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Water Extract of Kudzu Root (*Pueraria radix*) Decreases Apolipoprotein B_{100} and B_{48} Production *in vitro*

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Abstract

We have previously demonstrated that kudzu root extracts have a hypocholesterolemic effect on rats fed diets high in fat and cholesterol. To further elucidate the mechanism involved, in this study we investigated the effect of water extracts of kudzu root, *Pueraria radix,* on the production of apolipoprotein B_{100} (Apo B_{100}) in $HepG_2$ liver cells and secretion of apolipoprotein B_{48} (Apo B_{48}) in $Caco_2$ cells. Human cell lines, $HepG_2$ liver cells and $Caco_2$ intestinal epithelial cells, were grown with various concentrations (0%, 0.5%, 1.0%, 1.5%, 2.0%) of water extracts of kudzu root in the media. The kudzu root extract decreased Apo B_{100} production and secretion. Treatment of Hep G_2 cells with the kudzu root extract also significantly decreased the intracellular total and free cholesterol concentration, and also decreased esterified cholesterol but was only significant at the highest dose of 2%. Apo B_{48} production, but not secretion, from enterocytes was lowered by the kudzu root extracts. This research provided evidence that the hypocholesterolemic properties of kudzu root may be a consequence of decreased production and secretion of Apo B_{100} in the liver and Apo B_{48} in the intestine.

Key words: *Pueraria radix*, kudzu, Apo B_{100}, Apo B_{48}, Hep G_2 cells, $Caco_2$ cells, cholesterol

INTRODUCTION

Atherosclerosis is a common insidious disease, and a major cause of morbidity and mortality in industrialized societies (1,2). Therapeutic strategies for reducing the incidence of mortality and morbidity of atherosclerosis are the subject of ongoing intense research; current strategies include improving dietary habits, lifestyle changes, and early diagnosis and treatment of dyslipidemias (3). Hyperlipoproteinemia is one of several risk factors known to promote atherogenesis, both in human subjects and in experimental animals (4). The development of atherosclerosis and coronary heart disease (CHD) is associated with high circulating levels of cholesterol-rich lipoproteins of hepatic and intestinal origin. There is abundant evidence that disorders of lipid metabolism result in an increased risk for atherosclerosis, and that lowering low density lipoprotein (LDL)-cholesterol reduces morbidity and mortality from CHD (5).

Apo B_{100} is a liver derived, major structural protein for the assembly and secretion of LDL and very low density lipoprotein (VLDL). Increased expression of apolipoprotein B (Apo B) lipoproteins is associated with increased atherosclerotic risk because hepatic VLDL is the major precursor for LDL the major protein for transporting cholesterol to peripheral tissue (6). The rate of VLDL production is dependent on Apo B_{100}, therefore the regulation of Apo B_{100} secretion can be an important modulator of cholesterol metabolism.

Apo B_{48} is an excellent marker of postprandial lipoproteins because it is associated exclusively in humans with intestinally derived chylomicrons and their remnants, circulating particles that have been described as atherogenic (7) Intestinal Apo B_{48} is believed to be a key regulator of chylomicron synthesis and secretion (8).

Kudzu (*Pueraria lobata*) is a perennial leguminous vine that is native to eastern Asia where it is used in traditional oriental medicinal plant (9). Kudzu tea has a sweet and acrid taste, and is used as an antipyretic, anti-diarrhetic, and anti-emetic agent. Crude root extracts have been used for the treatment of hypertension and alcohol intoxication (10). However, few studies have investigated the efficacy, active constituents, or mechanisms of action of kudzu remedies.

Water extracts of Kudzu root have been shown to lower circulating cholesterol concentrations in rats fed diets high in fat and cholesterol (11). In chronically alcoholic rats, Kudzu extracts lowered VLDL and LDL cholesterol levels and reduced alcohol induced peroxidative damage (12,13). It is unknown how this root affects lipoprotein metabolism.

