The medicinal plant goldenseal is a natural LDL-lowering agent with multiple bioactive components and new action mechanisms

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Abstract Our previous studies have identified berberine (BBR), an alkaloid isolated from the Chinese herb huanglian, as a unique cholesterol-lowering drug that upregulates hepatic low density lipoprotein receptor (LDLR) expression through a mechanism of mRNA stabilization. Here, we demonstrate that the root extract of goldenseal, a BBR-containing medicinal plant, is highly effective in upregulation of liver LDLR expression in HepG2 cells and in reducing plasma cholesterol and low density lipoprotein cholesterol (LDL-c) in hyperlipidemic hamsters, with greater activities than the pure compound BBR. By conducting bioassay-driven semi-purifications, we demonstrate that the higher potency of goldenseal is achieved through concerted actions of multiple bioactive compounds in addition to BBR. We identify canadine (CND) and two other constituents of goldenseal as new upregulators of LDLR expression. We further show that the activity of BBR on LDLR expression is attenuated by multiple drug resistance-1 (MDR1)-mediated efflux from liver cells, whereas CND is resistant to MDR1. This finding defines a molecular mechanism for the higher activity of CND than BBR. We also provide substantial evidence to show that goldenseal contains natural MDR1 antagonist(s) that accentuate the upregulatory effect of BBR on LDLR mRNA expression.■ These new findings identify goldenseal as a natural LDL-c-lowering agent, and our studies provide a molecular basis for the mechanisms of action.— Abidi, P., W. Chen, F. B. Kraemer, H. Li, and J. Liu. **The medicinal plant goldenseal is a natural LDL-lowering agent with multiple bioactive components and new action mechanisms.** *J. Lipid Res.* 2006. **47:** 2134–2147.

Supplementary key words low density lipoprotein cholesterol • canadine • berberine • mRNA stabilization • multiple drug resistance-1 • extracellular signal-regulated kinase activation • hypercholesterolemia

Coronary heart disease is the major cause of morbidity and mortality in the Western population (1). Increased plasma low density lipoprotein cholesterol (LDL-c) level is postulated to be the primary risk factor for the development of coronary heart disease and atherosclerosis (2, 3).

In humans, >70% of LDL-c is removed from plasma by low density lipoprotein receptor (LDLR)-mediated uptake in the liver (4). Hence, the expression level of hepatic LDLR directly influences plasma cholesterol levels; therefore, regulation of liver LDLR represents a key mechanism by which therapeutic agents could intervene in the development of coronary heart disease and atherosclerosis.

Hepatic LDLR expression is predominantly regulated at the transcriptional level through a negative feedback mechanism by intracellular cholesterol pools. This regulation is controlled through specific interactions of the sterol-regulatory element of the LDLR promoter (5, 6) and a family of sterol-regulatory element binding proteins (7–9). The activation of LDLR transcription through the depletion of intracellular cholesterol is the principal working mechanism of the current cholesterol-lowering statin drugs (10). Statins are specific inhibitors of HMG-CoA reductase, the rate-limiting enzyme in cellular cholesterol biosynthesis. The depletion of the regulatory cholesterol pool in the liver results in an increased expression of LDLR and an enhanced uptake of LDL particles from the circulation. Since the development of lovastatin as the first HMG reductase inhibitor several decades ago, statin therapy has been the primary treatment choice for hypercholesterolemia (11–16), because of its high efficacy and improved clinical outcomes. Nevertheless, there is still a need to develop additional cholesterol-lowering agents to treat hyperlipidemia (1, 17).

Our interest in the discovery of new LDLR modulators from natural resources has led to the identification of berberine (BBR), an alkaloid isolated from the Chinese herb huanglian, as a novel upregulator of hepatic LDLR

Abbreviations: BBR, berberine; CND, canadine; ELSD, evaporative light-scattering detection; ERK, extracellular signal-regulated kinase; HC, high-cholesterol; HDT, hydrastine; HDTN, hydrastinine; LDL-c, low density lipoprotein cholesterol; LDLR, low density lipoprotein receptor; MDR, multiple drug resistance; OM, oncostatin M; PMT, palmatine; siRNA, small interfering RNA; TC, total cholesterol; TG, triglyceride; VRPM, verapamil.

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This article is available online at http://www.jlr.org

(18, 19). By conducting studies in human hepatoma-derived cell lines, we showed that BBR strongly increases LDLR mRNA and protein expression. Our studies further revealed that BBR upregulates LDLR expression by extending the half-life of LDLR mRNA without affecting gene transcription. Thus, BBR uses a mechanism of action different from statins. A placebo-controlled clinical study conducted in China showed that oral administration of BBR in 32 hypercholesterolemic patients at a daily dose of 1 g for 3 months reduced plasma total cholesterol (TC) by 29%, triglyceride (TG) by 35%, and LDL-c by 25% without side effects (18). These in vitro and clinical studies suggested a possible use of BBR in the treatment of hyperlipidemia.

BBR is not only present in the Chinese herb huanglian (*Coptis chinesis*) but is also an indigenous component of other members of the plant family Ranunculaceae, such as goldenseal (*Hydrastis canadensis* L.) (20). Goldenseal is native to the eastern region of North America, and its products are extracts from the dried root of the plant. Goldenseal is among the top five herbal products currently on the U.S. market and has been used to treat a variety of illnesses, such as digestive disorders, urinary tract infection, and upper respiratory inflammation (21). However, the effects of goldenseal in regulating plasma lipid and cholesterol levels have never been studied. In this investigation, we examined the effects of goldenseal in regulating hepatic LDLR expression and LDL-c metabolism in the human hepatoma-derived cell line HepG2 and in hypercholesterolemic hamsters.

MATERIALS AND METHODS

Analysis and quantitation of alkaloid components in goldenseal

BBR chloride, (−)-canadine (CND), β-hydrastine (HDT), hydrastinine (HDTN), and palmatine (PMT) were purchased from Sigma, and stock solutions of 10 mg/ml in DMSO were prepared and used as standards in HPLC, LC-MS, and evaporative light-scattering detection (ELSD). Goldenseal root extract Lot 1 was manufactured by Solgar Vitamin and Herb (Leonia, NJ), Lots 2, 8, and 9 were manufactured by Country Sun (Palo Alto, CA), Lot 3 was manufactured by Now Foods (Bloomingdale, IL), Lot 4 was manufactured by The Vitamin Shoppe (North Bergen, NJ), Lots 5 and 6 were manufactured by Nature's Way Products, Inc. (Springville, UT), and Lot 7 was manufactured by Gala Herbs (Brevard, NC). Lots 1–6 were in powder form and were extracted with ethanol. Lots 7–9 were in 60% grain alcohol. The ethanol extract of goldenseal was diluted in methanol and subjected to HPLC, ELSD, and LC-MS to determine the alkaloid contents. Chemical analyses were performed by Combinix, Inc. (Mountain View, CA).

Quantitation of LDLR mRNA expression by Northern blot analysis and real-time PCR

Isolation of total RNA from HepG2 and from hamster livers and analysis of LDLR and GAPDH mRNA by Northern blot were performed as described previously (18, 22). Differences in hybridization signals of Northern blots were quantitated with a PhosphorImager. For comparative real-time PCR assays, the reverse transcription was conducted with random primers using Moloney murine leukemia virus (Promega) at 37°C for 1 h in a volume of 25 μl containing 1 μg of total RNA. Real-time PCR was performed on the cDNA using the ABI Prism 7900-HT Sequence Detection System and Universal MasterMix. Human and hamster LDLR and GAPDH PreDeveloped TaqMan Assay Reagents (Applied Biosystems) were used to assess mRNA expression in HepG2 cells and hamster livers. Multiple drug resistance-1 (MDR1) mRNA expression in HepG2 cells was assayed similarly using the PreDeveloped probes from Applied Biosystems.

LDL uptake assay

HepG2 cells on six-well culture plates were treated with compounds for 18 h. Fluorescent DiI-LDL (Biomedical Technologies, Stoughton, MA) at a concentration of 6 μg/ml was added to the cells at the end of treatment for 4 h, and cells were trypsinized. The mean red fluorescence of 2×10^4 cells was measured using FACScan (filter 610/20 DF, BD LSRII; Becton Dickinson).

Transient transfection and dual luciferase reporter assays

HepG2 cells were transfected with plasmid DNA (100 ng/well) using FuGENE 6 transfection reagent. The DNA ratio of pLDLR234Luc (22) to renilla luciferase reporter pRL-SV40 was 90:10. Twenty hours after transfection, medium was changed to 0.5% FBS and drugs were added for 8 h followed by cell lysis. The luciferase activity in cell lysate was measured using the Dual Luciferase Assay System from Promega. Triplicate wells were assayed for each transfection condition.

Semipurification of goldenseal alkaloid components

One milliliter of goldenseal liquid extract was subjected to flash chromatography over a silica gel column with a chloroform-methanol 90–50% gradient as an eluting solvent. Twenty-six 15 ml fractions were collected. A total of 200 μl of each fraction was used directly to measure the fluorescence intensity with a fluorescent microplate reader (Spectra MaxGemini; Molecular Devices, Sunnyvale, CA) at 350 nm excitation and 545 nm emission (23). The rest of the fraction was evaporated under N_2, and residues in each fraction were dissolved in 250 μl of DMSO. Ten microliters from each fraction was diluted with 90 μl of ethanol and applied to HPLC, ELSD, and LC-MS.

