Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine

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Abstract **The alkaloid drug berberine (BBR) was recently described to decrease plasma cholesterol and triglycerides (TGs) in hypercholesterolemic patients by increasing expression of the hepatic low density lipoprotein receptor (LDLR). Using HepG2 human hepatoma cells, we found that BBR inhibits cholesterol and TG synthesis in a similar manner to the AMP-activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide 1-β-ribofuranoside (AICAR). Significant increases in AMPK phosphorylation and AMPK activity were observed when the cells were incubated with BBR. Activation of AMPK was also demonstrated by measuring the phosphorylation of acetyl-CoA carboxylase, a substrate of AMPK, correlated with a subsequent increase in fatty acid oxidation. All of these effects were abolished by the mitogen-activated protein kinase kinase inhibitor PD98059. Treatment of hyperlipidemic hamsters with BBR decreased plasma LDL cholesterol and strongly reduced fat storage in the liver. These findings indicate that BBR, in addition to upregulating the LDLR, inhibits lipid synthesis in human hepatocytes through the activation of AMPK. These effects could account for the strong reduction of plasma TGs observed with this drug in clinical trials.**—Brusq, J-M., N. Ancellin, P. Grondin, R. Guillard, S. Martin, Y. Saintillan, and M. Issandou. **Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine.** ***J. Lipid Res.*** **2006.** 47: **1281–1288.**

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Coronary heart disease is the most important cause of morbidity and mortality in developed countries. Among the different risk factors, increased LDL cholesterol level has been identified as a major cause of coronary heart disease, and it has been extensively demonstrated in clinical trials that treatment of dyslipidemic patients with drugs that decrease LDL cholesterol levels significantly reduces the risk for coronary heart disease (1–4). Statins represent the major class of hypolipidemic drugs on the market. They act through the inhibition of HMG-CoA reductase, a pivotal enzyme in the cholesterol biosynthetic pathway, thus leading to a reduction of cholesterol concentration and a subsequent increase in expression of the low density lipoprotein receptor (LDLR), the main receptor involved in the hepatic clearance of LDL cholesterol (5–8).

Recently, ezetimibe, a new LDL cholesterol-lowering therapy described as a cholesterol absorption inhibitor, was developed, and it is now commercialized as a monotherapy or in combination with statins (9, 10). One interest of combination therapy is to reach the goals recommended by the National Cholesterol Education Program Adult Treatment Panel III (11) and to limit the potential side effects observed with high doses of statins (12). Clearly, the discovery of new drugs that could be developed in combination with statins is still of interest, especially compounds targeting other lipid fractions, such as HDL cholesterol and triglycerides (TGs), or other risk factors, such as type II diabetes and hypertension.

Berberine (BBR), an alkaloid isolated from the Chinese herb *Coptis chinensis*, has been widely used as a drug to treat gastrointestinal infections. Recently, BBR has been described as a new cholesterol-lowering drug (13). In this study, BBR treatment of 32 type IIb dyslipidemic patients led to a 25% decrease in LDL cholesterol and a 35% decrease in TGs. This LDL cholesterol-lowering effect was attributed to the activity of BBR on hepatic LDLR expression via a new mechanism distinct from that of statins. Indeed, the authors demonstrated that in a human hepatoma cell line (HepG2) as well as in hyperlipidemic hamsters, BBR upregulated the expression of LDLR

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through stabilization of its mRNA involving an extracellular regulated kinase (ERK)-dependent mechanism (13, 14). The authors concluded that it could be worth developing this new drug in combination therapy with statins. However, statin treatment of hypercholesterolemic patients with TG levels < 200 mg/dl often shows a more pronounced effect on LDL cholesterol than on TGs (15). Thus, even if the mechanism of action described by Kong et al. (13) is consistent with a LDL cholesterol-lowering effect, it is more difficult to explain the TG-lowering effect simply through upregulation of the LDLR. The aim of our study was to identify additional mechanisms by which BBR could exert its hypotriglyceridemic effect in humans. Using both in vitro and in vivo models, we demonstrated that BBR was able to inhibit cholesterol and TG synthesis through the activation of AMP-activated protein kinase (AMPK), leading to a decrease in hepatic fat content.