Hep G_2 is a hepatocellular carcinoma cell line that synthesizes and secretes lipoproteins and cholesterol in the

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same manner as normal hepatocytes. $Caco_2$ cells are human colonic carcinoma cells that show morphological and biochemical properties consistent with fully differentiated small intestinal enterocytes (14). $Caco_2$ cells are suitable to use as a model to study the synthesis and secretion of chylomicrons in the intestine.

The aim of this study was to investigate the effect of the water extract of kudzu root on the production of Apo B_{100} in Hep G_2 liver cells and secretion of Apo B_{48} in $Caco_2$ cells.

MATERIALS AND METHODS

Preparation of the water extract of kudzu root

Kudzu roots, *Pueraria lobata* (*Pueraria radix*), were collected in Korea and dried at room temperature in the shade. The dried roots were chopped, extracted with boiling distilled water, filtered, and then concentrated under reduced pressure with a vacuum rotary evaporator at 6°C. The concentrated extract was freeze-dried for storage until used.

Hep G_2 cell culture

Hep G_2 cells were grown under 5% CO_2 at 37°C in Dulbecco's modified Eagle's media (DMEM) containing 10% (v/v) fetal calf serum (v/v) and 2% penicillin-streptomycin-glutamine. Cells were preincubated in serum free medium supplemented with 1% bovine serum albumin (BSA) then incubated for 24 h in the various amounts (0%, 0.5%, 1.0%, 1.5%, 2.0%) of kudzu extract.

3 mL of cold PBS was added to each flask and the Hep G_2 cells scraped off the sides into the PBS buffer. The cells were then pelletized by centrifugation (1,400 g at 4°C for 5 minutes). The pellets were resuspended in 800 μL of solubilization buffer and unsolubilized cell matter removed by pelleting by centrifugation at low speed for 5 minutes. The protein content of the solubilized cell supernatant was determined.

$Caco_2$ cell culture

$Caco_2$ cells were incubated for 24 hours at 37°C in DMEM with 20% fetal calf serum (v/v), 2% penicillin-streptomycin-glutamine and 1% non-essential amino acids. Cells were seeded at a density of 1×10^6 per 75 cm^2 flask. The media was replaced every 2 days. Cells were subcultured from flasks, at 90% confluency, onto polycarbonate microporous membranes (0.4 μm pore size, 24.5 mm diameter inserts) and plated at a density of 2×10^4 cells/6.5 mm. Fully differentiated $Caco_2$ cells were used for all experiments (13 days post-subculturing). 'Treatment' media was added to the apical well after washing the cells with phosphate buffered saline (PBS). DMEM supplemented with 2% penicillin-streptomycin-glutamine and 1% non-essential amino acids was placed in the basolateral well.

Cells were incubated for 24 hour with varied concentrations (0%, 0.5%, 1.0%, 1.5%, 2.0%) of kudzu extract.

The $Caco_2$ cells were harvested in PBS with a rubber scraper and pelleted at 2000 rpm for 5 minutes at 4°C. The supernatant was removed and the cell pellet was resuspended in solubilization buffer for 2 hours. Samples were pelleted at 9000 rpm for 5 minutes at 4°C and the supernatant collected. The protein content of the solubilized cell supernatant was measured. The supernatant was used to measure Apo B_{48} and assayed for cholesterol concentrations.

Cholesterol analysis

An aliquot of cells was used to determine total cholesterol and free cholesterol concentrations by gas chromatography/mass spectrometry (Hewlett-Packard HP 5890) and another small aliquot was used to measure cell protein using the Lowry method (15).

Apo B_{100} and Apo B_{48} quantitation

Apo B_{100} and Apo B_{48} were quantitated in solubilized cells and in the basolateral media. A Western Blotting technique was utilized with an enhanced chemiluminescence (ECL) detection system.

