Regulation of hepatic cholesterol biosynthesis by berberine during hyperhomocysteinemia

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Wu N, Sarna LK, Siow YL, O K. Regulation of hepatic cholesterol biosynthesis by berberine during hyperhomocysteinemia. Am J Physiol Regul Integr Comp Physiol 300: R635–R643, 2011. First published December 22, 2010; doi:10.1152/ajpregu.00441.2010.—Hyperhomocysteinemia, a condition of elevated plasma homocysteine levels, is a metabolic disorder associated with the dysfunction of multiple organs. We previously demonstrated that hyperhomocysteinemia stimulated hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase leading to hepatic lipid accumulation and liver injury. The liver plays an important role in cholesterol biosynthesis and overall homeostasis. HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis. Hepatic HMG-CoA reductase is a major target for lowering cholesterol levels in patients with hypercholesterolemia. The aim of the present study was to examine the effect of berberine, a plant-derived alkaloid, on hepatic cholesterol biosynthesis in hyperhomocysteinemic rats and to identify the underlying mechanism. Hyperhomocysteinemia was induced in Sprague-Dawley rats by feeding a high-methionine diet for 4 wk. HMG-CoA reductase activity was markedly elevated in the liver of hyperhomocysteinemic rats, which was accompanied by hepatic lipid accumulation. Activation of HMG-CoA reductase was caused by an increase in its gene expression and a reduction in its phosphorylation (an inactive form of the enzyme). Treatment of hyperhomocysteinemic rats with berberine for 5 days inhibited HMG-CoA reductase activity and reduced hepatic cholesterol content. Such an inhibitory effect was mediated by increased phosphorylation of HMG-CoA reductase. Berberine treatment also improved liver function. These results suggest that berberine regulates hepatic cholesterol biosynthesis via increased phosphorylation of HMG-CoA reductase. Berberine may be therapeutically useful for the management of cholesterol homeostasis.

hypercholesterolemia, an elevation of cholesterol levels in the blood, has been identified as one of the primary risk factors for coronary and peripheral arterial disease (26). The liver is central to the regulation of hepatic and systemic cholesterol levels. It regulates the export and clearance of cholesterol in the circulation through synthesis of VLDL and expression of LDL receptor, respectively. The 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in the biosynthesis of cholesterol and catalyzes the reduction of HMG-CoA to mevalonate. Regulation of HMG-CoA reductase activity is the primary approach for controlling de novo cholesterol synthesis. Regulation of HMG-CoA reductase can be achieved by multiple mechanisms, including transcription, translation, enzyme degradation rate, phosphorylation-dephosphorylation, and feedback inhibition by cholesterol levels (8, 24, 36, 38). Inhibitors of HMG-CoA reductase such as statins, which mainly target hepatic enzymes, are frequently used to treat patients with hypercholesterolemia.

Hyperhomocysteinemia, a condition of elevated plasma homocysteine levels, is a metabolic disorder associated with the dysfunction of multiple organs (16, 30). A positive correlation between plasma homocysteine and cholesterol levels has been found in hyperhomocysteinemic patients and animal models (35, 44, 48). Consistent with these findings, patients or animals with severe hyperhomocysteinemia also develop hepatic steatosis (fatty liver) (34, 44). Our previous study demonstrated that homocysteine stimulated the production and secretion of cholesterol in the liver and hepatocytes, a response attributed to enhanced HMG-CoA reductase gene expression (48). Collectively, increases in hepatic HMG-CoA reductase expression and activity play causative roles in the disruption of cholesterol homeostasis during hyperhomocysteinemia.