MATERIALS AND METHODS

Lipid synthesis

HepG2 cells obtained from the American Type Culture Collection were plated on 24-well plates. Cells were incubated for 6 h with vehicle or compounds at the indicated doses in the presence of 74 kBq of [^{14}C]acetate (Amersham), 18.5 kBq of [^{14}C]oleate (Perkin-Elmer), or 18.5 kBq of [^{14}C]glycerol (Perkin-Elmer) in RMPI 1640 medium supplemented with 2% lipoprotein-deficient serum. In some experiments, cells were preincubated for 30 min without or with 10 μM PD98059. At the end of the incubation, intracellular lipids were extracted with isopropanol and secreted lipids were extracted from supernatant by a mixture of cyclohexane-isopropanol (3:2, v/v). Both secreted and intracellular lipids were separated by thin-layer chromatography as described previously (16). Cholesterol and TGs were identified using purified standards, and the radioactivity associated with each individual lipid was quantified using a Phosphoscreen (Storm; Molecular Dynamics). Data are expressed as percentages of control values for cholesterol and TGs.

Measurements of Phosphorylated-acetyl-CoA carboxylase

HepG2 cells, plated on 96-well plates, were incubated for 6 h with the compounds at the indicated doses. Phosphorylation of acetyl-coenzyme A carboxylase (ACC) was performed using In Cell Western Protocol (Li-Cor Biosciences). Cells were fixed in 4% formaldehyde, permeabilized in PBS containing 0.1% Triton X-100, and then blocked with Li-Cor Odyssey Blocking buffer for 90 min. The antiphospho-ACC antibody (rabbit polyclonal IgG; Cell Signaling) was added to the cells overnight at 4°C. After several washes, a secondary antibody (IRDye 800 conjugated affinity purified anti-rabbit IgG; Rockland) was added for 1 h at room temperature. Fluorescence was quantified using the Odyssey Infrared Imaging system from Li-Cor Biosciences.

Measurements of AMPK phosphorylation and AMPK activity

HepG2 cells, plated on 24-well plates, were incubated for 6 h with the compounds at the indicated doses. At the end of the incubation, cells were lysed in Laemmli buffer containing phosphatase inhibitors (5 mM sodium pyrophosphate and 50 mM sodium fluoride). After SDS-PAGE and electrotransfer, AMPK was quantified using rabbit anti-α subunit (Cell Signaling) and AMPK threonine 172 phosphorylation was quantified using rabbit anti-pT172 antibody (Cell Signaling). Immununodetection was performed with the Odyssey Procedure (Li-Cor Biosciences) using an IRDye800 coupled anti- rabbit IgG secondary antibody (Rockland).

For AMPK activity, treated HepG2 cells were lysed using 1% Triton X-100, and AMPK was immunoprecipited by Sepharose-coupled rabbit anti-AMPKα1 antibody (Abcam). AMPK activity was then determined by phosphorylation of the synthetic SAMS peptide, as described previously (17), in the presence or absence of 50 μM AMP.

Fatty acid oxidation assay

Fatty acid oxidation was measured in HepG2 cells plated on 96-well plates and incubated for 6 h with the compounds at the indicated doses and then challenged for 30 min with 3 kBq of [^{14}C]palmitate (50 μM in fatty acid-free albumin). Incubation was stopped with 5% perchloric acid, and acid-soluble metabolites were separated and measured by scintillation counting (18, 19).

In vivo activity

All experimental protocols were performed in accordance with the policies of the Institutional Animal Care and Use Committee. Animals were fed an appropriate diet and had free access to water. Male Syrian golden hamsters (Janvier) were fed for 2 weeks with a high-fat diet (0.12% cholesterol and 10% coconut oil). Then, animals were treated orally twice a day for 10 days with BBR at 100 mg/kg/day or vehicle (0.5% methylcellulose and 1% Tween 80, pH 7). At the end of the treatment, lipoproteins were analyzed as described (20). Quantification of lipid molecular species in the liver was performed by gas-liquid chromatography. Part of the liver was weighted and then crushed using an Ultra Turax in 1 ml of methanol and 5 mM EGTA (2:1, v/v). Aliquots corresponding to an equivalent of 0.5 mg of liver were evaporated, and then the protein pellets were dissolved in 0.5 ml of NaOH (0.1 M) overnight and measured with the Bio-Rad assay. Lipids corresponding to an equivalent of 1 mg of tissue were extracted in chloroform-methanol-water (2.5:2.5:2.1, v/v/v) in the presence of the internal standards: 6 μg of stigmasterol, 4 μg of diacylglycerol-1,3-dimyristoyl, 4 μg of cholesteryl heptadecanoate, and 6 μg of triheptadecanoyl glycerol. The chloroform phase was filtered through glass wool, evaporated to dryness, and dissolved in 20 μl of ethyl acetate. Two microliters of the lipid extract was analyzed by gas-liquid chromatography on the FOCUS Thermo Electron system using Zebron-1 Phenomenex fused silica capillary columns. Quantification of fatty acid methyl ester (FAME) molecular species in the liver was done by gas-liquid chromatography. Lipids corresponding to an equivalent of 1 mg of liver were extracted according to the method of Bligh and Dyer in the presence of nonadecanoic acid (2 μg) as an internal standard. The lipid extract was transmethylated with a mixture of acetyl chloride in methanol (1:20, v/v) for 1 h at 55°C. After evaporation to dryness, the FAMEs were extracted with 2 ml of petroleum ether and 2 ml of water. The organic phase was evaporated to dryness and dissolved in 20 μl of ethyl acetate. One microliter of FAME was analyzed by gas-liquid chromatography using Famewax RESTEK fused silica capillary columns.