The solubilized cell samples and media samples were separated by electrophoresis on a 3~8% SDS polyacrylamide gradient gel at 150 volts for 60 minutes and electrotransferred onto polyvinyladine di-fluoride (PVDF) membranes at 30 volts for 90 minutes; the membranes were 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. After rinsing again with TBST, the membranes were incubated for an hour in a 1:30000 dilution of secondary antibody (anti-rabbit horse radish peroxidase conjugated) in TBST. After again washing with TBST, the PVDF membranes were incubated in the ECL solution for one minute, exposed to x-ray film in a Kodak X-Omatic cassette and developed in Agfa-Gaevert Radioprint X-ray developer; Apo B_{100} and Apo B_{48} were quantified by densitometry.

RESULTS

The effect of kudzu water extract Apo B_{100} production and secretion in Hep G_2 cells

Incubation of Heg G_2 cells with kudzu root extract (*Pueraria radix*) resulted in a 49.1% (1.0%). 50.0% (1.5%) and 62.2% (2.0%) decrease in Apo B_{100} concentrations, and in the media Apo B_{100} concentrations were decreased by 23.7% (0.5%), 34.5% (1.0%), 61.8% (1.5%) and 66.2% (2.0%) (Fig. 1). There was a similar pattern for the decreased concentrations of cellular and secreted Apo B_{100}.

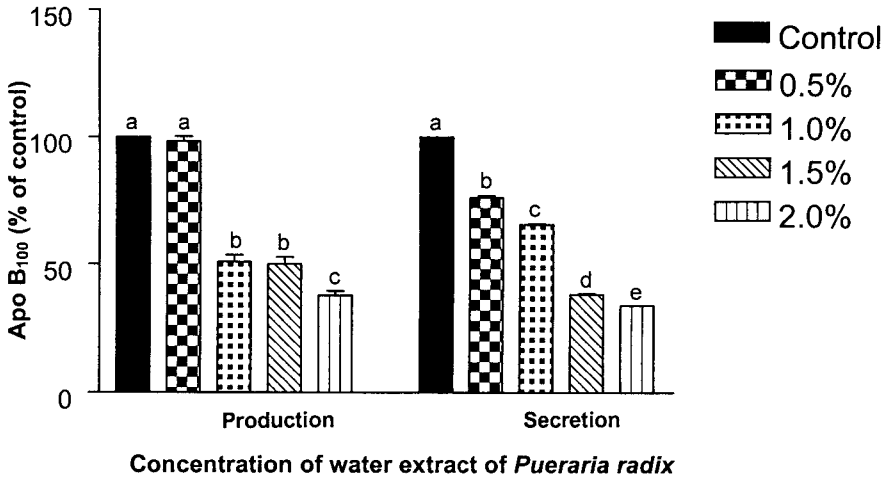

Fig. 1. Effects of several concentrations of kudzu root extract on Apo B_{100} production and secretion in Hep G_2 cells. Hep G_2 cells were incubated kudzu root extracts for 24 h in media containing FCS. Apo B_{100} concentrations in the cells and secreted into the media are expressed as a percentage of control ± SEM (n=4). Different letters above bars indicate significance at $p<0.05$

Effects of kudzu root extracts on cholesterol concentrations in Hep G_2 cells

As shown in Fig. 2, treatment of the cells with kudzu root extracts significantly decreased the intracellular total cholesterol and non-esterified cholesterol. The maximal decline in cholesterol was seen with the 1.5% concentration of the extract. The kudzu root extract also decreased the intracellular concentrations of cholesterol esters, but was only significant at the highest dose of 2% (Fig. 2).

The effect of kudzu root extract on Apo B_{48} production and secretion in $Caco_2$ cells

Kudzu root extract also decreased the Apo B_{48} concentration in $Caco_2$ cells but the effect was only significant at the 1.5% and 2.0% concentrations (Fig. 3). However, there were no significant differences in the Apo B_{48} levels in the media incubated with the water extract.

