Berberine improves lipid dysregulation in obesity by controlling central and peripheral AMPK activity

Woo Sik Kim,1* Yun Sok Lee,1* Seung Hun Cha,2* Hyun Woo Jeong,1 Sung Sik Choe,1 Mi-Ran Lee,3 Goo Taeg Oh,3 Hye-Sun Park,4 Ki-Up Lee,4 M. Daniel Lane,2 and Jae Bum Kim1

1Institute of Molecular Biology and Genetics, Seoul National University, Seoul 151-742, Korea; 2Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; 3Division of Life and Pharmaceutical Sciences, Center for Cell Signaling Research, Ewha Womans University, Seoul 120-750; and 4Asan Institute for Life Sciences, and Department of Internal Medicine, University of Ulsan College of Medicine, Seoul, Korea 138-736

Submitted 17 November 2008; accepted in final form 26 January 2009

Berberine (BBR) is a naturally occurring structurally characterized compound present in many herbs and has a variety of pharmacological properties including glucose lowering (9, 15, 41) and antimicrobial activities (17). We and others (9, 19, 38) have reported that BBR reduces body weight, causes a significant improvement in glucose tolerance, and improves insulin action in obese and/or diabetic subjects by activating AMPK. A large body of evidence indicates that BBR enhances insulin sensitivity and reduces hyperlipidemia (5, 9, 16, 38). Thus it is plausible to speculate that this mechanism might be applied therapeutically to treat fatty liver and hyperlipidemia. These

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
findings prompted us to address the question of whether BBR might alter whole body lipid metabolism to ameliorate the lipid dysregulation associated with obesity by direct and/or indirect regulation of AMPK in peripheral tissues, concomitantly with the resetting of the metabolic gene expression. In the present study, we show that BBR would relieve fatty liver and hyperlipidemia by modulating both peripheral and central AMPK activity to increase whole body energy homeostasis.

MATERIALS AND METHODS

Mouse experiments. All experiments were approved by the Seoul National University Animal Experiment Ethics Committee and the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee. Obese and diabetic C57BLKS/J-Lepr<sup>db</sup>/Lepr<sup>db</sup> male mice were housed at 22 ± 2°C, 55 ± 5% relative humidity, with a light-dark cycle of 12 h. Food (Purina Mills) and water were provided ad libitum. At 9 wk of age, BBR (Wako, Osaka, Japan) was intraperitoneally (5 mg/kg body weight) injected into the mice for 3 wk between 0900 and 1000. Thereafter, the liver, kidney, spleen, heart, lung, and skeletal muscle were dissected. After dissection, the specimens were immediately frozen in liquid nitrogen and stored at −80°C. For intracerebroventricular injection, BBR (5 μg in 2 μl of RPMI 1640; Invitrogen) or vehicle was injected into the lateral ventricle under isoflurane anesthesia. After intracerebroventricular injection, the mice were given free access to food. Two hours after intracerebroventricular injection of BBR, hypothalamus and skeletal muscle (gastrocnemius muscle) were removed for analysis within 30–60 s after death. Respiratory quotient (RQ) and energy expenditure are measured as described previously (25).

Blood parameters. Blood samples were collected from mice for the measurement of plasma levels of triglyceride, cholesterol, and free fatty acid. Liver damage was assessed by measurement of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Hepatic fatty acid. Liver extracts from BBR- or vehicle-treated db/db mice were prepared using a TGN lysis buffer (50 mmol/l Tris·HCl pH 7.5, 150 mmol/l NaCl, 1% Tween 20, 0.2% Nonidet P-40, 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l sodium Na3VO4, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1 μg/ml pepstatin), and triglyceride and total cholesterol levels were measured according to the manufacturer’s instructions. Triglyceride and cholesterol contents were measured from liver samples prepared as described previously (3). Determination of malonyl-CoA was as described previously (13).

Quantitative real-time PCR analysis. mRNA expression of several metabolic genes was quantified by real-time PCR with SYBR Green I (BioWhittaker Molecular Applications). The primer sequences used for the real-time PCR analyses are available on request.