RESULTS

BBR inhibits lipid synthesis and secretion in HepG2 cells

We first evaluated the impact of BBR on TG and cholesterol synthesis by incubating HepG2 cells for 6 h with

[^{14}C]acetate in the presence of increasing concentrations of BBR. As depicted in **Fig. 1A**, BBR dose-dependently inhibited both cholesterol and TG synthesis, leading to an IC_{50} of ~15 μg/ml for both lipids. Regarding the secreted fraction of lipids, Fig. 1B shows that the inhibition of lipid synthesis translates into a similar reduction of cholesterol and TG secretion, with IC_{50} of 10.4 and 5.8 μg/ml, respectively. These potencies are in total accordance with those obtained with BBR for LDLR upregulation (13). Moreover, these inhibitions were observed 6 h after the addition of the drug, and this time has been shown to be the optimal time point for LDLR upregulation (13). In addition, it was previously described that the effects of BBR on LDLR upregulation were dependent on the mitogen-activated protein kinase kinase (MAPK/ERK) cascade (13). As illustrated in Fig. 1C, D, preincubation of the cells with the MAPK inhibitor PD98059 at 10 μM blunted the BBR-mediated inhibition of cholesterol (63% vs. 97% of control) and TG (64% vs. 90% of control) synthesis. These results confirm that, as observed for LDLR upregulation, BBR effects on lipid synthesis are mediated by the MAPK/ERK pathway. Similar results were obtained in the secreted fraction (data not shown). In parallel, cells were incubated with 5-tetradecyloxy-2-furan-carboxylic acid (TOFA; at 2.5 μM), an ACC inhibitor (16). In contrast to BBR, TOFA specifically decreased TG synthesis (Fig. 1D) via a MAPK-independent pathway. In summary, we found that in HepG2 cells, BBR inhibits TG and cholesterol synthesis by a signaling pathway involving the MAPK/ERK cascade.

BBR activates AMPK

The AMPK has been proposed to act as a fuel gauge in mammalian cells. Among the large number of AMPK protein targets, HMG-CoA reductase and ACC are well identified (21, 22). These two enzymes, involved in cholesterol and fatty acids synthesis, are inactivated by AMPK-mediated phosphorylation, leading to cholesterol and TG synthesis inhibition. We decided to compare the effects of BBR with those induced by the AMPK activator 5-aminoimidazole-4-carboxamide 1-β-ribofuranoside (AICAR) on lipid synthesis. AICAR is phosphorylated in cytosol by adenine kinase and then is converted in AICA-ribotide, which mimics AMP and activates AMPK in various cells, including HepG2 (23). TG synthesis was determined using [^{14}C]acetate or [^{14}C]glycerol incorporation, whereas TG assembly was assessed by the incorporation of [^{14}C]oleate.