BBR uptake assay

HepG2 cells were seeded on six-well culture plates at a density of 0.8×10^6 cells/well in medium containing 10% FBS. The next day, cells were incubated with medium containing 0.5% FBS. BBR at a concentration of 15 μg/ml or goldenseal with an equivalent amount of BBR was added to the cells for the indicated times. At the end of treatment, cells were washed with cold PBS and trypsinized. Cell suspensions in PBS were placed on ice to minimize efflux activity. The mean green fluorescence of 2×10^4 cells was measured using FACScan (filter 525/50HQ, BD LSRII; Becton Dickinson).

MDR direct dye efflux assay

The MDR Direct Dye Efflux Assay kit (No. ECM910; Chemicon International, Inc., Temecula, CA) was used to measure MDR1 activity. HepG2 cells seeded on six-well culture plates were incubated in efflux buffer (RPMI + 2% BSA) and 0.2 μg/ml $DiOC_2$ in the absence or presence of the tested compounds at 37°C for 2 h. Cells were washed with cold PBS and trypsinized. Cell suspensions in PBS were placed on ice to minimize efflux activity. The mean green fluorescence of 2×10^4 cells was measured using

FACScan (filter 530/30DF, BD LSRII; Becton Dickinson). The weak green fluorescence of goldenseal constituted <1% of the fluorescent signal of DiOC$_2$ and was ignored.

Small interfering RNA transfection

Predesigned small interfering RNAs (siRNAs) targeted to human MDR1 (No. 51320) and a negative control with a scrambled sequence (No. 4618G) were obtained from Ambion. HepG2 cells were seeded on six-well culture plates and were transfected with siRNA using the Silencer™ siRNA Transfection II Kit (Ambion) according to the given instructions. After 3 days, transfected cells were untreated or treated with BBR, CND, or goldenseal for 6 h before RNA isolation.

Goldenseal in vivo studies

Thirty-three male Golden Syrian hamsters at 6–8 weeks of age were purchased from Charles River Laboratories and were housed in cages (three animals per cage) in an air-conditioned room with a 12 h light cycle. Animals had free access to autoclaved water and food. After 1 week on a regular rodent chow diet, 27 hamsters were switched to a rodent high-cholesterol (HC) diet containing 1.25% cholesterol (product D12108, Research Diet, Inc., New Brunswick, NJ) and 6 hamsters were fed a control normal diet containing 0.37% fat and no cholesterol (product D12102; Research Diet, Inc.). After 21 days, hamsters on the HC diet were randomly divided into three groups (n = 9 per group) and were given goldenseal at 125 µl/day or BBR at 1.8 mg/day intraperitoneally once per day at 9 AM. The control group received an equal volume of vehicle (20% hydroxypropyl-β-cyclodextrin). Goldenseal grain alcohol extract Lot 9 was dried under a nitrogen stream and resuspended in 2× volume of 20% hydroxypropyl-β-cyclodextrin to a final BBR concentration of 3.6 mg/ml. BBR chloride was dissolved in the same vehicle solution. Four hours after the last drug treatment, all animals were euthanized. At the time of dissection, body weight, liver weight, and gross morphology of the liver were recorded. Livers were immediately removed, cut into small pieces, and stored at −80°C for RNA isolation, protein isolation, and cholesterol content measurement. Animal use and experimental procedures were approved by the Institutional Animal Care and Use Committee of the Department of Veterans Affairs Palo Alto Health Care System.

Histopathology assessment

For histological examination, pieces of liver tissues were either immersed in OCT solution under liquid N$_2$ and stored at −80°C for Oil Red O staining or fixed in 10% paraformaldehyde at room temperature for hematoxylin and eosin staining. Tissue sections were processed and stained at Stanford University's Histology Research Core Laboratory using routine laboratory procedures. After staining, tissue sections were evaluated by a veterinary pathologist and an experienced scientist independently.

Serum isolation and cholesterol determination

Blood samples (0.2 ml) were collected from the retro-orbital plexus using heparinized capillary tubes under anesthesia (2–3% isoflurane and 1–2 l/min oxygen) after an 8 h fast (7 AM to 3 PM) before and during the drug treatments. Serum was isolated at room temperature and stored at −80°C. Standard enzymatic methods were used to determine TC, TG, LDL-c, HDL-c, and FFA levels with commercially available kits purchased from Stanbio Laboratory and Wako Chemical GmbH (Neuss, Germany). Each sample was assayed in duplicate.

Measurement of hepatic cholesterol

One hundred milligrams of frozen liver tissue was thawed and homogenized in 2 ml of chloroform-methanol (2:1). After homogenization, lipids were further extracted by rocking samples for 1 h at room temperature, followed by centrifugation at 5,000 g for 10 min. One milliliter of lipid extract was dried under a nitrogen stream and redissolved in 1 ml of ethanol. TC and TG were measured using commercially available kits.

HPLC analysis of lipoprotein profiles

Twenty microliters of each serum sample from hamsters on a normal diet (n = 6), an HC diet (n = 9), and an HC diet treated with goldenseal (n = 9) was pooled. Cholesterol and TG levels of each of the major lipoprotein classes, including chylomicron, VLDL, LDL, and HDL, in the pool sera were analyzed by HPLC (24) at Skylight Biotech, Inc. (Tokyo, Japan).

Western blot analysis of phosphorylated extracellular signal-regulated kinase in liver tissues and in HepG2 cells

Approximately 90–100 mg of hamster liver tissue from each animal was pooled from the same treatment group (n = 9) and homogenized in 5 ml of buffer containing 20 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 1 mM CaCl$_2$, and cocktails of phosphatase inhibitors (Sigma) and protease inhibitors (Complete Mini; Roche Diagnostic). Total homogenate was centrifuged at 800 g for 5 min to pellet nuclei, and the supernatant was filtered through muslin cloth. The filtrate was subjected to 100,000 g centrifugation for 1 h at 4°C to obtain the cytosolic fraction. After protein quantitation using the BCA™ protein assay reagent (Pierce), 50 µg of protein from each pooled sample was subjected to SDS-PAGE, followed by Western blotting using anti-phosphorylated extracellular signal-regulated kinase (ERK; Cell Signaling) antibody and antibody against total ERK (Santa Cruz Biotechnology). To analyze ERK activation in HepG2 cells, cells seeded on six-well culture plates in 0.5% FBS Eagle's Minimum Essential Medium (EMEM) were treated with 10 µg/ml of each alkaloid as well as goldenseal (1.5 µl/ml; Lot 8) for 2 h, and cell lysates were collected as described previously (18).

Statistical analysis

Significant differences between control and treatment groups or between BBR- and CND-treated samples were assessed by Student's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Goldenseal strongly upregulates LDLR expression in HepG2 cells

Goldenseal contains three major isoquinoline alkaloids, BBR, CND, and HDT, as well as some minor alkaloid components such as HDTN (20, 21, 25, 26) (**Fig. 1A**). Although PMT exists in *Coptis*, Oregon grape root, and several other BBR-containing plants (20), CND and HDT are the only native components of goldenseal (26–28). Goldenseal root extract typically contains 2.5–6% total alkaloids (27).