Western blot analysis. Western blot analysis was carried out as described previously (19). To summarize, protein samples were subjected to SDS-PAGE and blotted onto polyvinylidene difluoride (Millipore) membranes. Blotted membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h and then incubated with primary antibodies against AMPK (Cell Signaling Technology), phosphor-AMPK (Thr172; Cell Signaling Technology), ACC (Upstate), phosphor-ACC (Upstate), carnitine palmitoyltransferase-1 (CPT-1; Astellas Pharma), GAPDH (Ab Frontier), and medium chain acyl-CoA dehydrogenase (mCAD; Santa Cruz Biotechnology) for 16 h at 4°C. After three washes in Tris-buffered saline containing 0.1% Tween 20, the membranes were incubated with anti-mouse, anti-rabbit, or anti-goat IgG antibodies conjugated with horseradish peroxidase for 2 h. Immunoreactive signals were detected with the WEST-1 Western blot detection system (Intron, Kyungki-Do, Korea) and quantified with a LuminoImager (LAS-3000) and Science Lab 2001 Image Gauge software (Fuji Photo Film).

RESULTS

BBR alleviates dyslipidemia and fatty liver in obese mice. Since obesity and insulin resistance are closely associated with fatty liver, we further examined whether BBR would ameliorate fatty liver damage. In the present study, we show that BBR would relieve fatty liver and hyperlipidemia by modulating both peripheral and central AMPK activity to increase whole body energy homeostasis.

![Fig. 1. Effects of berberine (BBR) on body weight, food intake, and energy expenditure in db/db mice. A: effect of BBR on body weight. Vehicle (Veh) or BBR was given into db/db mice every day for 3 wk. Data are means ± SD (n = 5), *P < 0.05; **P < 0.005. B: effect of BBR on food intake. Vehicle (Veh) or BBR was given in db/db mice. C: effect of BBR on respiratory quotient (RQ) and energy expenditure from vehicle-treated and BBR-treated db/db mice. RQ and energy expenditure were measured as described in MATERIALS AND METHODS. Data are means ± SD (n = 5). **P < 0.05.](http://ajpendo.physiology.org/doi/10.1210/ajpendo.2009-0021)
BBR stimulates AMPK to increase fatty acid oxidation in the liver of obese mice. Because recent studies (5, 9, 19) have shown that BBR stimulates AMPK activity, we hypothesized that improvement of fatty liver in obese animals results from the increase of hepatic AMPK activity. To test this idea, we investigated the phosphorylation levels of AMPK and ACC in liver of db/db mice with or without BBR treatment. BBR increased the phosphorylation levels of hepatic AMPK and ACC in db/db mice (Fig. 3A). Since it has been well established that peripheral AMPK activation could promote fatty acid oxidation by inactivating ACC (14), we determined whether BBR-induced AMPK phosphorylation in the liver of obese animals indeed causes reduction of hepatic fat contents. As illustrated in Fig. 3B, BBR substantially increased fatty acid oxidation in the liver of db/db mice. In accordance with in vivo results, we observed that BBR enhanced phosphorylation levels of AMPK and ACC and stimulated AMPK activity in cultured FAO hepatocyte cells (Supplemental Fig. S2, A and B). Furthermore, in high glucose challenged FAO hepatocyte cells exhibiting increased lipid accumulation (39), BBR treatment accelerated fatty acid oxidation and decreased cellular triglyceride and cholesterol contents (Supplemental Fig. S2, C-E). Together, these results suggest that BBR could increase fatty acid oxidation in the liver via AMPK activation, which would lead to the removal of excess hepatic fat accumulation.

BBR regulates the expression of genes involved in lipid metabolism in the liver of obese mice. The observation that BBR reduced the levels of hepatic triglyceride and cholesterol in obese animals led us to examine the idea whether BBR