HepG2 cells were incubated for 6 h with [^{14}C]acetate, [^{14}C]glycerol, or [^{14}C]oleate in the presence of BBR (15 μg/ml) or AICAR (2 mM). As observed in **Fig. 2A**, BBR

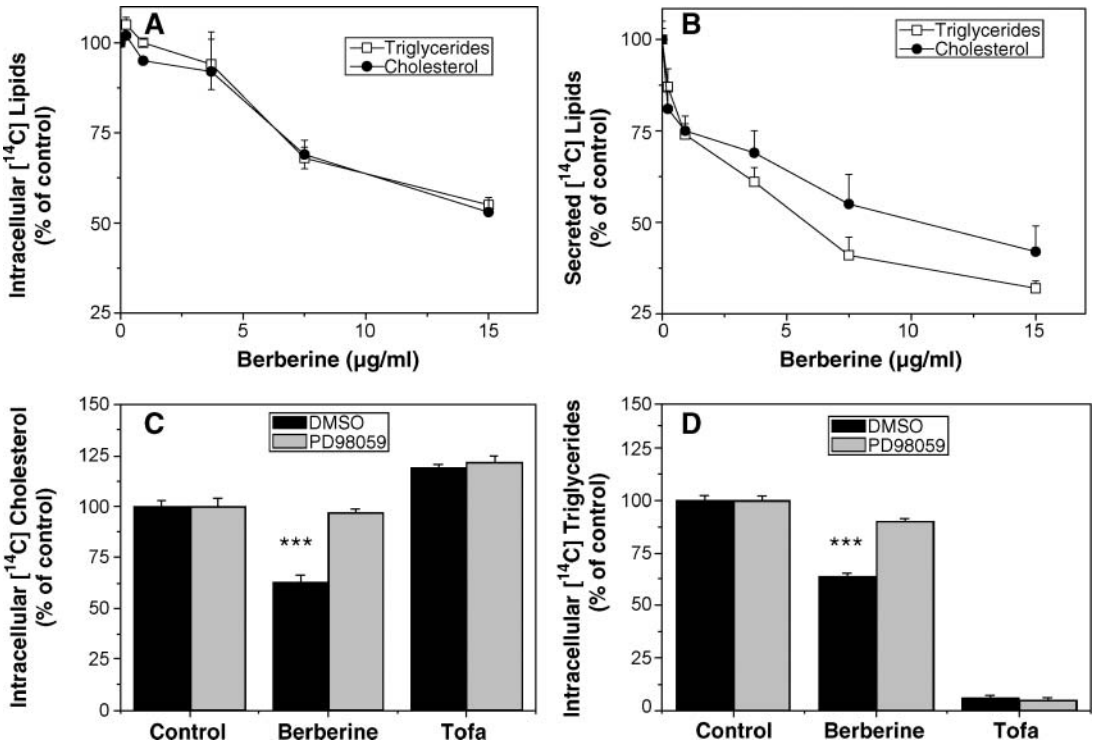

Fig. 1. Inhibition of lipid synthesis by berberine (BBR). A, B: HepG2 cells were incubated for 6 h in the absence or presence of BBR and labeled with [^{14}C]acetate. Intracellular (A) or secreted (B) ^{14}C-lipids were extracted and analyzed as indicated in Materials and Methods. Neosynthesized triglycerides (TGs; open squares) and cholesterol (closed circles) were quantified. Values are expressed as percentages of control and are means ± SEM (n = 5 from two independent experiments). C, D: HepG2 cells were preincubated for 30 min without (DMSO) or with 10 μM PD98059 and then further incubated for 6 h without (control) or with 10 μg/ml BBR or 2.5 μM 5-tetradecyloxy-2-furan-carboxylic acid (Tofa) and labeled with [^{14}C]acetate. Intracellular [^{14}C]cholesterol (C) and [^{14}C]TGs (D) were quantified. Values are expressed as percentages of control and are means ± SEM (n = 11 for BBR, n = 5 for Tofa, from four and two independent experiments, respectively). *** $P < 0.001$.