Berberine is an isoquinoline alkaloid found in barberry and goldenseal plants, Oregon grapes, and the traditional Chinese herb golden thread. Extracts prepared from berberine-containing plants have been used widely as alternative or traditional medicines in humans and animals (13, 32). Recent evaluation of berberine has uncovered several diverse pharmacological properties, including anti-cancer, anti-inflammatory response, and anti-oxidative properties, which have attracted increasing attention to this natural compound (11, 33, 41). Our laboratory has demonstrated that berberine reduces NADPH oxidase-mediated oxidative stress (39). Berberine also improves insulin sensitivity through activation of AMP-activated protein kinase (AMPK) (29). Berberine displays promising lipid-lowering capabilities, as it can affect plasma cholesterol clearance by stabilizing and upregulating LDL receptor expression in hypercholesterolemic patients and animals (1, 27). Although there is striking evidence that berberine lowers cholesterol through mechanisms independent of statins, its ability to regulate cholesterol biosynthesis has yet to be considered.

In the present study, we examined the effect of berberine on HMG-CoA reductase in hyperhomocysteinemic rats that displayed increased cholesterol biosynthesis in the liver. We also investigated the mechanism by which berberine administration regulated hepatic HMG-CoA reductase activity.

MATERIALS AND METHODS

Animal model. Male Sprague-Dawley rats (Charles River Laboratories) aged 8 wk were fed for 4 wk with 1 control diet consisting of Lab Diet Rodent Diet 5001 (PMI Nutrition International) that contained 0.67% (wt/wt) methionine; 2 high-methionine diet consisting of a regular diet plus 1.7% (wt/wt) methionine (45, 48); and 3 high-methionine diet for 4 wk and daily intraperitoneal injection of...
berberine (5 mg/kg body wt ip) for 5 days before euthanasia. Serum homocysteine concentrations were measured by using the IMx homocysteine assay based on the fluorescence polarization immunoassay technology (Abbott Diagnostics Division) (46, 47). Lipid in the liver tissue was extracted according to the Folch method (14). Cholesterol levels in the serum and liver tissue were determined using enzymatic kits (Wako Chemicals) after fasting. Liver function was examined by measuring alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum samples with an enzymatic kit (Diagnostic Chemical Limited). All procedures were performed in accordance with the Guide in the Care and Use of Experimental Animals published by Canadian Council on Animal Care and approved by the University of Manitoba Protocol Management and Review Committee.

Cell culture. HepG2 cells, commonly used as hepatocyte model to study metabolic regulation (15, 23, 46), were purchased from American Type Culture Collection. Cells were cultured in DMEM supplemented with 10% FBS.

Real-time PCR analysis. Total RNAs were isolated from liver tissue with TRIzol reagent (Invitrogen). HMG-CoA reductase mRNA was determined by real-time PCR analysis using the iQ5 real-time PCR detection system (Bio-Rad). In brief, 2 μg of total RNA was converted to cDNA by reverse transcriptase. The real-time PCR reaction mixture contained 0.4 μM of 5’ and 3’ primers and 2 μl of cDNA product in iQ-SYBR Green supermix reagent (Bio-Rad). Crossing threshold values were normalized to GAPDH. The mRNA expression was expressed as percentage change relative to control. The primers (Invitrogen) used in this study were rat HMG-CoA reductase, 5’-GATTTCCAAGGGTGAGGGA-3’ (forward), 5’-TTATGGCAGCAGGTTTCTTG-3’ (reverse) (GenBank accession no. NM_013134); and rat GAPDH, 5’-TCAAGAAGGTGGTGAGGCAG-3’ (forward), 5’-AGTTGGAAGATGGGAGTTG-3’ (reverse) (GenBank accession no. NM_017008).