Fig. 2. BBR relieves fatty liver and decreases plasma lipid contents in db/db mice. A: morphology of the liver from vehicle (left)-treated and BBR (right; 5 mg·kg\(^{-1}\)·day\(^{-1}\))-treated db/db mice. B: weights of several tissues from vehicle-treated and BBR-treated db/db mice. Data are means ± SD (n = 5). *P < 0.05. C: hepatic triglyceride and cholesterol levels from vehicle-treated mice and BBR-treated mice. Data are means ± SD (n = 5). *P < 0.05. D: histological analysis of liver from vehicle-treated and BBR-treated db/db mice. Samples were stained with Oil Red O (top) or hematoxylin and eosin (bottom). E-F: plasma triglyceride (E), cholesterol (F), nonesterified fatty acids (NEFA; G), alanine aminotransferase (H), and aminotransferase (I) levels were measured from plasma of db/db mice treated with vehicle or BBR (5 mg·kg\(^{-1}\)·day\(^{-1}\)). Data are means ± SD (n = 5). *P < 0.05.
might affect the expression of genes of lipid metabolism in the liver. To test this, we investigated the expression of lipogenic genes in BBR-treated db/db mice. As illustrated in Fig. 4A, BBR remarkably elevated the expression of key fatty acid oxidation genes such as acyl-CoA oxidase (ACO), CPT-1α, mCAD, and peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) in liver of db/db mice. In addition, BBR increased the expression of uncoupling protein 2 (UCP2; Fig. 4A), which is thought to be associated with energy expenditure by dissipating the chemiosmotic gradient in mitochondria (10, 11, 28).

On the contrary, BBR reduced the expression of many lipogenic genes including ADD1/SREBP-1c, PPAR-γ, ACC, stearoyl-CoA desaturase, and fatty acid synthase (Fig. 4B). Despite these changes, BBR did not alter the expression of genes involved in VLDL synthesis and secretion, such as apoB, apoE, and microsomal triglyceride transfer protein, precluding the possibility that BBR might increase secretion of lipids from the liver of obese mice (Fig. 4C). BBR also downregulated several hepatic proinflammatory genes, including TNF-α, IL-6, and serum amyloid A3 (SAA3; Fig. 4D), which have been proposed to play a role in the development of steatohepatitis (12). Consistent with these gene expression profiles, BBR increased CPT-1 and mCAD proteins, which are associated with fatty acid oxidation, in the liver of db/db mice (Supplemental Fig. S3A). These findings suggest that BBR would ameliorate fatty liver by enhancing fatty acid oxidation.
and by repressing lipid biosynthesis and inflammatory responses in the liver of obese animals.

**BBR promotes muscular AMPK to increase fatty acid oxidation in obese mice.** Since BBR has been reported to increase AMPK phosphorylation in L6 myotubes (9, 19), we investigated whether BBR might also stimulate fatty acid oxidation in the skeletal muscle of obese animals through AMPK activation as it does in the liver of db/db mice. We examined the levels of AMPK and ACC phosphorylation in the skeletal muscle of db/db mice in the absence or presence of BBR administration. As shown in Fig. 5A, BBR potently elevated the phosphorylation of AMPK and ACC in the skeletal muscle of db/db mice. Furthermore, BBR greatly increased the expression of fatty acid oxidation genes, including ACO, mCAD, CPT-1, UCP2, and PGC-1α, in the skeletal muscle of db/db mice (Fig. 5B), concomitantly with the increase of the CPT-1 protein level (Supplemental Fig. S3B). Similar to in vivo data, BBR increased phosphorylation levels of AMPK and ACC and promoted fatty acid oxidation in C2C12 myotubes (Supplemental Fig. S4, A and B). BBR-treated myotubes also elevated the expression of fatty acid oxidation genes such as ACO, CPT-1, and mCAD (Supplemental Fig. S4C). These results indicate that muscular AMPK activation by BBR would contribute to the alleviation of fatty liver by enhancing fatty acid oxidation, leading to an improvement of whole body energy metabolism.

**Central administration of BBR regulates the level of malonyl-CoA and the expression of fatty acid oxidation genes in skeletal muscle of obese mice.** Centrally administered effectors that perturb AMPK and ACC phosphorylation and malonyl-CoA levels in the hypothalamus rapidly trigger neural signals to skeletal muscle that alter fatty acid oxidation (7, 8, 14, 21, 24). These findings provide a clue to test the effect of centrally administered BBR on such parameters. BBR, administered by intracerebroventricular injection, rapidly increased hypothalamic dephospho-AMPK (inactive) and dephospho-ACC (active) levels (Fig. 6A), provoking an increase in hypothalamic malonyl-CoA (Fig. 6B) and a decrease in food intake and body weight within several hours (Supplemental Fig. S5). Consistent with previous findings on the effects of elevating hypothalamic malonyl-CoA concentration (7, 8), intracerebroventricular BBR rapidly (∼2 h) lowered the level of muscular malonyl-CoA (Fig. 6C) and promoted the expression of key genes involved in fatty acid oxidation, i.e. CPT-1b, mCAD, PPARα, PGC-1α, and UCP3, in skeletal muscle (Fig. 6D). These results are consistent with the findings that BBR activates peripheral AMPK and induces fatty acid oxidation and thereby the dissipation of excess lipid metabolites in liver and muscle of obese animals.