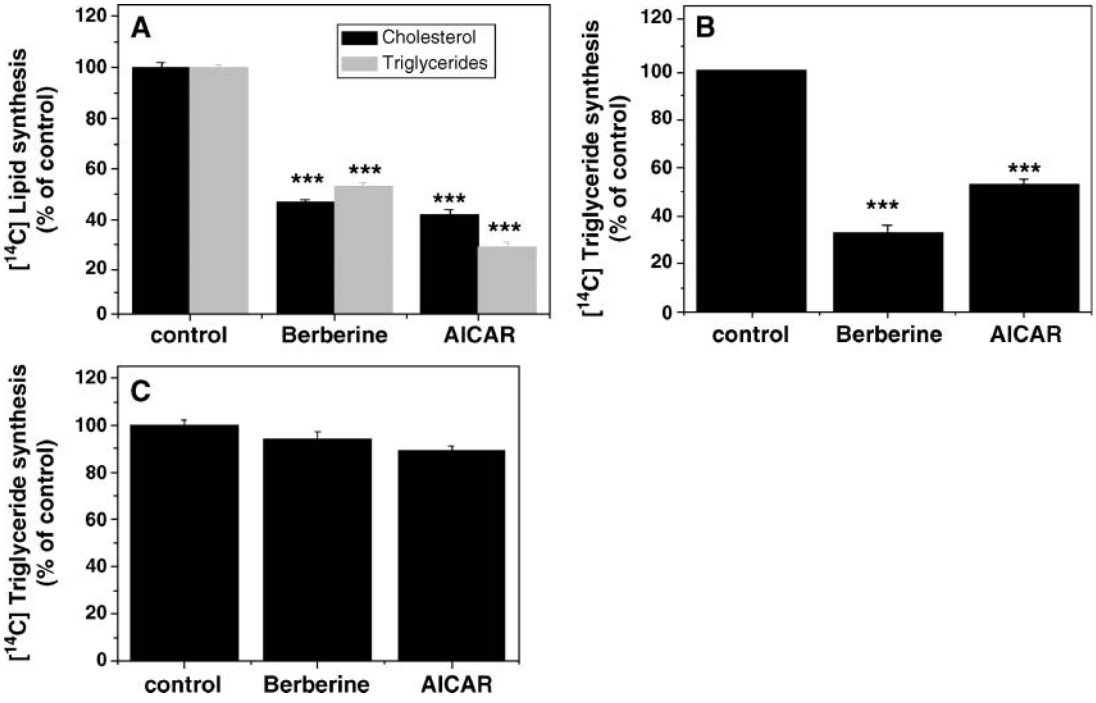

Fig. 2. Similar profiles induced by BBR and 5-aminoimidazole-4-carboxamide 1-β-ribofuranoside (AICAR) on lipid synthesis. HepG2 cells were incubated for 6 h in the absence (control) or presence of 15 μg/ml BBR or 2 mM AICAR and labeled with [^{14}C]acetate (A), [^{14}C]glycerol (B), or [^{14}C]oleate (C). Intracellular ^{14}C-lipids were extracted and analyzed as indicated in Materials and Methods. Values are expressed as percentages of control and are means ± SEM (n = 9, from three independent experiments). *** $P < 0.001$.

and AICAR inhibited [^{14}C]acetate incorporation into cholesterol by 53% and 58%, respectively, and into TG by 47% and 71%, respectively. When cells were incubated with [^{14}C]glycerol, BBR and AICAR inhibited TG synthesis by 67% and 47%, respectively (Fig. 2B). However, as shown in Fig. 2C, neither BBR nor AICAR prevented [^{14}C]oleate incorporation into TG, suggesting that TG assembly was not affected during the treatment. Moreover, these results suggest that BBR and AICAR induce similar profiles on lipid synthesis, suggesting that both compounds could regulate lipid metabolism by a common mechanism.

Therefore, we studied the possibility that BBR could activate AMPK. It is currently accepted that ACC phosphorylation levels into the cells represent a marker of AMPK activity (21). As shown in **Fig. 3A**, BBR dose-dependently increased ACC phosphorylation, with a half-maximal effect obtained at 2 μg/ml. Moreover, BBR-induced ACC phosphorylation was completely blocked in the presence of PD98059. In parallel, we showed that AICAR also increased ACC phosphorylation by 2.5-fold, an effect that was not affected by the presence of PD98059 (Fig. 3B).

AMPK activation leads to an increase in fatty acid oxidation, dependent on the inhibition of ACC by AMPK (24, 25). Figure 3C shows that BBR achieved a significant dose-dependent increase in fatty acid oxidation (half-maximal effect obtained at 10 μg/ml). As observed for the phosphorylation of ACC, this effect was totally inhibited in the presence of the MAPK inhibitor. In parallel, we showed that AICAR also increased fatty acid oxidation by 16-fold and that, as shown previously for ACC phosphorylation, this effect was not affected by the presence of PD98059 (Fig. 3D). We conclude that in HepG2 cells, BBR, but not AICAR, activates AMPK through a MAPK/ERK-dependent pathway, leading to the activation of fatty acid oxidation. This latter effect, associated with a decrease in TG synthesis, could explain the TG-lowering effect of BBR observed in patients and suggests pleiotropic effects of BBR on lipid metabolism.