Western immunoblot analysis. The protein levels of phosphorylated and total HMG-CoA reductase, phosphorylated AMPK, and total AMPK, LDL receptor protein, and as nuclear sterol regulatory element binding protein-2 (SREBP-2) were determined by Western

![Fig. 1. Cholesterol level in rat serum and liver tissue. Serum and liver tissues were collected from rats fed a regular diet (control), a high-methionine diet (HHcy) for 4 wk, a high-methionine diet plus berberine administration (HHcy+BBR), or a regular diet plus berberine administration (BBR). Total or free cholesterol levels in serum (A) and liver tissue (B) were measured. Results were expressed as means ± SE; n = 6, each performed in duplicate. *P < 0.05 when compared with control value and #P < 0.05 when compared with HHcy value.](http://ajpregu.physiology.org/)

![Fig. 2. HMG-CoA reductase mRNA, protein level, and enzyme activity in rat liver. Livers were collected from rats fed a regular diet (control), a high-methionine diet (HHcy) for 4 wk, a high-methionine diet plus berberine administration (HHcy+BBR), or a regular diet plus berberine administration (BBR). A: HMG-CoA reductase mRNA levels were determined by real-time PCR. B: protein levels were determined by Western immunoblotting. C: HMG-CoA reductase activity was measured. Results were expressed as means ± SE; n = 6, each performed in duplicate. *P < 0.05 when compared with control value. #P < 0.05 when compared with HHcy value.](http://ajpregu.physiology.org/)
immunoblot analysis. In brief, liver proteins (60 μg) were separated by electrophoresis on a 10% SDS polyacrylamide gel. Partitioned proteins were transferred to a nitrocellulose membrane. The membrane was probed with either rabbit anti-HMG-CoA reductase polyclonal antibody (Millipore), rabbit anti-phospho-HMG-CoA reductase (Ser-872) polyclonal antibody (Millipore), rabbit anti-AMPK antibody (Cell Signaling Technology), rabbit anti-phospho-AMPK (Thr-172) antibody (Cell Signaling Technology), chicken anti-LDL receptor antibody (Millipore), or rabbit anti-SREBP-2 antibody (Santa Cruz Biotechnology). HRP-conjugated anti-rabbit or anti-chicken IgG antibody (Cell Signaling Technology) was used as the secondary antibody. The corresponding protein bands were visualized using enhanced chemiluminescence reagents and analyzed with a gel documentation system (Bio-Rad Gel Doc1000). The same membranes were reprobed with rabbit anti-β-actin monoclonal antibody to confirm equal loading of proteins for each sample.

Determination of HMG-CoA reductase activity. HMG-CoA reductase activity was determined by radioisotope assay using [3-14C]HMG-CoA (PerkinElmer) as a substrate (46, 48). In brief, the assay mixture contained liver microsomes (0.25 mg proteins), 20 mM glucose 6-phosphate, 2.5 mM NADP, 1 unit glucose-6-phosphate dehydrogenase, 8 mM dithiothreitol, 1.2 mM EDTA, and 0.004 μCi [3-14C]HMG-CoA (PerkinElmer) in a phosphate buffer (pH 7.4). The reaction was carried out at 37°C for 60 min followed by the addition of mevalonolactone and HCl. Radiolabeled HMG-CoA and mevalonolactone were separated by thin-layer chromatography in chloroform-acetone (2:1, vol/vol). The location of mevalonolactone on the chromatographic plate was visualized after staining with iodine vapor. The radioactivity associated with mevalonolactone was measured with a scintillation counter.

EMSA. To determine whether HMG-CoA reductase was regulated by SREBP-2, a major transcription factor for the reductase, the SREBP/DNA binding activity was measured by EMSA. In brief, nuclear proteins were prepared from rat liver as previously described (2, 47). Nuclear proteins (10 μg) were incubated with excess 32P-end-labeled oligonucleotides containing a consensus sequence specific for SREBP/DNA binding site (5′-GGATGTCCATATTAGGACATCT-3′) (Santa Cruz Biotechnology). The reaction mixture was then separated in 6% nondenaturing polyacrylamide gel and dried on a piece of filter paper followed by autoradiography.