To determine the activity of goldenseal in the regulation of LDLR expression, we first performed HPLC analysis on goldenseal ethanol extracts obtained from six different commercial suppliers. HPLC/ultraviolet photodiode array detector (UV-DAD) spectroscopic comparisons with standard solutions were used to confirm the presence of BBR,
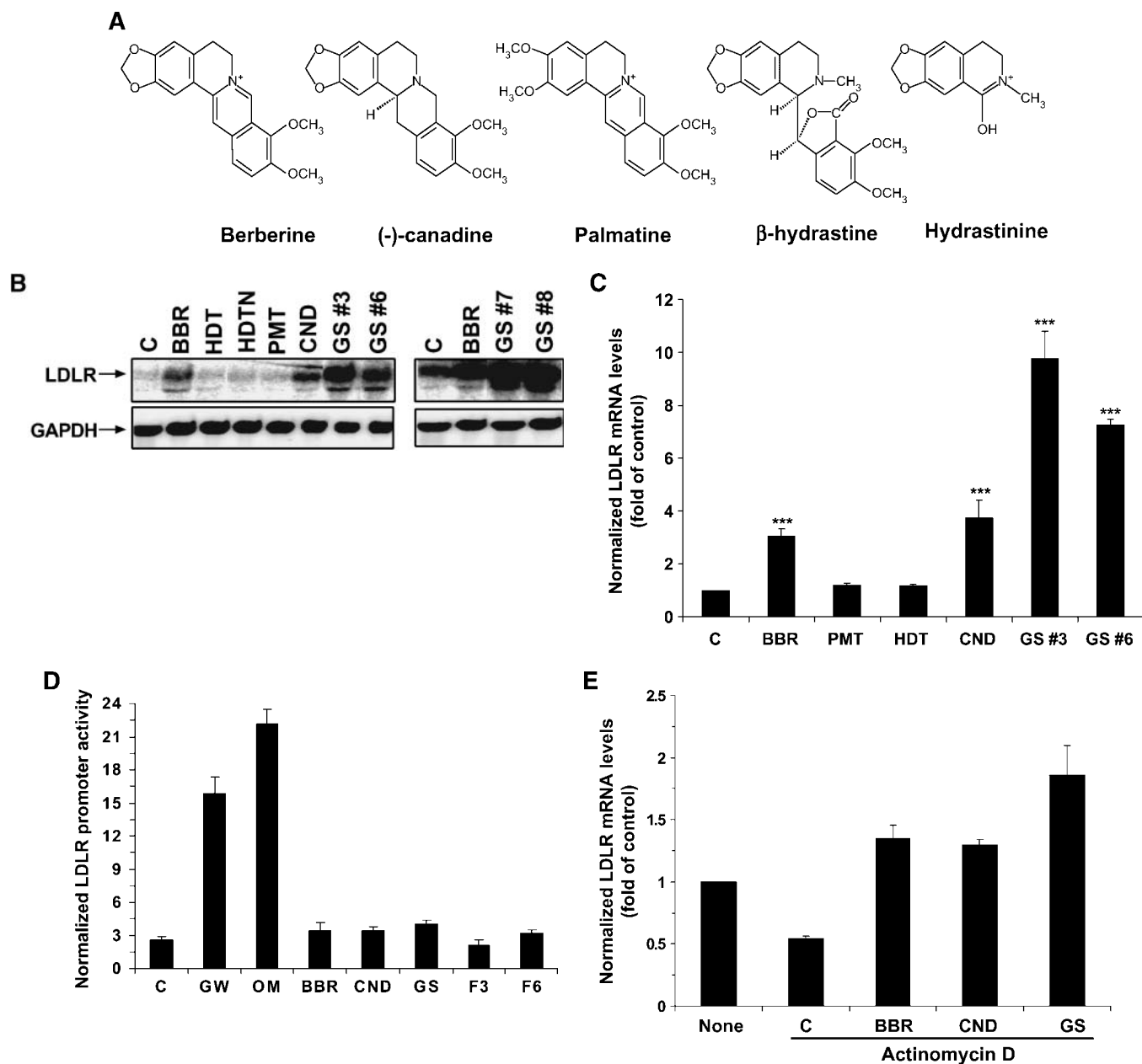
Fig. 1. Upregulation of low density lipoprotein receptor (LDLR) expression by goldenseal, canadine (CND), and berberine (BBR) in HepG2 cells. A: Chemical structures of BBR, CND, palmatine (PMT), hydrastine (HDT), and hydrastinine (HDTN). B: Northern blot analysis of LDLR mRNA expression. HepG2 cells cultured in Eagle's Mimumum Essential Medium (EMEM) containing 0.5% FBS were treated with each compound at a dose of 20 μg/ml or with goldenseal (GS) Lots 3 and 6 at a dose of 10 μl/ml for 8 h (left panel). In the right panel, HepG2 cells were treated with 20 μg/ml BBR, 5 μl/ml Lot 7, or 2.5 μl/ml Lot 8 for 8 h. Total RNA was isolated, and 15 μg per sample was analyzed for LDLR mRNA by Northern blot. Membranes were stripped and hybridized to a human GAPDH probe. The results shown are representative of three experiments. C, control. C: Real-time quantitative RT-PCR analysis. Effects of goldenseal and each alkaloid on LDLR mRNA expression in HepG2 cells were independently examined with quantitative real-time PCR assays. LDLR mRNA levels were corrected by measuring GAPDH mRNA levels. The abundance of LDLR mRNA in untreated cells was defined as 1, and the amounts of LDLR mRNA from drug-treated cells were plotted relative to that value. The results shown are representative of three to five independent experiments in which each sample was assayed in triplicate. The results are means ± SD. * $P < 0.001$. D: Analysis of LDLR promoter activity. HepG2 cells were cotransfected with pLDLR234Luc and pRL-SV40. After an overnight incubation, GW707 (2 μM), oncostatin M (OM; 50 ng/ml), BBR (15 μg/ml), CND (15 μg/ml), goldenseal (2.2 μl/ml; Lot 8), F3 (3 μl/ml), and F6 (3 μl/ml) were added to cells for 8 h before cell lysis. Firefly luciferase and renilla luciferase activities were measured. The data shown are representative of two separate experiments in which triplicate wells were assayed. The results are means ± SD. E: Regulation of LDLR mRNA stability by goldenseal. HepG2 cells were untreated or treated with actinomycin D at a dose of 5 μg/ml for 30 min before the addition of BBR (15 μg/ml), CND (15 μg/ml), or goldenseal (2.2 μl/ml). Total RNA was harvested after 4 h, and expression levels of LDLR mRNA were determined by real-time quantitative RT-PCR. The abundance of LDLR mRNA in cells cultured without actinomycin D was defined as 1, and the amounts of LDLR mRNA from actinomycin D-treated cells without or with herbal drugs were plotted relative to that value. The results shown are representative of two independent experiments in which each sample was assayed in triplicate. The results are means ± SD.

CND, HDT, and HDTN as well as the absence of PMT. Concentrations of CND and HDT in sample extracts were determined using single-point calibration, and concentrations of BBR in sample extracts were calculated using a standard curve. We further verified the identities of BBR, CND, and HDT in extracts by LC-MS analysis. **Table 1** lists the concentrations of alkaloids in different lots of goldenseal extracts. After these comprehensive quantitative analyses, HepG2 cells were treated for 8 h with goldenseal extract Lots 3 and 6 at a concentration of 10 μl/ml (equivalent to a BBR concentration of ~15 μg/ml) and with each alkaloid at a concentration of 20 μg/ml. Northern blot analysis showed that HDT, HDTN, and PMT have no effects, but CND and BBR are both strong inducers of LDLR mRNA expression (Fig. 1B, left panel). Interestingly, goldenseal extract Lots 3 and 6 with lower BBR concentrations produced the greatest increase of LDLR mRNA levels. The Northern blot results were independently confirmed by real-time quantitative RT-PCR (Fig. 1C). A 9.8-fold increase in the level of LDLR mRNA was achieved by goldenseal extract Lot 3, which contains 15 μg/ml BBR and 0.9 μg/ml CND, whereas the pure compound BBR at a concentration of 20 μg/ml only produced a 3-fold increase in LDLR mRNA expression. Strong activities of goldenseal Lots 7 and 8 on LDLR expression were demonstrated by Northern blot (Fig. 1B, right panel). Similar experiments were repeated three to four times using goldenseal extracts from all six different suppliers. In all assays, goldenseal extracts outperformed the pure compound BBR in the upregulation of LDLR mRNA expression. At comparable concentrations of BBR, the activity of goldenseal extract is typically two to three times higher than that of pure BBR. To confirm the higher potency of goldenseal on LDLR expression, we measured the DiI-LDL uptake of HepG2 cells untreated or treated for 15 h with BBR (10 μg/ml) or goldenseal containing an equivalent amount of BBR (1.5 μl/ml of Lot 8). The LDLR-mediated ligand uptake in HepG2 cells was increased 2.5-fold by BBR and 4.9-fold by goldenseal compared with untreated cells.

Our previous studies demonstrated that BBR does not activate LDLR gene transcription but has a stabilizing effect on LDLR mRNA (18, 19). To determine whether mRNA half-life prolongation is the primary mechanism through which goldenseal increases LDLR expression, HepG2 cells were transfected with the LDLR promoter

luciferase construct pLDLR234Luc along with the normalizing reporter pRL-SV40Luc. After transfection, cells were treated for 8 h with BBR or CND at a concentration of 15 μg/ml or with 2.2 μl/ml goldenseal Lot 8 along with two known activators of the LDLR promoter, cytokine oncostatin M (OM; 50 ng/ml) and the compound GW707 (2 μM). OM activates LDLR transcription through a sterol-independent regulatory element of the LDLR promoter (29), and GW707 is a sterol-like compound that increases LDLR transcription through sterol-regulatory element-1 (30, 31). Figure 1D shows that LDLR promoter activity was strongly increased by GW707 and OM but was not affected at all by goldenseal, CND, or BBR. To further corroborate this finding, HepG2 cells were untreated or treated with actinomycin D for 30 min before the addition of BBR, CND, or goldenseal, and total RNA was isolated after a 4 h treatment. Real-time quantitative RT-PCR showed that inhibition of transcription by actinomycin D reduced the abundance of LDLR mRNA but did not prevent the upregulatory effects of these agents on LDLR mRNA expression. Under the same condition of transcriptional suppression, LDLR mRNA was increased 2.5-fold by BBR or CND and 3.4-fold by goldenseal compared with controls (Fig. 1E). Collectively, these results illustrate that goldenseal extract is highly effective in the upregulation of LDLR expression through mRNA stabilization, with a greater activity than the pure compound BBR.

Goldenseal increases LDLR expression through the concerted action of multiple bioactive components in addition to BBR

We were interested in seeking the molecular mechanisms that confer the higher potency of goldenseal, a crude BBR-containing mixture, than the pure compound BBR. To this end, we first compared the dose-dependent effects of CND and BBR in the modulation of LDLR mRNA expression by Northern blot analysis (**Fig. 2A**) and by quantitative real-time RT-PCR (Fig. 2B). Within similar concentration ranges, CND increased levels of LDLR mRNA to higher extents than BBR, indicating that CND is a more potent inducer of LDLR expression.