Next, we evaluated the in cell activity of AMPK. After incubation of HepG2 cells with BBR, we monitored both the phosphorylation of the AMPK on threonine 172 and AMPK using specific antibodies. As shown in **Fig. 4A**, BBR significantly increased the phosphorylation of AMPK without altering the expression of AMPK (data not shown). Furthermore, preincubation of the cells with the MAPK inhibitor PD98059 at 10 μM suppressed the effects of BBR on AMPK phosphorylation.

To reinforce the activation of AMPK by BBR, HepG2 cells were treated with BBR and the enzyme was immunoprecipitated. AMPK activity was quantified using SAMS peptides (17). The assay was done in the presence or absence of 50 μM AMP (Fig. 4B). As expected for the AMPK, addition of AMP in the in vitro assay elicited a 2.3-fold increase in AMPK basal activity. Furthermore, cells treated with BBR exhibited an increase in AMPK activity, as demonstrated using the AMPK assay performed without or with AMP (1.7- and 1.6-fold, respectively, vs. nonstimulated cells).

Inhibition of lipid synthesis by BBR translates into a diminution of hepatic lipid and fatty acid content in vivo

Hamsters fed a diet enriched in cholesterol and fatty acids display a lipoprotein profile comparable to that of

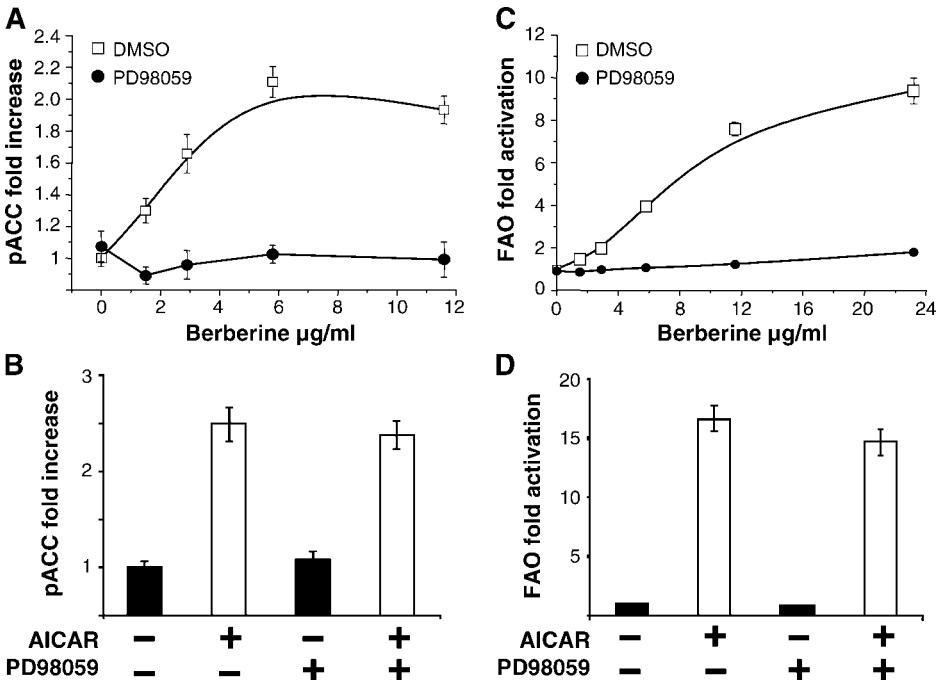

Fig. 3. Stimulation of acetyl-coenzyme A carboxylase (ACC) phosphorylation and fatty acid oxidation by BBR and AICAR. HepG2 cells were preincubated for 30 min without (DMSO) or with 10 µM PD98059 and then further incubated for 6 h with increasing doses of BBR or 2 mM AICAR. At the end of the incubation, phosphorylation of ACC (A, B) and fatty acid oxidation (C, D) were measured as indicated in Materials and Methods. Values are expressed as fold increase over untreated cells and are means ± SEM (n = 6, from two independent experiments).

humans (20). We investigated the effects of BBR on the hepatic lipid content in fat-fed hamsters. After 10 days of treatment with BBR (100 mg/kg/day), the serum LDL cholesterol fraction was significantly reduced by 39% compared with that in control animals. The HDL cholesterol fraction was unchanged (**Table 1**). The extent of the LDL cholesterol-lowering effect was comparable to that described previously (−42%) for the same dose of BBR in the same animal model (13).

In addition, BBR significantly decreased hepatic lipid content, with reductions of TG, cholesterol, and cholesteryl ester of 23, 27, and 41%, respectively. Interestingly, we also observed that hepatic fatty acid levels were significantly reduced by 16%. Thus, in addition to decreasing plasma LDL cholesterol, BBR induces a significant reduction of the hepatic fat content.