Determination of AMP-activated protein kinase activity. A portion of liver tissue was homogenized in a buffer containing 50 mM Tris·HCl (pH 8.5), 250 mM sucrose, 50 mM NaF, 1 mM EGTA, 1

Fig. 3. Effects of berberine treatment on SREBP/DNA binding activity. Livers were collected from rats fed a regular diet (control), a high-methionine diet (HHcy) for 4 wk, a high-methionine diet plus berberine administration (HHcy+BBR), or a regular diet plus berberine administration (BBR). A: nuclear SREBP-2 protein levels were determined by Western immunoblotting analysis. B: SREBP/DNA binding activity was determined by EMSA. Results were expressed as means ± SE; n = 6, each performed in duplicate. *P < 0.05 when compared with control value.

Fig. 4. Phosphorylation of HMG-CoA reductase in rat liver. Livers were collected from rats fed a regular diet (control), a high-methionine diet (HHcy) for 4 wk, a high-methionine diet plus berberine administration (HHcy+BBR), or a regular diet plus berberine administration (BBR). Western immunoblotting analysis was performed to measure the protein levels of phosphorylated HMG-CoA reductase (pHMG-CoA reductase) and total HMG-CoA reductase. Results were expressed as means ± SE (n = 6, each performed in duplicate). *P < 0.05 when compared with control value. #P < 0.05 when compared with HHcy value.
mM EDTA, 10 mM β-mercaptoethanol, 10 mM benzamidine, 0.5 mM PMSF, 1 μg/ml pepstatin, and 0.5 μg/ml leupeptin. After centrifugation for 30 min at 4°C, the supernatant was collected and used for measuring the AMPK enzyme activity. The AMPK enzyme activity was measured using a commercial AMPK activity assay kit (MBL International).

Protein dephosphorylation. Liver proteins (0.1 mg) were incubated with calf intestinal alkaline phosphatase (100 milliunits) for 60 min at 37°C in a dephosphorylation buffer (New England Biolabs) (49). The dephosphorylated proteins were used for Western immunoblotting analysis and HMG-CoA reductase activity assay.

Statistical analysis. Results were analyzed by a two-tailed Student’s t-test or using one-way ANOVA followed by Newman-Keuls post hoc test using Prism 4 (GraphPad Software). P values less than 0.05 were considered statistically significant.

RESULTS

Effect of berberine on hyperhomocysteinemia-induced HMG-CoA reductase activation in the liver. Hyperhomocysteinemia was induced in rats fed a high-methionine diet for 4 wk. The serum homocysteine level was ∼4-fold higher in hyperhomocysteinemic rats than that of the control group (24.88 ± 2.29 μM vs. 4.11 ± 0.59 μM). There was a significant increase in the cholesterol level in the serum (Fig. 1A) and in the liver (Fig. 1B) of hyperhomocysteinemic rats. The HMG-CoA reductase mRNA expression (Fig. 2A) and the protein levels (Fig. 2B) were significantly increased in the liver of hyperhomocysteinemic rats. The hepatic activity of HMG-CoA reductase was higher in hyperhomocysteinemic rats than those in the control group (Fig. 2C).

Administration of berberine once a day (5 mg·kg⁻¹·day⁻¹ ip) for 5 days to hyperhomocysteinemic rats reduced cholesterol levels in the serum and in the liver (Fig. 1). In accordance, HMG-CoA reductase activity in the liver was significantly reduced in berberine-treated rats (Fig. 2C). However, berberine treatment did not affect the mRNA or protein levels of HMG-CoA reductase in the liver of hyperhomocysteinemic rats (Fig. 2, A and B). Berberine treatment did not affect HMG-CoA reductase expression or enzyme activity in control rats (Fig. 2). These results suggested that administration of berberine could attenuate hepatic HMG-CoA reductase activity, as well as reduce cholesterol levels in the liver and circulation of hyperhomocysteinemic rats.