The effect of water extract of kudzu root on cholesterol content in $Caco_2$ cells

Intracellular total and esterified cholesterol were significantly decreased by incubation with kudzu extracts at concentrations greater than 1.0% (Fig. 4). Free cholesterol was unchanged by the extract at any concentration.

DISCUSSION

We previously reported that kudzu root extract lowers VLDL and LDL cholesterol concentrations in rats. One possible explanation for the cholesterol lowering effect is that kudzu root extract attenuates the secretion of apo B_{100} in liver and the production of Apo B_{48} in intestines. Therefore, this study investigated the hypothesis that kudzu root exerts its hypocholesterolemic effect by downregulating the production and secretion of Apo B_{100} and Apo B_{48}.

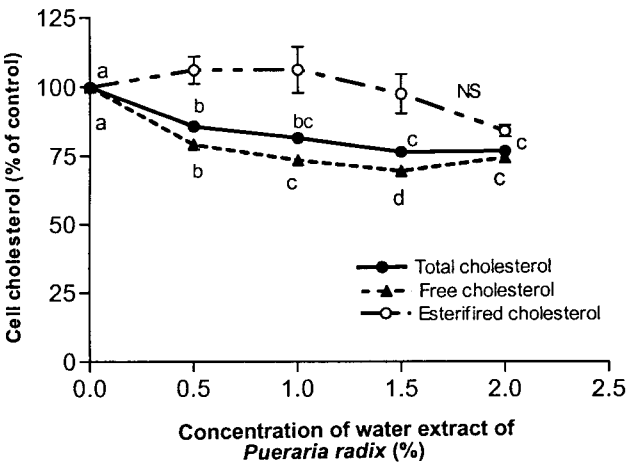

Fig. 2. The effect of kudzu root extracts on intracellular total cholesterol, free cholesterol and esterified cholesterol concentrations in Hep G_2 cells. Hep G_2 cells were incubated with water extracts of kudzu root for 24 hours in media containing FCS Cholesterols in the cells are expressed as a percentage of control ± SEM (n=3). Different letters indicate significance of differences at $p<0.05$. NS: not significant at $p<0.05$.

It is well established that disorders of lipid metabolism can result in an increased risk for atherosclerosis, and that lowering of LDL-cholesterol reduces morbidity and mortality from CHD (5). This study investigated the hypocholesterolemic effect of kudzu root extract on cholesterol metabolism in a human hepatoma cell line (Hep G_2) an established model for studying the regulation of human hepatic cholesterol metabolism (16).

Treatment of cells with kudzu root extract significantly decreased intracellular total and free cholesterol concentrations as well as esterified cholesterol at the highest dose of 2%. Apo B_{100} production and secretion was also decreased in a dose dependent manner that mirrored the ef-