Our quantitative HPLC analyses of goldenseal obtained from different suppliers indicated that the amount of CND in goldenseal is significantly lower than that of BBR, with BBR-to-CND ratios ranging from 10:1 to 60:1 (Table 1). This implied that CND alone could not account for the 2- to 3-fold higher activity of goldenseal in the upregulation of LDLR expression. A bioassay-driven semipurification procedure was used to detect possible LDLR upregulators accompanying BBR and CND in goldenseal. One milliliter of goldenseal ethanol extract was subjected to flash chromatography over a silica gel column with chloroform-methanol in a 90–50% gradient as the eluting solvent, and 26 fractions of 15 ml were collected. After evaporation of the solvent, residues in each fraction were dissolved in 250 μl of DMSO and subjected to fluorescence spectroscopy, HPLC, and LC-MS analyses. Based upon the retention time and mass spectrometric characteristics of standard solutions, CND was found in fraction 2, HDT was

TABLE 1. Alkaloid concentrations in different lots of goldenseal

Lot	BBR	Canadine	HDT
Lot 3	1.48	0.09	2.42
Lot 6	1.50	0.14	3.08
Lot 7	4.47	0.11	3.28
Lot 8	6.87	0.26	5.07
Lot 9	7.23	0.27	11.70

BBR, berberine; HDT, hydrastine. Values shown are mg/ml. Concentrations of each alkaloid in different lots of goldenseal root ethanol extract were estimated by comparing the sample peak area of HPLC-ultraviolet light adsorption with the peak area of the standard solution.
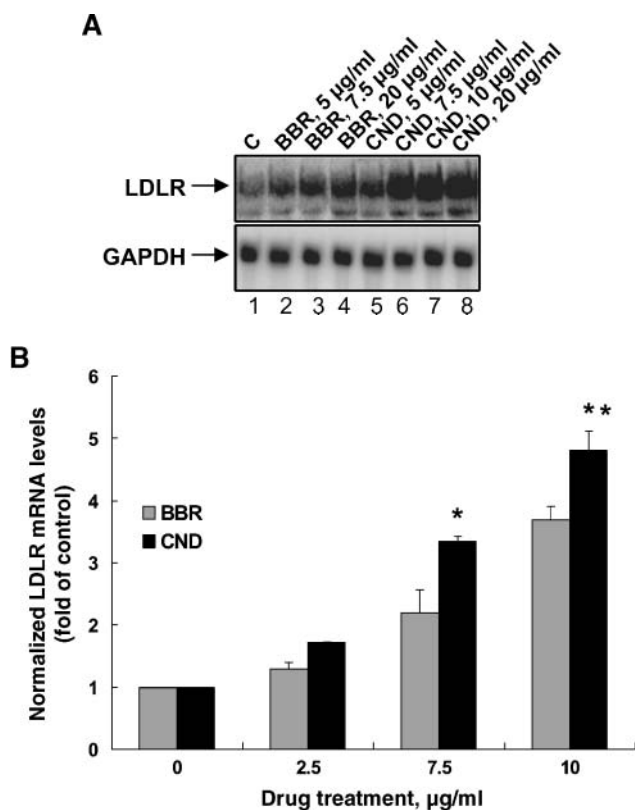
A

LDLR →

GAPDH →

C BBR, 5 µg/ml BBR, 7.5 µg/ml BBR, 20 µg/ml CND, 5 µg/ml CND, 7.5 µg/ml CND, 10 µg/ml CND, 20 µg/ml

1 2 3 4 5 6 7 8

B

Fig. 2. Comparison of dose-dependent effects of CND and BBR on LDLR mRNA expression. HepG2 cells were treated with CND or BBR for 8 h at the indicated concentrations, and total RNA was isolated for analysis of LDLR mRNA and GAPDH mRNA expression by Northern blot (A) and real-time PCR (B). C, control. The results are means ± SD. * $P < 0.05$, ** $P < 0.01$ versus BBR.

eluted in fractions 2–5, and BBR was identified in fractions 16–20. The majority of the fluorescent material was coeluted with BBR (**Fig. 3A**). Fractions not containing BBR or CND were tested for LDLR-modulating activity. HepG2 cells were treated with each fraction at concentrations of 1.5 and 3 µl/ml for 8 h. BBR and goldenseal were included in this assay as positive controls. The abundance of LDLR mRNA was determined by real-time RT-PCR (Fig. 3B). The LDLR mRNA level was strongly increased by fraction 3 (F3) up to 4.3-fold in a dose-dependent manner and was also modestly increased by fraction 6 (F6). We subsequently tested the effects of F3 and F6 on pLDLR234Luc promoter activity. The results showed that similar to BBR and CND, F3 and F6 do not stimulate LDLR transcription (Fig. 1D).

We further characterized the components of F3 using the method of HPLC-coupled ELSD. This method detects signal strengths directly proportional to an analyte's mass in the sample, which provides assessments of relative amounts of compounds (32). The ELSD procedure detected five single peaks in F3, and the second peak was identified as HDT, which constituted 92% of the mass in F3 (**Table 2**). Based upon the reference concentration of HDT, we estimated concentrations of these compounds in the stock solution ranging from the lowest, 40 µg/ml F3-5, to the highest, 190 µg/ml F3-3. Because F3 stock was

added to HepG2 cells at a 1:333 dilution and was able to increase LDLR mRNA expression, we estimate that the effective concentrations of these compounds were likely in the range of 20–600 ng/ml. These data suggest that the compound(s) in F3 are more potent LDLR modulators than BBR. Together, these results indicate that goldenseal increases LDLR expression through a concerted action of multiple bioactive compounds in addition to BBR and that these compounds appear to have greater activities than BBR.

The MDR1 transporter pgp-170 significantly attenuates the activity of BBR but has little effect on goldenseal or CND to upregulate LDLR expression

A comparison of time-dependent effects of BBR and goldenseal on LDLR mRNA expression revealed that goldenseal increased the cellular level of LDLR mRNA with faster kinetics than BBR (**Fig. 4A**). To determine whether the difference in kinetics results from different rates of uptake of BBR and its related compounds, HepG2 cells were incubated with 15 µg/ml BBR, CND, or HDT or with goldenseal Lot 8 (2.2 µl/ml) for 2 h. Cells were washed with cold PBS and collected through trypsinization. Green fluorescence intensities of BBR in samples were determined by FACS. CND and HDT are not fluorescent and only produced weak background signals, similar to untreated control cells. Interestingly, at an equivalent BBR concentration, cells treated with goldenseal had 2.2-fold higher fluorescence than BBR (Fig. 4B). To further examine the kinetics of BBR uptake, HepG2 cells were incubated with BBR or goldenseal for different times from 0 to 60 min before FACS analysis. Although the fluorescence intensity increased slowly in a linear manner in BBR-treated cells, it accumulated rapidly in goldenseal-treated cells (Fig. 4C). At 5 min of incubation, the fluorescence intensity had already increased ~13-fold in goldenseal-treated cells but had increased only ~2-fold in BBR-treated cells. It is possible that some other minor components of goldenseal are fluorescent and contribute to the higher fluorescence intensity in goldenseal-treated HepG2 cells; however, our column separation profile indicated that the majority of the fluorescent signal is derived from BBR (Fig. 3A).

It was reported that the weak antimicrobial action of BBR is caused by an active efflux of BBR from bacteria by multidrug resistance pumps (33–35). It is possible that the exclusion of BBR by the MDR1 transporter pgp-170 in HepG2 cells is responsible for its low intracellular accumulation. To test this hypothesis, the uptake of BBR and goldenseal for 2 h in HepG2 cells was measured in the absence and presence of a known MDR1 inhibitor, verapamil (VRPM) (36–38), at a dose of 0.6 µM. The green fluorescence intensity in BBR-treated cells was increased significantly by VRPM, as demonstrated by direct examination with fluorescence microscopy (**Fig. 5A**). FACS analysis indicated that blocking MDR1 activity with VRPM resulted in a 49% increase of fluorescence intensity in BBR-treated cells but only an 8% increase in goldenseal-treated cells (Fig. 5B). To directly assess the functional role of MDR1 in BBR-mediated LDLR mRNA upregulation, cells were
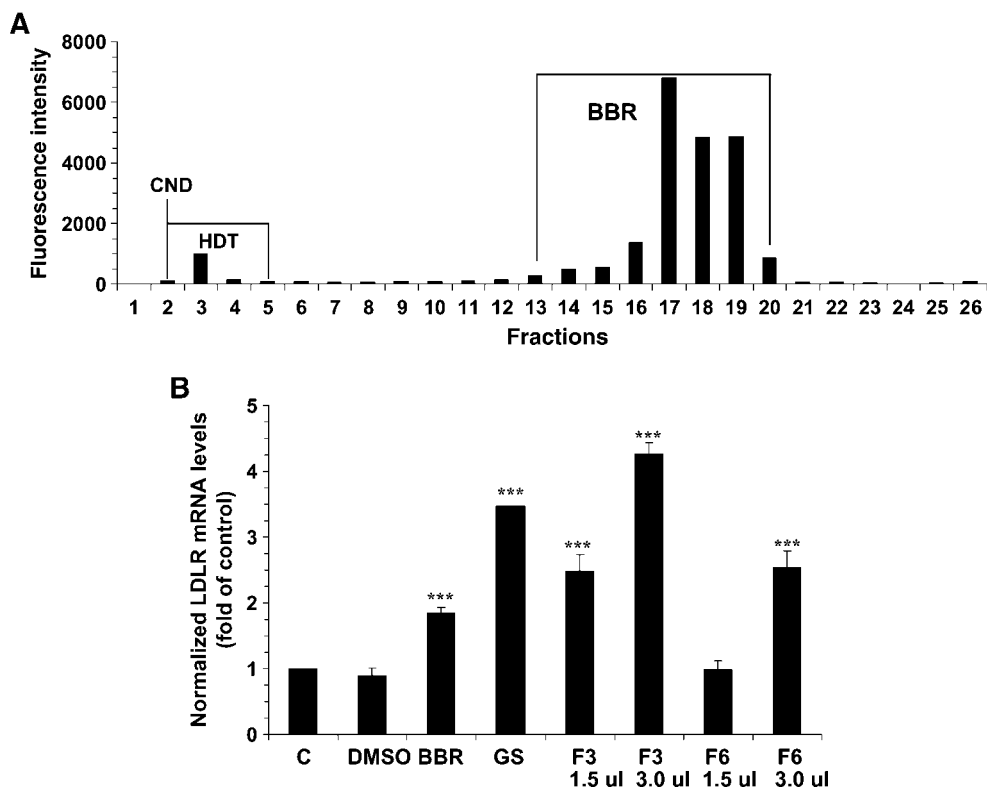
Fig. 3. Separation of goldenseal extract by silica gel column chromatography and detection of LDLR modulation activity in column eluates. A: One milliliter of goldenseal extract was separated into 26 fractions by silica gel column chromatography using chloroform-methanol as the elution solvent. The fluorescence intensity of 200 μl from each fraction was measured by a fluorescent microplate reader at 350 nm excitation and 545 nm emission. The presence of CND, HDT, or BBR in eluates was determined by HPLC and LC-MS with standard solutions of each compound as the reference. B: HepG2 cells were treated for 8 h with 1.5 or 3 μl of each fraction after evaporation of the solvent and redissolving in DMSO. BBR (15 μg/ml) and goldenseal (GS; 2.2 μl/ml; Lot 8) were used in these experiments as positive controls. The inducing effects of F3 and F6 on LDLR mRNA expression were consistently observed in four separate experiments. C, control. The results are means ± SD. *** $P < 0.001$ versus control.