Regulation of HMG-CoA reductase activity by berberine. HMG-CoA reductase can be regulated at the transcriptional and the posttranslational levels (6, 24). SREBP-2, a sterol-sensitive transcription factor, plays a major role in regulating the biosynthesis of cholesterol. Upon stimulation, SREBP enters the nucleus and binds to the sterol regulatory element in the promoter region to activate the transcription of the target genes, such as HMG-CoA reductase (21). The nuclear level of SREBP-2 (Fig. 3A) and SREBP/DNA binding activity (Fig. 3B) were significantly elevated in the liver of hyperhomocysteinemic rats, which, in turn, might have contributed to the increased expression of HMG-CoA reductase in the liver. However, berberine treatment did not affect the nuclear levels of SREBP protein nor the SREBP/DNA binding activity. To investigate the mechanism by which berberine reduced HMG-CoA reductase activity, we performed dephosphorylation experiments on liver proteins from rats given berberine treatment for 5 days.
CoA reductase enzyme activity but did not affect its mRNA expression and protein level, the phosphorylation status of HMG-CoA reductase protein was examined. In general, phosphorylation of HMG-CoA reductase leads to inactivation of the enzyme, while dephosphorylation activates it (22, 36). In the present study, the level of phosphorylated HMG-CoA reductase protein was significantly lower in the liver of hyperhomocysteinemic rats compared with the control group (Fig. 4). Administration of berberine to hyperhomocysteinemic rats increased the phosphorylated form of hepatic HMG-CoA reductase to control levels (Fig. 4). It appeared that hyperhomocysteinemia increased the mRNA level of HMG-CoA reductase and decreased the protein level of phosphorylated HMG-CoA reductase (inactive form of the enzyme) in the liver, leading to an elevation of hepatic HMG-CoA reductase activity. Berberine treatment resulted in an increase in the level of phosphorylated HMG-CoA reductase (inactive form), which, in turn, caused a significant reduction of HMG-CoA reductase activity.

Next, to confirm that changes in the phosphorylation status of HMG-CoA reductase played an important role in regulating its activity, proteins were prepared from the liver of berberine-treated hyperhomocysteinemic rats. An aliquot of proteins was incubated with calf intestinal phosphatase. Phosphatase treatment resulted in a significant decrease in the level of phosphorylated HMG-CoA reductase (inactive form), while it did not affect the level of total reductase protein (Fig. 5A). Upon phosphatase treatment, the activity of HMG-CoA reductase was markedly increased (Fig. 5B).

Inhibition of HMG-CoA reductase activity by berberine via activation of AMPK. Although HMG-CoA reductase can be phosphorylated in vitro by several protein kinases, AMPK is thought to be the major kinase targeting the enzyme in the liver (36). Next, to examine whether AMPK was involved in berberine-induced phosphorylation of hepatic HMG-CoA reductase, Western immunoblotting analysis was performed to measure the phosphorylated AMPK (active form) and total AMPK in the liver tissue. No significant difference in the total AMPK protein levels between the control and hyperhomocysteinemic rats was observed (Fig. 6A). However, the level of phosphorylated AMPK was markedly decreased in the liver of hyperhomocysteinemic rats (Fig. 6A). In line with the decrease in the level of phosphorylated AMPK, the enzyme activity of AMPK was also reduced in the liver of hyperhomocysteinemic rats (Fig. 6B). Administration of berberine increased the level of phosphorylated AMPK, although it did not affect the protein level of total AMPK in the liver of hyperhomocysteinemic rats (Fig. 6B). The enzyme activity of AMPK was restored to the control level upon berberine treatment (Fig. 6B).

Effects of berberine on AMPK and HMG-CoA reductase activity in hepatocytes. It is recently reported that AMPK can be activated by a number of compounds, including berberine and biguanides, such as metformin via inhibition of the mitochondrial respiratory chain (20). In the present study, the effects of berberine and metformin on AMPK and HMG-CoA reductase activity were examined using HepG2 cells. Western immunoblotting analysis was performed to measure the protein levels of phosphorylated AMPK (pAMPK) and total AMPK proteins. AMPK activities were determined in rat liver tissues. Results were expressed as means ± SE (n = 6, each performed in duplicate). *P < 0.05 when compared with control value. #P < 0.05 when compared with HHcy value.
Reductase activities were examined in HepG2 cells. The activity of AMPK (Fig. 8A) and the protein level of phosphorylated HMG-CoA reductase (Fig. 8B) were significantly decreased, while the activity of HMG-CoA reductase (Fig. 8C) was markedly increased in cells incubated with homocysteine. However, incubation with berberine or metformin effectively increased the AMPK activity and the phosphorylated HMG-CoA reductase protein level in these cells (Fig. 8, A and B). As a consequence, the activity of HMG-CoA reductase was reduced in cells incubated with berberine or metformin (Fig. 8C).