**DISCUSSION**

Numerous reports (5, 9, 19, 32) of both in vivo and ex vivo studies have shown that BBR can bring about improvements in metabolic disorders, including obesity, insulin resistance and hyperlipidemia, by stimulating AMPK activity. For example, we (19) have shown that chronic BBR administration reduces body weight and improves insulin sensitivity in obese animals through AMPK activation in adipose tissue. It has also been reported that BBR increases glucose uptake in muscle cells by activating AMPK and p38 MAPK (9). While the present report was in preparation, it was reported that BBR stimulates AMPK activity and fatty acid oxidation in HepG2 cells and relieves hyperlipidemia in hamsters fed a high-fat diet (5). This study proposed that BBR might ameliorate fatty liver in obese animals by regulating energy metabolism especially through the activation of hepatic and muscular AMPK. On the other hand, it has been shown that BBR lowers blood cholesterol by stabilizing hepatic LDLR in an ERK-sensitive manner (16). In spite of these findings, the following has not been clearly elucidated: 1) whether BBR increases fatty acid oxidation by modulating metabolic gene expression in liver and skeletal muscle of obese animals; 2) whether lipid-lowering effect of BBR in obese animals leads to the improvement of liver functions; and, more importantly, 3) whether BBR activates peripheral AMPK indirectly through neural signaling.

In the present study, we demonstrated that BBR ameliorates lipid dysregulation including fatty liver in obese subjects. In obese mice, BBR augmented energy expenditure and reduced the RQ ratio. When we recalculated energy expenditure by normalizing with kidney weights, which had not been significantly changed by BBR, we obtained the similar result (data not shown) as shown in Fig 1C, implying that BBR could increase energy expenditure. Because BBR selectively diminished fat mass of obese animals, we cannot completely exclude the possibility that body weight loss would affect energy expenditure regardless of the effect of BBR. Furthermore, BBR reversed macro- and micro-alterations of hepatic structure in obese mice with reduced plasma ALT and AST levels, implying that BBR could improve liver function in obese mice. Although there are some differences between in vivo and in vitro system, the effect of BBR on the activation of AMPK and fatty acid oxidation was blunted by an AMPK inhibitor, com C, in cultured cells (Supplemental Figs. S2C and S4C), implying that AMPK might be a player to dissipate stored fat contents on BBR. Therefore, these findings suggest that BBR would reverse dyslipidemia and fatty liver by stimulating fatty acid oxidation and thereby inducing AMPK-mediated fatty acid oxidation. Thus, we believe that BBR may provide a clue to treat hyperlipidemia and obesity.
acid oxidation and resetting metabolic programs through directly modulating hepatic and muscular AMPK activity.

As a major regulator of energy expenditure, AMPK has been shown to coordinate metabolic programs that increase energy expenditure and decrease energy storage by modulating the activities of the key transcriptional regulators such as ADD1/SREBP1c and PGC-1α (40, 42). Accordingly, metformin has been shown to block expression of lipogenic genes by suppressing the activity of ADD1/SREBP1c through AMPK activation in primary hepatocytes (40). On the other hand, exercise and AMPK activators, such as 5-aminoimidazole-4-carboxamide and metformin, improve fatty liver by stimulating PGC-1α expression, a crucial factor in the transcriptional regulation of mitochondrial biogenesis and fatty acid oxidation (29–31). Consistently, we observed that BBR stimulated the expression of fatty acid oxidative genes, while BBR suppressed that of lipogenic genes concomitant with activation of AMPK and fatty acid oxidation. These results support the concept that BBR prevents dyslipidemia and fatty liver by directly promoting the activation of hepatic AMPK and fatty acid oxidation through upregulating fatty acid oxidation genes and downregulating lipid/energy storage genes as proposed by Brusq et al. (5).