DISCUSSION

Kong et al. (13) described that BBR increased LDLR expression in HepG2 cells. They also demonstrated that this effect was not attributable to a transcriptional upregulation but rather to LDLR mRNA stabilization. Finally, they concluded that the LDL cholesterol-lowering effect (−25%) of BBR observed in hypercholesterolemic patients could be driven by LDLR upregulation. In addition, BBR in this patient population also displayed a robust and unexpected TG-lowering effect (−35%), in light of the mechanism of action described above. Statins, which also decrease LDL cholesterol levels through LDLR upregulation, have some significant TG-lowering effects, but in general the impact on TG is lower than that observed for LDL cholesterol (15). Thus, to better understand this additive beneficial TG-lowering effect, we decided to explore in more detail the effects of BBR on lipid synthesis and secretion by human hepatocytes. We found that in HepG2 cells, BBR strongly reduced TG and cholesterol synthesis and secretion. The IC_{50} values obtained for these lipid parameters are in accordance with the potency described for LDLR upregulation in this cellular model. In addition, as described for LDLR upregulation, we demonstrated here that lipid synthesis inhibition induced by BBR was also sensitive to a MEK inhibitor, suggesting that both phenomena, LDLR upregulation and lipid synthesis inhibition, are driven by a common pathway that requires MAPK/ERK activation.

Because this inhibition of TG synthesis could explain, at least in part, the TG-lowering effect observed in hypercholesterolemic patients treated for 3 months with BBR, we decided to dissect out the mechanism that could trigger this effect. We concluded that activation of AMPK induced by BBR could be responsible for this profile. Several reasons led us to postulate this assumption. Indeed, AMPK has been proposed to play a key role in the regulation of lipid metabolism (21). Two enzymes involved in cholesterol and fatty acid synthesis, HMG-CoA reductase and ACC, are considered the primary targets of

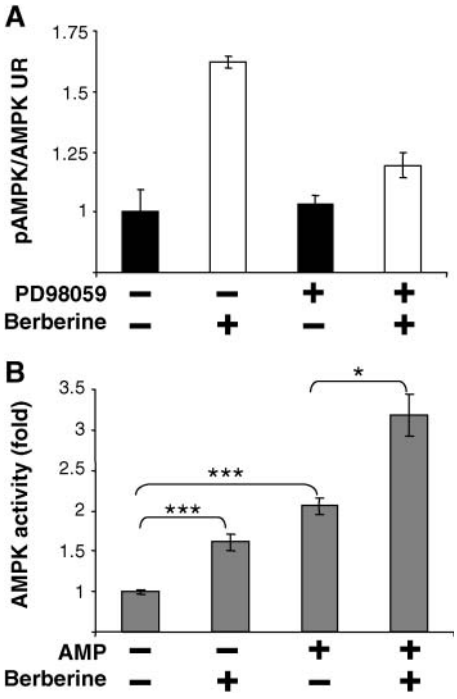

Fig. 4. Stimulation of AMP-activated protein kinase (AMPK) phosphorylation and AMPK activity by BBR. A: HepG2 cells were preincubated for 30 min without (DMSO) or with 10 μM PD98059 and then further incubated for 6 h with BBR at 24 μg/ml. Phosphorylation of AMPK and total cellular AMPK were measured by Western blot, as indicated in Materials and Methods. The ratio of phosphorylated AMPK (pAMPK) to AMPK signal was determined, and data are expressed as fold increase over untreated cells. Data are means ± SD of duplicates from one representative experiment. Similar results were obtained in another independent experiment. B: HepG2 cells were incubated for 6 h without or with BBR at 14 μg/ml. After immunoprecipitation, AMPK activity was quantified with or without AMP, as described in Materials and Methods. Values are expressed as fold increase over untreated cells and are means ± SEM (n = 5, from two independent experiments). * $P < 0.05$, *** $P < 0.001$.