**Effect of berberine on hepatic LDL receptor levels during hyperhomocysteinemia.** Apart from its biosynthesis via HMG-CoA reductase, hepatic cholesterol homeostasis is also regulated by LDL receptor-mediated uptake of lipoproteins from the circulation (9). To investigate whether changes in the level of LDL receptor might also contribute to the cholesterol-lowering effect of berberine, Western immunoblotting analysis was conducted to measure LDL receptor protein in the liver tissue. There was no significant difference in the protein level of LDL receptor between the control and hyperhomocysteinemic rats (Fig. 9). Berberine administration had no significant effect on the LDL receptor protein level in the liver (Fig. 9).
Effect of berberine on liver function. Lipid accumulation has been associated with liver dysfunction (40). Therefore, levels of serum transaminases, which are indices of liver function, were measured in hyperhomocysteinemic rats with or without berberine treatment. Serum ALT and AST levels, which were elevated during hyperhomocysteinemia, were significantly diminished by berberine, indicating its beneficial effect on improving liver function (Fig. 10). Taken together, these results indicated that berberine treatment could lower cholesterol levels in the liver and the circulation via inhibition of HMG-CoA reductase activity, while at the same time, ameliorating liver function during hyperhomocysteinemia.

DISCUSSION

The de novo synthesis of cholesterol in the liver plays an important role in regulating hepatic lipid homeostasis and blood cholesterol levels. The HMG-CoA reductase catalyzes the rate-limiting reaction in cholesterol biosynthesis in mammalian cells. Increased hepatic cholesterol biosynthesis can lead to impaired lipid homeostasis and liver dysfunction (7, 17). In hyperhomocysteinemia, both liver and serum cholesterol levels are elevated due to homocysteine-mediated activation of hepatic HMG-CoA reductase (48). Results obtained from the present study indicated two potential mechanisms that might have contributed to hepatic HMG-CoA reductase activation during hyperhomocysteinemia: transcriptional regulation and posttranslational modification of the enzyme. An increase in HMG-CoA reductase gene expression caused an elevation in the enzyme protein level and was associated with an increase in cholesterol biosynthesis in the liver of hyperhomocysteinemic rats. In addition to transcriptional regulation, decreased phosphorylation of HMG-CoA reductase also led to an activation of the enzyme. Several lines of evidence obtained in the present study suggested that the inhibitory effect of berberine on homocysteine-induced HMG-CoA reductase activation was mediated predominantly via posttranslational modification of the enzyme. First, both mRNA and protein levels of HMG-CoA reductase were found to be elevated in the liver of hyperhomocysteinemic rats. Administration of berberine to those rats had no effect on either the gene expression or protein levels of the reductase in the liver tissue. Berberine treatment was, however, effective in attenuating HMG-CoA reductase enzyme activity in the liver of hyperhomocysteinemic rats. Second, although the
protein level of total HMG-CoA reductase (the sum of nonphosphorylated and phosphorylated forms) was not altered by berberine treatment, the level of the phosphorylated enzyme (inactive form) was markedly increased in the berberine-treated group. These results suggested that increased phosphorylation of the enzyme might account for a decrease in HMG-CoA reductase activity in the liver of hyperhomocysteinemic rats treated with berberine.