treated with BBR, CND, or goldenseal in the absence or presence of VRPM and levels of LDLR mRNA were determined. The results showed that VRPM did not increase the activity of goldenseal or CND but enhanced the activity of BBR on LDLR mRNA expression in a dose-dependent manner (Fig. 5C, D). The fact that the activity of CND was not affected at all by VRPM suggests that CND is not a substrate of MDR1.

To further examine the inhibitory role of pgp-170 on BBR activity, HepG2 cells were transfected with siRNA of MDR1 or a control siRNA for 3 days. Western blot analysis of MDR1 abundance showed a significant reduction of MDR1 protein level by the transfection of MDR1 siRNA (Fig. 5F, inset). Thus, the siRNA-transfected cells were treated with BBR for 2 h to measure BBR uptake or for 6 h for RNA isolation. FACS analysis showed that the cellular retention of BBR in MDR1 siRNA-transfected cells was increased by 47% ($P < 0.01$) compared with that in mock-transfected cells (Fig. 5E). Quantitative RT-PCR showed that the mRNA level of MDR1 was decreased by 69% in control cells and by 71% in BBR-treated cells compared with nonspecific siRNA-transfected cells (mock). Reduction of MDR1 expression by siRNA did not affect LDLR mRNA level in control cells; however, it caused a 45% increase ($P < 0.001$) in the activity of BBR to increase LDLR mRNA level (Fig. 5F). As expected, the activity of CND or goldenseal on LDLR expression was not affected by MDR1 siRNA transfection (data not shown). Together, these re-

TABLE 2. Analysis of components of F3 by HPLC coupled with evaporative light-scattering detection

Compound	Retention Time	Peak Area	Percentage Area
HDT (10 mg/ml)	1.7	1,621,945	100.0
F3-1 (unknown, 0.15 mg/ml)	1.58	24,813	2.5
F3-2 (HDT, 5.7 mg/ml)	1.78	926,022	91.5
F3-3 (unknown, 0.19 mg/ml)	11.09	32,185	3.2
F3-4 (unknown, 0.12 mg/ml)	12.78	20,780	2.1
F3-5 (unknown, 0.04 mg/ml)	13.62	7,805	0.8

HPLC-coupled evaporative light-scattering detection was used to analyze the components of F3. The standard solution of HDT was used as a reference. The amount of mass in each peak was estimated by comparing the peak area of each peak with the peak area of HDT.
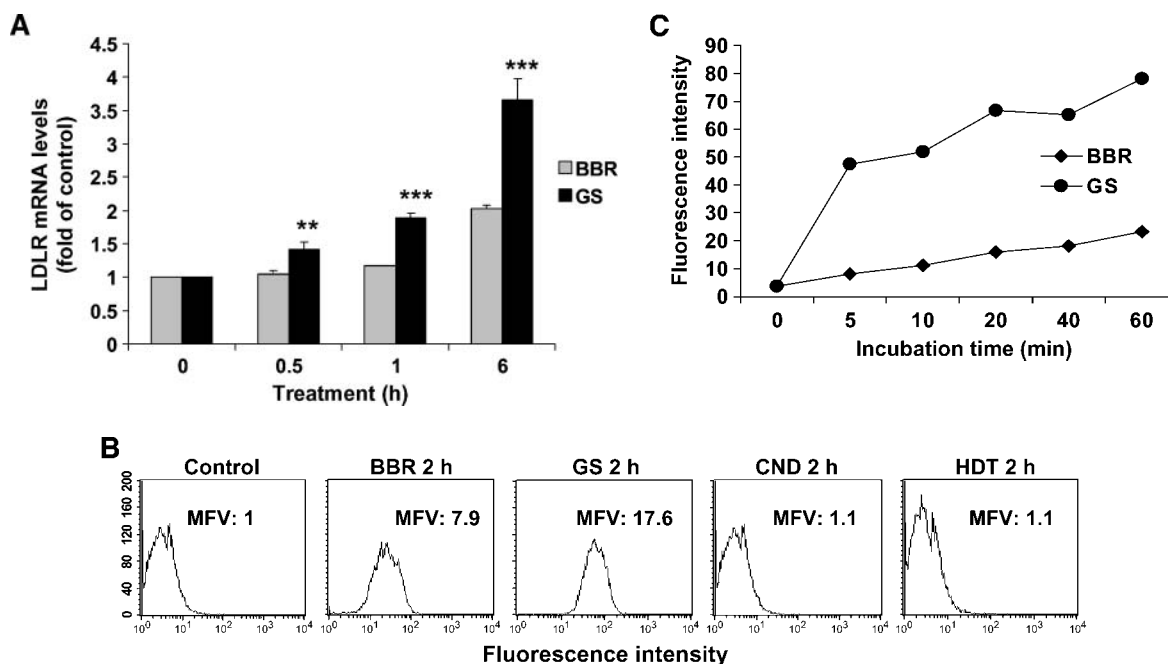
Fig. 4. Kinetics studies of LDLR expression and uptake of BBR in HepG2 cells. A: Time-dependent inductions of LDLR mRNA expression by goldenseal (GS) and BBR. HepG2 cells were incubated with BBR (15 μg/ml) or goldenseal (2.2 μl/ml; Lot 8) for the times indicated. The abundance of LDLR mRNA was determined by quantitative real-time PCR assays. The results are means ± SD. ** $P < 0.01$, *** $P < 0.001$ versus BBR. B: FACS analysis of intracellular accumulation of BBR. HepG2 cells were incubated with 15 μg/ml BBR, CND, or HDT or with goldenseal at 2.2 μl/ml for 2 h at 37°C. Thereafter, cells were washed with cold PBS and trypsinized. Intracellular fluorescence signals were analyzed by FACS. The mean fluorescence value (MFV) of untreated cells was defined as 1, and the mean fluorescence value in drug-treated cells were plotted relative to that value. C: Kinetics of BBR uptake. Cells were incubated with BBR (15 μg/ml) or goldenseal (2.2 μl/ml) at 37°C. At the times indicated, medium was removed and cells were collected by trypsinization and subjected to FACS analysis. The results shown are representative of three to five experiments.

sults clearly demonstrate that MDR1 attenuates the activity of BBR on LDLR expression by actively excreting BBR from cells.

The fact that BBR in goldenseal is not excreted by MDR1 suggests that goldenseal may contain natural MDR inhibitor(s). $DiOC_2$, a known fluorescent small molecule, has been widely used as a specific substrate of MDR1 (39), and the efflux of $DiOC_2$ from cells is attenuated by the MDR1 inhibitor VRPM. We incubated HepG2 cells with $DiOC_2$ in the absence or presence of VRPM (50 μM) or goldenseal (2.5 μl of Lot 8) for 2 h, and the retention of $DiOC_2$ was measured by FACS. The summarized results from three separate experiments showed that the efflux of $DiOC_2$ was inhibited by goldenseal to a similar degree as by VRPM (**Fig. 6**). These results, using a known transporter substrate in direct functional assays of MDR1, independently confirmed our finding that goldenseal contains a natural MDR1 antagonist(s) that accentuates the upregulatory effect of BBR on LDLR mRNA expression.