AMPK and are phosphorylated by the enzyme, leading to cholesterol and TG synthesis inhibition (19). When compared with the well-known AMPK activator AICAR, BBR induced a similar profile on lipid synthesis, with inhibition of cholesterol and TG synthesis without any impact on TG assembly. These effects may be explained by data showing that BBR induces an increase in the level of phosphorylated ACC, considered a marker of AMPK activity. The half-maximal effect was obtained at 2 μg/ml, similar to the concentration required to inhibit lipid synthesis. In addition, as observed for lipid synthesis, the effect of BBR on the phosphorylation of ACC was completely abolished with the MEK inhibitor (PD98059). Phosphorylation of ACC led to a decrease in malonyl-CoA that relieves the inhibition of fatty acid oxidation (21). As observed for the phosphorylation of ACC, stimulation of fatty acid oxidation by BBR occurred in the same dose range and was also blunted by the MEK inhibitor. Furthermore, after BBR treatment, AMPK activity as well as phospho-AMPK levels increase inside the cells, as demonstrated by immunoprecipitation and Western blot experiments.

TABLE 1. BBR reduces plasma LDL cholesterol and lipid mass accumulation in liver of fat-fed hamsters

Sample	Plasma Lipids						Hepatic Lipids						Hepatic Fatty Acids	
	VLDL Cholesterol		LDL Cholesterol		HDL Cholesterol		Cholesterol		Cholesteryl Ester		Triglycerides			
	g/l	*% of control*	*g/l*	*% of control*	*g/l*	*% of control*	*nmol/mg*	*% of control*	*nmol/mg*	*% of control*	*nmol/mg*	*% of control*	*nmol/mg*	*% of control*
Control	0.3 ± 0.1	100%	1.5 ± 0.3	100%	2.2 ± 0.3	100%	39.4 ± 7	100%	387 ± 120	100%	25.7 ± 4	100%	710 ± 101	100%
BBR	0.3 ± 0.1	100%	0.9 ± 0.2 ($P < 0.05$)	61%	2.2 ± 0.3	100%	28.8 ± 2	73% ($P < 0.05$)	229 ± 54	59% ($P < 0.05$)	19.8 ± 5	77% ($P < 0.1$)	596 ± 72	84% ($P < 0.1$)

BBR, berberine. Hamsters were orally dosed twice a day during 10 days with vehicle (control) or with 50 mg/kg BBR. Plasma and hepatic lipids and fatty acids were quantified as indicated in Materials and Methods. Values are means ± SD of five animals.

Together, these data suggest that BBR induces the activation of AMPK, which translates into the phosphorylation of ACC, leading to the subsequent increase in fatty acid oxidation, decrease in fatty acid synthesis, and, finally, TG synthesis. Whether or not BBR activates AMPK by altering the AMP/ATP ratio or by an alternative mechanism is still unknown. Interestingly, we showed that this AMPK activation occurred through the MAPK/ERK pathway, thus confirming a putative link between this pathway and AMPK, as demonstrated previously (26). This finding could be of great interest because AMPK activation has been proposed as a valuable approach to target lipid disorders (22) and because antidiabetic drugs such as rosiglitazone and metformin have been described to act, at least partially, through AMPK activation (27). These results suggest that the TG-lowering effect of BBR observed in patients may be independent of LDLR upregulation, thus demonstrating pleiotropic effects of BBR.

To reinforce the physiological relevance of these cellular observations, we investigated the impact of BBR on hepatic lipid content in fat-fed hamsters. First, we confirmed the extent of the LDL cholesterol-lowering effect described previously for the same dose of BBR in the same animal model (13). In addition, we found that BBR treatment significantly decreased neutral lipid content as well as free fatty acid level. These findings suggest that the inhibition of lipid synthesis that we demonstrated in vitro on HepG2 cells translates into hepatic fat storage diminution in vivo. We speculate that this decrease in hepatic lipid content could lead to the improved liver function demonstrated by Kong et al. (13) in their clinical study. Indeed, BBR improved liver function in patients, based on alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transpeptidase activities; this property, in addition to circulating lipid effects, could be of great interest for combination therapies with statins or other lipid-modifying drugs.

In conclusion, we demonstrate that BBR, via AMPK activation, inhibits cholesterol and TG synthesis in hepatic cells. LDLR upregulation (13, 14), AMPK activation, and, finally, lipid synthesis inhibition are abolished when the MAPK/ERK pathway is blocked, suggesting that all of these events are linked to a similar mechanism of action. The precise mechanism by which BBR induces an ERK-dependent activation of AMPK remains to be elucidated. When administered to animal models, the effects of BBR on lipid homeostasis translate into a significant reduction in hepatic fat storage, confirming that BBR could represent a promising new approach to reduce LDL cholesterol and TGs as a monotherapy or in combination with other existing drugs.

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