in the media.

When human Hep G₂ liver cells were cultured in the green tea and *Pueraria radix* tea, LDL receptor binding activity and protein were significantly increased compared to control. The increase in LDL receptor binding activity was therefore most likely due to an increase in the number of LDL receptors available to bind LDL on the outer surface of the Hep G₂ cells (28). The clearance of the lipoproteins from the circulation is mediated by the LDL receptor; the LDL receptor pathway is therefore one of the mechanisms by which the blood concentration of cholesterol is controlled (29).

In conclusion, green tea and *Pueraria radix* tea were observed to decrease the intracellular cholesterol concentration of Hep G₂ cells and upregulate their LDL receptors. Upregulation of the LDL receptor may play a role in the hypocholesterolemic effect *in vivo*.

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