Goldenseal effectively decreases serum cholesterol and LDL-c levels

To determine whether the strong induction of hepatic LDLR expression renders goldenseal an effective agent in reducing LDL-c from plasma, hypercholesterolemic hamsters were used as an animal model to examine the cholesterol-lowering activity of goldenseal. Twenty-seven

Golden Syrian male hamsters on an HC diet were divided into three treatment groups. One group was treated with BBR at a daily dose of 1.8 mg/animal (15 mg/kg); the second group was treated with goldenseal Lot 9 at a daily dose of 125 μl/animal, equivalent to a BBR dose of 0.9 mg/animal (7.5 mg/kg); the third group received an equal amount of vehicle (20% hydroxypropyl-β-cyclodextrin) as the control group. **Table 3** shows the plasma lipid levels of different groups after 24 days of drug treatment. Goldenseal at a daily dose of 125 μl/animal, with an equivalent BBR dose of 0.9 mg/day/animal, reduced plasma TC by 31.3%, LDL-c by 25.1%, TG by 32.6%, and FFA by 33.8% compared with the untreated group. This lipid reduction by goldenseal is nearly identical to the lipid-lowering effect of BBR at a daily dose of 1.8 mg. We performed an additional HPLC analysis of lipoprotein cholesterol and TG profiles (24) in pooled serum from untreated hamsters on a normal diet and an HC diet and from goldenseal-treated hamsters fed the HC diet. HC feeding markedly increased the serum levels of VLDL-c, LDL-c, and chylomicron-associated cholesterol in hamsters. Goldenseal treatment reduced cholesterol levels in these lipoproteins without decreasing HDL-c (**Fig. 7**, upper panel). The TG-lowering effect of goldenseal was also confirmed by the HPLC analysis (Fig. 7, lower panel).

To directly correlate the LDL-c-lowering effects of goldenseal with its ability to upregulate hepatic LDLR expression,
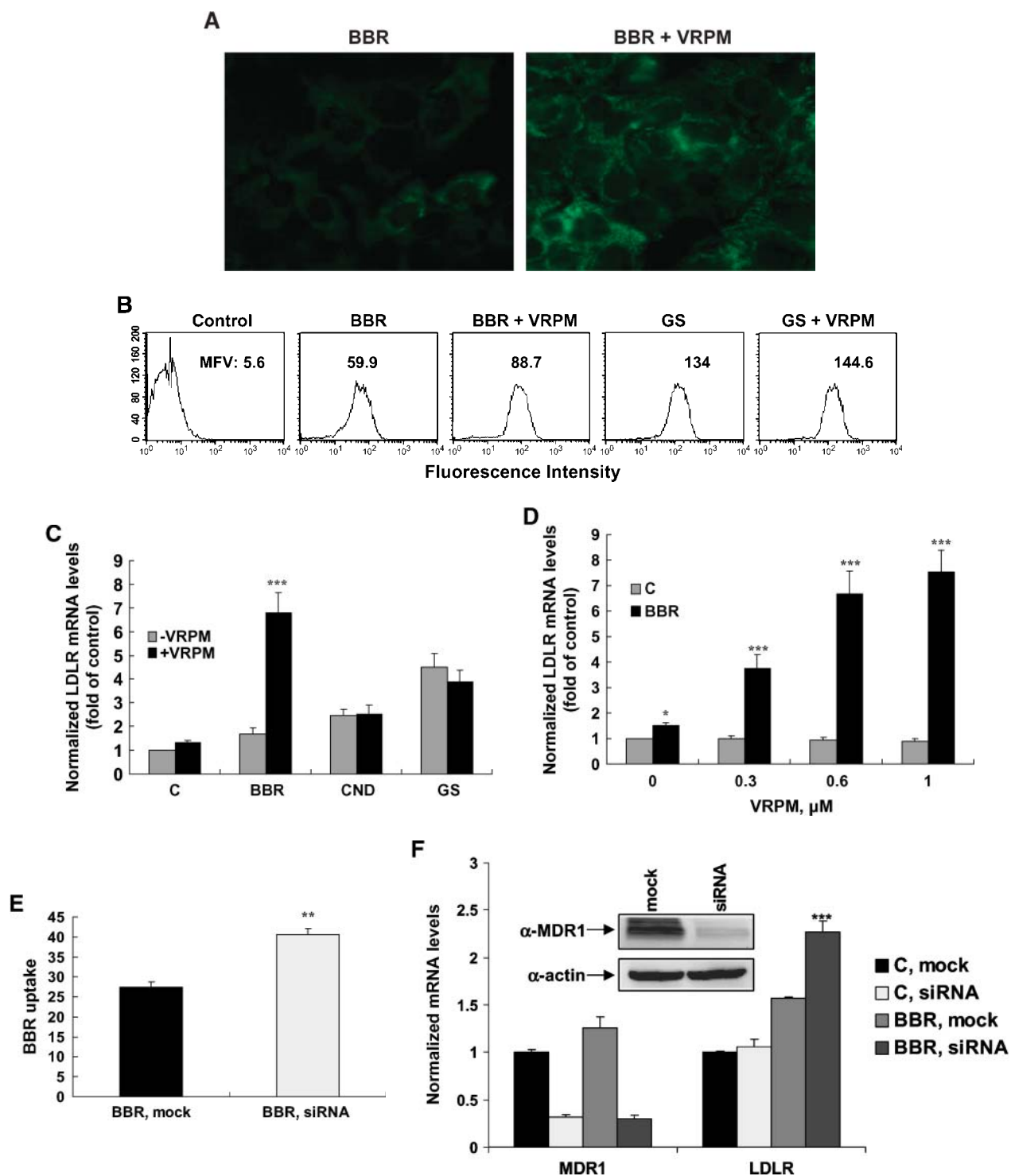
Fig. 5. Multiple drug resistance-1 (MDR1) attenuates BBR intracellular accumulation and BBR activity on LDLR mRNA expression. A, B: HepG2 cells were preincubated with 0.6 μM verapamil (VRPM) for 30 min before the addition of BBR (15 μg/ml) or goldenseal (GS; 2.5 μl/ml). After a 2 h drug treatment, the intracellular accumulation of BBR was examined with a fluorescence microscope (A) or was analyzed by FACS (B). MFV, mean fluorescence value. C: Cells were treated with BBR (10 μg/ml), goldenseal (2.5 μl/ml), or CND (10 μg/ml) in the absence or presence of 0.6 μM VRPM for 8 h. LDLR mRNA levels were determined by real-time PCR. C, control. The results are means ± SD. *** $P < 0.001$ versus without VRPM. D: HepG2 cells were treated with BBR (10 μg/ml) without or with the indicated concentrations of VRPM for 8 h. The results are means ± SD. *** $P < 0.001$ versus without VRPM. E: HepG2 cells were transfected with MDR1 small interfering RNA (siRNA) or a control siRNA for 3 days. The transfected cells were treated with BBR (15 μg/ml) for 2 h, and BBR uptake was measured by FACS. The results are means ± SD of three experiments. ** $P < 0.01$ versus mock-transfected cells. F: siRNA-transfected cells were treated with BBR (15 μg/ml) for 6 h. Total RNA was isolated, and the mRNA levels of MDR1, LDLR, and GAPDH were assessed by real-time quantitative RT-PCR. The results shown are representative of two independent experiments in which each sample was assayed in triplicate. The results are means ± SD. *** $P < 0.001$ versus mock siRNA. In the inset, total cell lysate was isolated from mock siRNA- or MDR1 siRNA-transfected cells, and cellular levels of MDR1 protein were assessed by Western blotting using mouse anti-MDR1 monoclonal antibody (sc-13131).
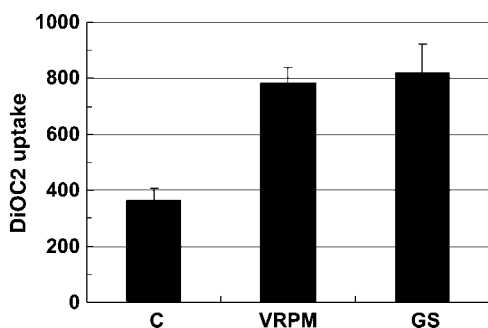
Fig. 6. Goldenseal inhibits MDR1 transport activity. HepG2 cells were incubated with 0.2 μg/ml $DiOC_2$ in the absence or presence of goldenseal (GS; 2.5 μl/ml; Lot 8) or VRPM (50 μM) for 2 h at 37°C. The retention of $DiOC_2$ was measured by FACS. C, control. Results are means ± SD of three experiments.

at the end of treatment animals from control and treated groups were euthanized and levels of liver LDLR mRNA were individually assessed by quantitative real-time RT-PCR using hamster-specific probes. The results represent means ± SD of six animals per group. We detected a 3.2-fold increase by goldenseal ($P < 0.0001$) and a 3.7-fold increase by BBR ($P < 0.0001$) in LDLR mRNA expression (**Fig. 8A**).

Activation of the ERK signaling pathway is a critical event in the BBR-mediated upregulation of LDLR expression (18, 19). We examined ERK phosphorylation in liver tissues of hamsters. Total cell lysates were prepared from 100 mg of liver tissue, and cell lysates from each treatment group (n = 9) were pooled. Western blot analysis with anti-phosphorylated ERK antibody demonstrated that levels of phosphorylated ERK were greatly increased in both goldenseal- and BBR-treated animals (Fig. 8B). We further examined ERK activation in HepG2 cells treated with different lots of goldenseal and with individual alkaloids of goldenseal. ERK phosphorylation was induced by goldenseal from different suppliers, and this activity was attributable to CND and BBR but not to HDT (Fig. 8C). Together,

these in vivo and in vitro data provide a solid link between ERK activation and LDLR upregulation by this medicinal plant.