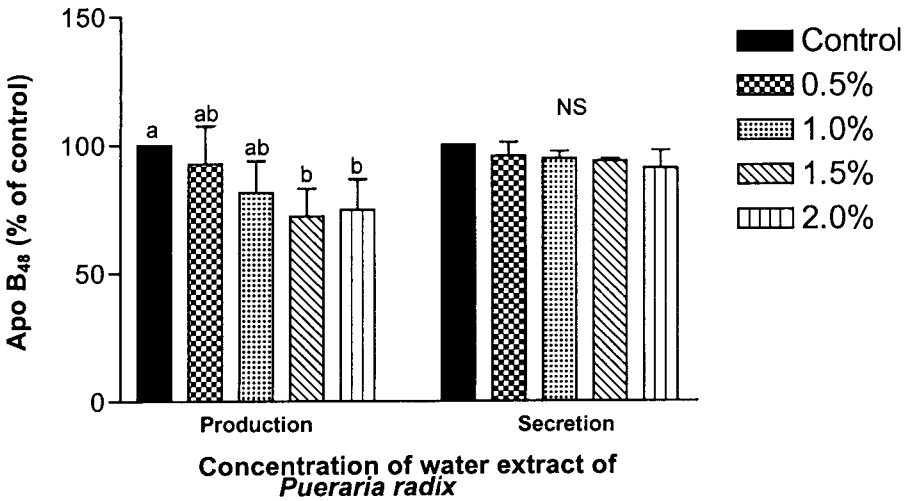

Fig. 3. Effects of of kudzu root extrats on Apo B_{48} production and secretion in $Caco_2$ cells. $Caco_2$ cells were incubated with kudzu root extracts for 24 hours in media containing FCS. Apo B_{48} concentrations in the cells and secreted into the media are expressed as a percentage of control ± SEM (n=3). Different letters above bars indicate significance at $p<0.05$. NS: not significant at $p<0.05$

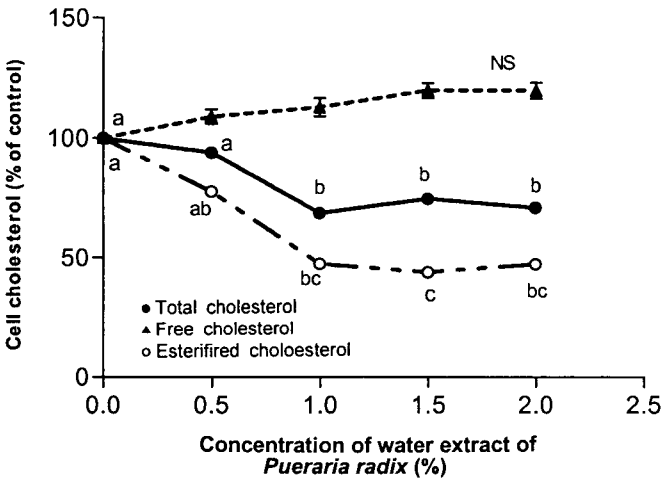

Fig. 4. The effect kudzu root extracts on intracellular total, free, and esterified cholesterol concentrations. $Caco_2$ cells were incubated with kudzu root extracts for 24 h in media containing FCS. Intracelluar cholesterols are expressed as a percentage of control ± SEM (n=3). Different letters indicate significance at $p<0.05$. NS: not significant at $p<0.05$.

fect on cholesterol concentrations.

Apo B_{100} is primarily synthesized and secreted by hepatocytes, and is closely associated with VLDL and LDL in plasma. Cholesterol appears to play an important role in the regulation of Apo B secretion by hepatocytes, and the rate of cholesterol synthesis may, in turn, regulate Apo B secretion by determining the availability of cholesteryl esters (17,18). In cell cultures, Apo B secretion has been shown to be stimulated by cholesterol and inhibited by HMG-Co A reductase inhibitors (19,20). Therefore, these results demonstrate that kudzu root extract significantly lowers the intracellular production of cholesterol, and since the rate of VLDL production is dependent on Apo B_{100}, the reduced production and secretion of Apo B_{100} might be responsible for a decreased production of VLDL in the liver.

Apo B_{48} is a structural protein associated with chylomicron particles and is an exclusive marker of chylomicrons since it is not associated with other lipoproteins (21). After ingesting a fat-containing meal, the dietary lipids (triglyceride and cholesterol) are packaged together with an Apo B_{48} protein into a chylomicron particle by the enterocyte (22). High concentrations of post-prandial triglyceride-rich lipoproteins (chylomicrons and VLDL remnants) may be causally related to the development of atherosclerosis (3). Therefore, decreasing the production of Apo B_{48} should decrease the secretion of chylomicrons from the intestine.

Kudzu root extract decreased Apo B_{48} production, but not Apo B_{48} secretion from enterocytes. Decreased intracellular cholesterol concentrations may induce reduced production of Apo B_{48}. However, the reason for the decreased production, but not secretion, of Apo B_{48} is unknown.

These results suggest that decreased production and secretion of Apo B_{100} and production of Apo B_{48} may be the mechanisms responsible for the hypocholesterolemic effect of kudzu root extract.

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