It is also important to note that BBR is capable of acting indirectly via the CNS to alter peripheral lipid metabolism, notably in skeletal muscle. Intracerebroventricular injection of low amounts of BBR (too low to act systemically) increased malonyl-CoA concentrations in hypothalamus and rapidly lowered the level of malonyl-CoA in skeletal muscle of both lean and obese mice (Fig. 6). It is well established that a “malonyl-CoA signal” is rapidly transmitted from the hypothalamus to muscle via the sympathetic nervous system to activate muscular AMPK (7, 8). In skeletal muscle, lowering malonyl-CoA and increasing the expression of mitochondrial oxidative enzyme levels via peripheral AMPK activation leads to an increase in fatty acid oxidation (7, 8). Thus the effect of centrally administered BBR would be expected to improve fatty liver by stimulating muscular AMPK activity and fatty acid oxidation and thereby contributing to improve lipid metabolism and systemic insulin sensitivity.

Since centrally administered BBR reduced the level of muscular malonyl-CoA, apparently by activating AMPK in obese mice, it appears that BBR is able to promote muscular AMPK activity both directly and indirectly. Although acute intracerebroventricular injection of BBR suppressed food intake within several hours, long-term intraperitoneal administration of BBR into obese db/db and ob/ob mice did not significantly reduce food intake, implying that BBR would improve fatty liver and reduce body weight in obese mice mainly by increasing energy dissipation rather than by decreasing food intake. In the hypothalamus, it is well known that suppression of AMPK and subsequent increase of malonyl-CoA would reduce food intake by promoting anorexigenic neuropeptide proopiomelanocortin and decreasing orexigenic NPY (37). Very recently, it has been reported that BBR could pass the blood brain barrier and reach the brain (36). In this aspect, we can not exclude the possibility that a certain portion of intraperitoneally administered BBR might be delivered into brain, leading to coordinate energy dissipation with peripheral tissue. Further studies are required to determine how much BBR is present in the brain after the long-term intraperitoneal administration of BBR and to elucidate whether such level of BBR might affect food intake after chronic treatment in obese animals.

It is evident that the effect of BBR on AMPK modulation is indirect, as it neither binds to nor directly activates purified AMPK (personal communication, D. Graeme Hardie). Moreover, recently, it has been shown that BBR stimulates AMPK by suppressing mitochondrial activity in cultured myotubes (32), indicating that BBR affects AMPK activity through
different intermediaries in liver/muscle vs. the CNS. The mechanisms by which BBR regulates AMPK activity in these tissues remain to be elucidated.

Low-grade chronic inflammation is one of the major characteristics of obesity and obesity-related metabolic disorders such as atherosclerosis, type II diabetes mellitus, and steatohepatitis. Furthermore, NAFLD is often linked to the progression into the steatohepatitis and cirrhosis with increasing local hepatic inflammation (22). In this aspect, it is interesting to note that BBR reduced inflammatory gene expression in liver (Fig. 4D) and adipose tissue of obese animals (unpublished data, H. W. Jeong et al.). Therefore, it is likely that BBR could improve metabolic diseases probably through its anti-inflammatory property as well.

In conclusion, we have shown that BBR-induced improvement of hyperlipidemia and fatty liver in obese animals is mediated by the direct and indirect activation of AMPK in peripheral tissues, notably liver and muscle, and that AMPK activation is required for its effect on increasing fatty acid oxidation. BBR-dependent alleviation of dyslipidemia in obesity was accompanied by changes in hepatic and muscular gene expression programs that enhance fatty acid oxidation and reduce lipogenesis. Taken together, our findings suggest that BRR might be useful as a therapeutic agent for the treatment of fatty liver and hyperlipidemia.

GRANTS

This work was supported by the Korea Science and Engineering Foundation grant funded by the Korean government (MEST; No. R0A-2004-000-10359-0, M108KL01006-08KL1201-00630, R11-2005-009-10102-0). H. W. Jeong and J. B. Kim were supported by a BK21 Research Fellowship from the Ministry of Education and Human Resources Development.

REFERENCES