Goldenseal reduces liver fat storage

The HC diet increases hepatic cholesterol content and fat storage (40, 41). To determine whether goldenseal treatment reduces the hepatic fat content in animals fed an HC diet, liver tissue sections from animals under different diets and treatment were examined by Oil Red O staining (**Fig. 9A–D**) and hematoxylin and eosin staining (Fig. 9E–H). Histological examinations showed that liver tissue from hamsters fed a normal diet displayed a normal lobular architecture with portal areas uniformly approximated. Oil Red O staining showed minimal and scattered lipid staining within small randomly distributed clusters of hepatocytes (Fig. 9A). In liver tissues taken from the control HC-fed hamsters, lipid was massively accumulated in the cytoplasm of hepatocytes (Fig. 9B). Treatment of hamsters with goldenseal significantly reduced lipid accumulation in hepatocytes (Fig. 9C). Restoration of hepatocyte morphology and reduction of liver steatosis were achieved by BBR application as well (Fig. 9D).

To quantitatively assess the effect of goldenseal in reducing lipid storage, hepatic cholesterol contents in HC-fed control and HC-fed and drug-treated hamsters were measured (Table 3). Hepatic TC and TG were reduced to 46.3% and 54.3% of HC-fed control values by goldenseal and were reduced to 68.7% and 70.6% of HC-fed control values by BBR. These data parallel the results of plasma lipid measurements, further demonstrating that goldenseal extract is highly effective at decreasing plasma lipid levels and reducing the hepatic accumulations of cholesterol and TG.

DISCUSSION

Goldenseal is an indigenous North American medicinal plant. The first medical use of goldenseal root extract was

TABLE 3. Goldenseal reduces plasma cholesterol and lipid accumulation in the liver of HC-fed hamsters

Lipids	Total Cholesterol		LDL-Cholesterol		HDL-Cholesterol		Triglyceride		FFA	
	mg/dl	% control	mg/dl	% control	mg/dl	% control	mg/dl	% control	mEq/l	% control
Plasma lipids										
HC, control	1,023 ± 47	100	597 ± 47	100	422 ± 38	100	1,379 ± 58	100	4.1 ± 0.17	100
HC, goldenseal	702 ± 27	68 ± 2.6[a]	447 ± 16	74 ± 2.8[a]	380 ± 70	90 ± 16.6	929 ± 37	67 ± 2.7[a]	2.7 ± 0.08	66 ± 2.0[a]
HC, BBR	716 ± 40	70 ± 3.9[b]	478 ± 34	73 ± 5.8	384 ± 36	91 ± 8.6	910 ± 114	66 ± 8[b]	2.6 ± 0.11	64 ± 2.7[a]
	μmol/g	% control					μmol/g	% control		
Hepatic lipids										
HC, control	82.9 ± 12.4	100					69.2 ± 9.29	100		
HC, goldenseal	38.5 ± 2.58	46 ± 3.1[a]					37.6 ± 3.7	54.3 ± 5.3[a]		
HC, BBR	57 ± 7.54	68.7 ± 9.0[a]					48.9 ± 6.83	70.6 ± 9.89[a]		

HC, high-cholesterol. Hamsters on the HC diet were administered vehicle (control), 125 μl/animal goldenseal (Lot 9), or 1.8 mg/animal BBR daily by intraperitoneal injection for 24 days. Plasma and hepatic lipids were measured as indicated in Materials and Methods. Values are means ± SEM of seven to nine animals. Differences in the hepatic lipid-lowering effects of goldenseal and BBR were significant ($P < 0.001$), whereas differences in their plasma lipid-lowering effects were not significant.
  [a] $P < 0.001$, compared with the untreated control group.
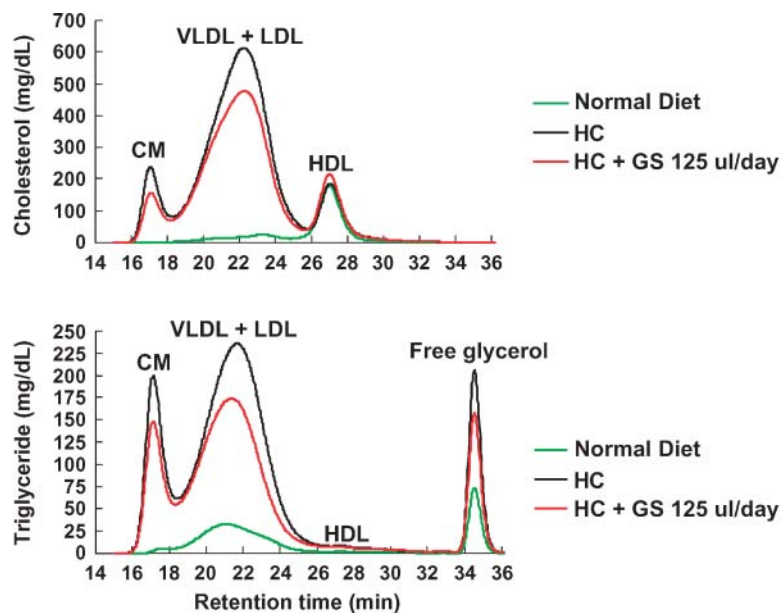 [b] $P < 0.01$ compared with the untreated control group.

Fig. 7. Plasma lipoprotein cholesterol profiles of control and goldenseal (GS)-treated animals. Serum from the normal diet group (n = 6), the high-cholesterol (HC) control group (n = 9), and the goldenseal group were pooled, and the pooled sera were subjected to HPLC analysis of lipoprotein profiles associated with total cholesterol (upper panel) and triglyceride (lower panel). CM, chylomicron.

reported in 1798 for the treatment of what was thought to be cancer and for inflamed eyes (42). Since then, goldenseal has been widely used by herbal practitioners as an antimicrobial and antisecretory agent for a variety of infections that affect the mucosa, such as respiratory and intestinal infections (20, 21). In this study, we demonstrate that goldenseal has strong activities in decreasing plasma cholesterol and LDL-c through its stimulating effect on hepatic LDLR
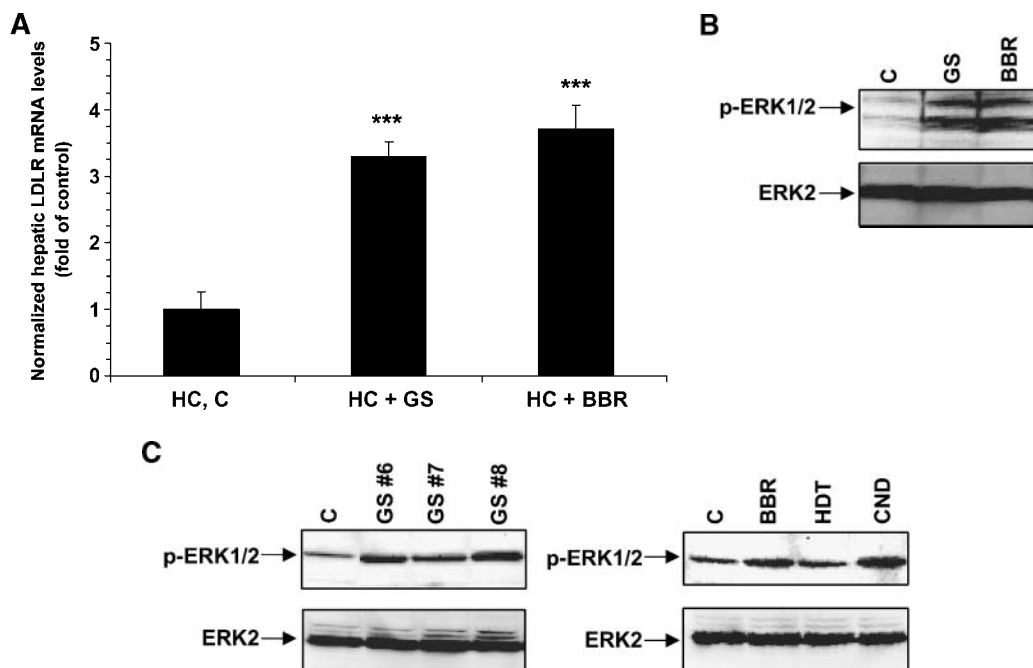