The equilibrium of enzyme phosphorylation-dephosphorylation plays an important role in regulating HMG-CoA reductase activity. It has been shown that dephosphorylation of HMG-CoA reductase enhances its activity, while phosphorylation at Ser-872 leads to enzyme inactivation (22). Several protein kinases i.e., AMPK (reductase kinase), PKC, and Ca2+/calmodulin-dependent kinase are able to phosphorylate HMG-CoA reductase in vitro (4–6). However, AMPK appears to be the major kinase that targets HMG-CoA reductase in the liver (19). The present study revealed that berberine treatment activated AMPK which, in turn, increased phosphorylation of HMG-CoA reductase in the liver. AMPK is thought to act as an enzymatic regulator of cellular energy homeostasis. AMPK is sensitive to the intracellular AMP/ATP ratio, which leads to a conformational change of AMPK (25). AMPK is activated upon phosphorylation, and, in turn, inactivates HMG-CoA reductase via phosphorylation of the enzyme (3, 10, 12). In the present study, the activity of AMPK was decreased in the liver of hyperhomocysteinemic rats. Administration of berberine effectively restored AMPK activity. In accordance, the level of phosphorylated HMG-CoA reductase (inactive form) increased to that of the control value. A recent study has identified several compounds, including berberine and metformin (a well-known antidiabetic biquanide), that activate AMPK via inhibition of mitochondrial respiratory chain complex I and ATP production (20). It is suggested that activation of AMPK may serve as a common mechanism for the antidiabetic effect by metformin and berberine (37, 42). Results from the present study demonstrated that incubation with metformin restored AMPK activity and inhibited homocysteine-induced HMG-CoA reductase activation in hepatocytes. The in vivo effect of metformin, however, remains to be examined in future studies. Taken together, these results suggested that the inhibitory effect of berberine on HMG-CoA reductase activity was mediated via the AMPK signaling pathway.

Over the past few decades, berberine has gained considerable attention because of its potential therapeutic application for the treatment of dyslipidemia, diabetes, and cardiovascular related disorders (27, 29, 31). Berberine has been reported to be an effective cholesterol-lowering compound in hypercholesterolemic animals and patients, as well as in type 2 diabetic animal models (27, 29). The principal focus of research investigating the lipid-lowering effect of berberine has been directed toward its regulation of LDL receptor, a mechanism distinct from statins (27). Berberine upregulates LDL receptor expression via two identified mechanisms, namely activation of ERK and JNK-dependent pathways (1, 27, 28). In the present study, hepatic LDL receptor protein level was not affected by hyperhomocysteinemia or berberine treatment.

Berberine’s hypolipidemic effect may not be the only means through which berberine protects against cardiovascular pathologies. For instance, berberine may be effective for the management of vascular oxidative disturbances through the regulation of redox homeostasis. Berberine is an effective inhibitor of NADPH oxidase-mediated oxidative stress, which has been shown to play a central role in the initiation and progression of atherosclerosis (18, 43). Berberine normalizes superoxide anion levels in macrophages by reducing gp91phox expression, significantly impairing NADPH oxidase activity (39). It was also shown to enhance superoxide anion clearance by increasing superoxide dismutase activity in these cells (39). Taken together, berberine’s extensive pharmacological effects and growing list of molecular targets support its application for the management of lipid, metabolic, and cardiovascular related disorders.

**Perspectives and Significance**

The present study has demonstrated that berberine regulates HMG-CoA reductase activity in the liver, while at the same time improving liver function. Activation of hepatic HMG-CoA reductase during hyperhomocysteinemia appears to be regulated at the transcriptional and the posttranslational levels. Berberine acts posttranslationally to modulate hyperhomocysteinemia-induced activation of HMG-CoA reductase via AMPK-mediated phosphorylation of the enzyme. Attenuation of HMG-CoA reductase activity by berberine effectively reduces cholesterol levels in the liver and in the circulation during hyperhomocysteinemia. Understanding the multiple mechanisms that regulate HMG-CoA reductase activation may lead to a better control of cholesterol homeostasis in metabolic disease.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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