Fig. 8. Upregulation of LDLR mRNA expression and activation of the extracellular signal-regulated kinase (ERK) pathway in hamsters by goldenseal. A: Hepatic LDLR mRNA expression. Four hours after the last drug treatment, all animals were euthanized and liver total RNA was isolated. Individual levels of LDLR mRNA in untreated, goldenseal (GS)-treated, and BBR-treated hamsters fed the HC diet were assessed by quantitative PCR. Results are means ± SD of six animals per group. C, control. *** $P < 0.001$ versus the HC control group. B: Western blot analysis of phosphorylated ERK. Cytosolic proteins were prepared from pooled liver samples of the same treatment group (n = 9), and 50 μg of protein from the pooled sample was subjected to SDS-PAGE. The membrane was blotted with anti-phosphorylated ERK antibody and subsequently blotted with anti-ERK2 antibody. C: Activation of ERK in HepG2 cells. HepG2 cells were treated with 2.5 μl/ml goldenseal obtained from three different suppliers or with 20 μg/ml BBR, HDT, or CND for 2 h. Total cell lysates were prepared, and 50 μg of protein per sample was analyzed for phosphorylated ERK by Western blot analysis.
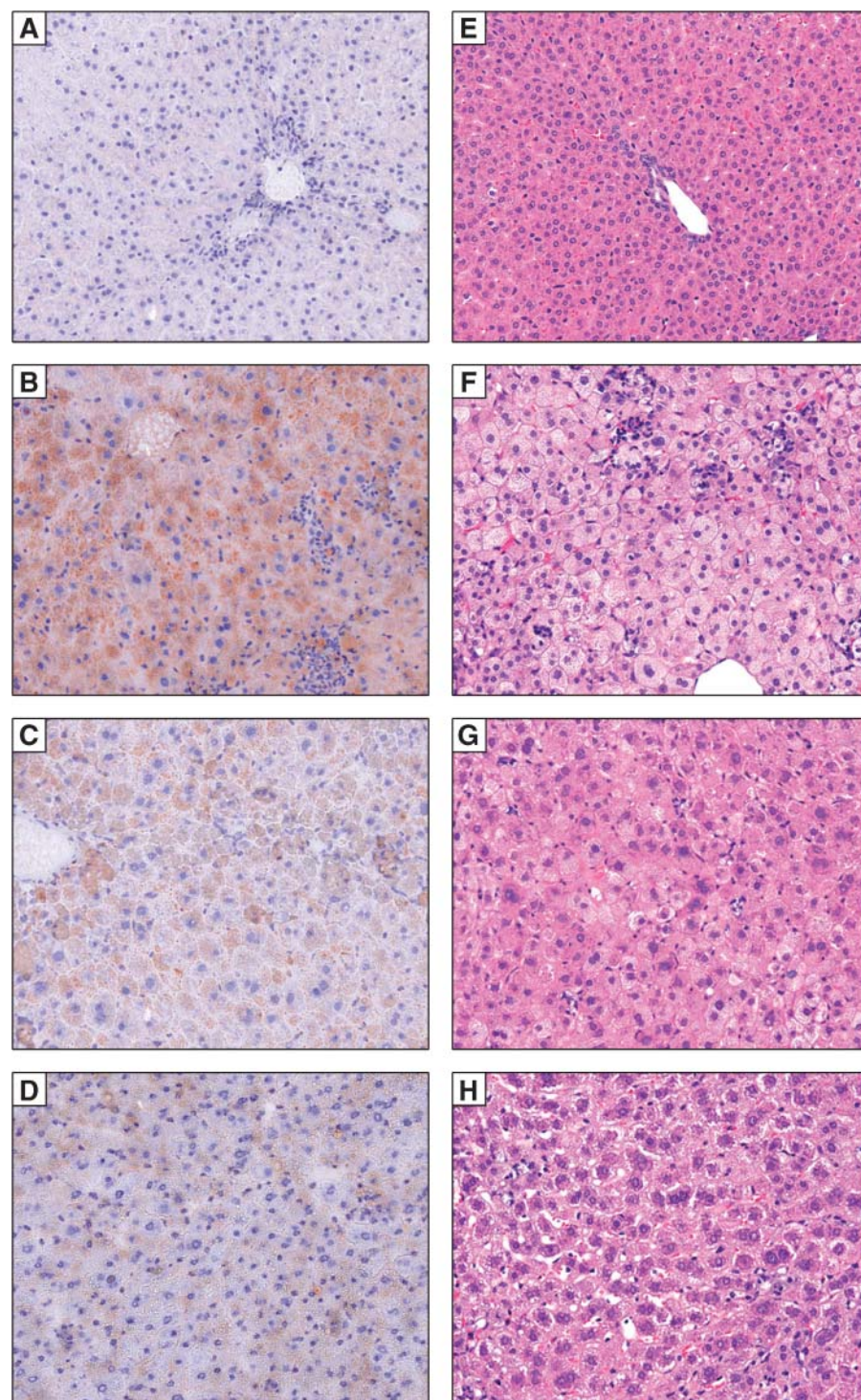
Fig. 9. Goldenseal administration reduces hepatic fat storage in hyperlipidemic hamsters. Frozen tissue sections of liver taken from a hamster fed a normal diet (A), an HC diet untreated (B), or an HC diet treated with goldenseal (C) or BBR (D) were stained with Oil Red O to detect lipid droplets and counterstained with Mayer's hematoxylin. Paraformaldehyde-fixed tissue sections of liver taken from a hamster fed a normal diet (E), an HC diet untreated (F), or an HC diet treated with goldenseal (G) or BBR (H) were stained with hematoxylin and eosin to show the tissue morphology. Photographs were taken at 200× magnification.

expression. To the best of our knowledge, this is the first report that goldenseal regulates cholesterol metabolism.

We initially observed that at equivalent concentrations of BBR, goldenseal root extract has higher activity in in-creasing LDLR expression in HepG2 cells than the pure compound BBR. To understand the underlying mecha-nisms, we used different and complementary chemical, biochemical, and molecular approaches. These studies al-

lowed us to discover several important factors that contribute to the higher activity of goldenseal in the modulation of LDLR expression.

First, we have identified CND, another major isoquinoline compound of goldenseal, as a new modulator of LDLR expression with greater activity than BBR. It is noteworthy that CND and PMT are structurally closely related to BBR, yet PMT has no regulatory activity on LDLR expression. On the other hand, both BBR and PMT have strong DNA binding affinities, whereas CND, a hydrogenated product of BBR, does not bind to DNA (43). It has been proposed that the quaternary ammonium and planar structure play critical roles in the DNA binding of BBR and PMT. The fact that CND lacks both critical features for DNA binding but shares the common activity with BBR in stabilizing LDLR mRNA provides the first evidence that separates the DNA binding property from the activity of mRNA stabilization in these isoquinoline compounds.

Second, we have demonstrated the presence of additional LDLR regulators in goldenseal extract. We showed that eluates F3 and F6 of silica gel columns loaded with goldenseal have LDLR-inducing activities that cannot be attributed to BBR or CND. At present, it is not clear whether the increased LDLR expression is caused by a single compound in F3 or F6 or results from a combined action of the mixture. Because neither F3 nor F6 increased LDLR promoter activity (Fig. 1D), the unknown compound(s) likely acts on the stability of LDLR mRNA. Thus, our studies demonstrate that goldenseal contains a group of natural compounds that have unique properties in stabilizing LDLR mRNA. Experiments to isolate and structurally characterize these unknown compounds are currently under way in our laboratory.

The third factor that contributes to the strong activity of goldenseal in increasing LDLR expression is the resistance to MDR1-mediated drug excretion. Using two different approaches, MDR1 inhibitors that inhibit the transport activity of MDR1 and siRNA that blocks the expression of MDR1, we demonstrated that pgp-170 actively excludes BBR from HepG2 cells, resulting in a lower efficacy of BBR in LDLR regulation. BBR and PMT, which are strong amphipathic cations, have been identified as natural substrates of the MDR NorA pump of microorganisms (33–35), and our data are consistent with these literature reports. The fact that BBR in goldenseal has a longer intracellular retention time, with greater influx and lesser efflux than BBR alone, suggested the existence of an MDR inhibitor(s) in goldenseal. Using $DiOC_2$, a well-characterized MDR1 substrate, we were able to show that, indeed, the MDR1-mediated efflux of $DiOC_2$ was inhibited by goldenseal at a concentration that elicited a response in LDLR expression. A previous study has identified an MDR inhibitor, 5′-methoxyhydnocarpin (34), in the leaves of *Berberis fremontii*, a BBR-producing plant. However, our LC-MS did not detect a peak corresponding to the molecular weight of 5′-methoxyhydnocarpin in goldenseal. It is likely that the inhibitor(s) produced by goldenseal is structurally different from the one made in *Berberis fremontii*. Our studies also revealed that CND is not a substrate of MDR1,

thereby providing a molecular explanation for the higher activity of CND than BBR. With its unique features of MDR1 resistance and lack of DNA binding, CND is possibly a better candidate in clinical use for cholesterol reduction, with potentially lower toxicity.

We demonstrated strong plasma TC and LDL-c reductions and a 3.2-fold increase in the hepatic LDLR mRNA level in goldenseal-treated hamsters fed the HC diet at half the equivalent dose of BBR. These in vivo results confirmed the higher potency of goldenseal observed in our in vitro studies. In addition to decreasing TC and LDL-c, goldenseal and BBR markedly reduced serum FFA and TG. A recent study has shown that in HepG2 cells, BBR induces the activation of acetyl-CoA carboxylase through AMP kinase-mediated phosphorylation, which led to a subsequent increase in fatty acid oxidation and decreases in fatty acid synthesis and TG synthesis (44). AMP kinase activation was shown to be the downstream event of the ERK/MEK pathway. In this study, we showed that goldenseal strongly activates ERK activation in liver tissue and in HepG2 cells. Thus, it is possible that activation of acetyl-CoA carboxylase by goldenseal acting through the ERK signaling pathway accounts for the strong reductions of FFA and TG in hamsters.

In conclusion, we have discovered that goldenseal, a Native American medicinal plant, has strong cholesterol- and lipid-lowering effects. Goldenseal reduces cholesterol and lipid accumulations in plasma, as well as in liver, through the actions of multiple bioactive compounds that work synergistically. This work opens the way for a potential alternative therapeutic intervention for hyperlipidemia.

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