Lipid-lowering effect of berberine in human subjects and rats


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ABSTRACT

Due to serious adverse effects and the limited effectiveness of currently available pharmacological therapies for obesity, many research efforts have focused on the development of drugs from natural products. Our previous studies demonstrated that berberine, an alkaloid originally isolated from traditional Chinese herbs, prevented fat accumulation in vitro and in vivo. In this pilot study, obese human subjects (Caucasian) were given 500 mg berberine orally three times a day for twelve weeks. The efficacy and safety of berberine treatment was determined by measurements of body weight, comprehensive metabolic panel, blood lipid and hormone levels, expression levels of inflammatory factors, complete blood count, and electrocardiograph. A Sprague-Dawley rat experiment was also performed to identify the anti-obesity effects of berberine treatment. The results demonstrate that berberine treatment produced a mild weight loss (average 5 lb/subject) in obese human subjects. But more interestingly, the treatment significantly reduced blood lipid levels (23% decrease of triglyceride and 12.2% decrease of cholesterol levels) in human subjects. The lipid-lowering effect of berberine treatment has also been replicated in the rat experiment (34.7% decrease of triglyceride and 5% decrease of cholesterol level). Cortisol, calcitriol, ACTH, TSH, FT4, and SHBG levels were not significantly changed following 12 weeks of berberine treatment. However, there was interestingly, an increase in calcitriol levels seen in all human subjects following berberine treatment (mean 59.5% increase, p = 0.11). Blood inflammatory factors (CRP, IL-6, TNFα, COX-2) and erythrocyte sedimentation rate (ESR) were not significantly affected by treatment with berberine. Tests of hematological, cardiovascular, liver, and kidney function following berberine treatment showed no detrimental side effects to this natural compound. Collectively, this study demonstrates that berberine is a potent lipid-lowering compound with a moderate weight loss effect, and may have a potential role in osteoporosis treatment/prevention.

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Introduction

The obesity epidemic has emerged as a severe health threat to the population worldwide. The Centers for Disease Control and Prevention (CDC) reported that 33.8% of U.S. adults and approximately 17% of children and adolescents aged 2–19 years were obese in 2010. The prevalence of obesity has tremendously increased the risk of many other disorders such as hyperlipidemia, type II diabetes, and osteoarthritis (Malnick and Knobler 2006). According to NIH guidelines (NIH 1998); a low calorie diet, increased physical activity, and behavior therapy are the fundamental treatments for obese individuals. If the combined lifestyle modification isn’t effective, pharmacological therapy should be considered for individuals with a BMI ≥30 kg/m² or patients with a BMI ≥27 kg/m² with concomitant obesity-related risk factors or diseases.

An unfortunate fact of obesity pharmacotherapy is that even though more than one dozen anti-obesity medications have been introduced into market, the majority of them have been withdrawn or discontinued due to serious adverse effects (Ioannides-Demos et al. 2011). The most recent examples are rimonabant and sibutramine which were withdrawn in 2009 and suspended in 2010 respectively. The currently available FDA-approved drugs for obesity treatment include amfepramone, phentermine, and orlistat. Amfepramone and phentermine are appetite suppressants...
approved only for short-term use due to safety issues. Orlistat is a fat absorption inhibitor approved for long-term use, however meta analyses support a significant although modest loss of only 2.4 kg weight in ≥12 months (Ioannides-Demos et al. 2011). Thus due to the serious adverse effects observed and the limited effectiveness of current pharmacological therapies for obesity, many research efforts have been concentrated on the development of drugs from natural products (Seo et al. 2011).

Berberine is an alkaloid originally extracted from Huanglian (Coptis chinensis), an ancient Chinese herb, which has been applied in traditional Chinese medicine for thousands of years to treat obesity and correlated disorders (Tan et al. 2011). Berberine has many well-established therapeutic benefits. Berberine can be used to treat gastrointestinal infections and also as an anti-inflammatory, anti-carcinoma, and an anti-diabetes drug (Vuddanda et al. 2010). In China berberine is used primarily as an antiidiabetic drug. Most recently berberine has been studied for its potential as a pharmacetical intervention for obesity. Our group has previously reported that berberine prevents fat accumulation in both murine and human adipocytes (Hu and Davies 2009; Hu et al. 2010). Our subsequent studies in a high-fat diet induced-obesity mouse model replicated the in vitro results of berberine’s prevention of fat accumulation (Hu and Davies 2010). As a natural extension of these previous studies, the aim of this work was to examine the safety, efficacy, and possible mechanisms of berberine on the treatment of obesity and associated disorders (e.g. hyperlipidemia and inflammation). Using a small Caucasian population, the safety of berberine was evaluated using hematological, cardiovascular, self-reports of side effects, and key liver and kidney function tests. The therapeutic efficacy of berberine was studied by measuring body weight, cholesterol, triglycerides, and the differential expression of inflammatory factors prior to and after 12 weeks of treatment. Potential mechanisms for berberine’s effects were studied by comparing measurements of hormone levels before and after berberine treatment. In addition, we also sought to replicate the anti-obesity and hyperlipidemia efficacy of berberine in Sprague-Dawley (SD) rats.

Materials and methods

Human subjects in this pilot study

This study was conducted in accordance with “good clinical practice” (GCP) and all applicable regulatory requirements including, where applicable, the October 1996 version of the Declaration of Helsinki. All applicable regulatory documents were reviewed by the Avera Institutional Review Board and prior approval was received before any implementation of the protocol procedures. A consent form was provided to each subject, which detailed the study title, physician, contact information, background, purpose, procedure, possible risks and side effects, benefits, cost, and compensation. Informed consents were obtained before the subject could participate in the study. The consents and process of obtaining informed consent were in accordance with all applicable regulatory requirements.

Subjects who provided signed consent forms and met the following inclusion criteria were enrolled: (1) Males and females 18 years and older; (2) BMI of 30 or greater; (3) Agree to keep diet, exercise and all current health habits (smoking and alcohol use) stable during participation in the study; (4) Willing to sign consent and follow study-related procedures.

The exclusion criteria were: (1) Gastroparesis or intestinal pseudo-obstruction; (2) Receiving medications known to affect gastric motility; (3) If currently on cholesterol-lowering, diabetes or thyroid medications, and the individual did not agree to be on a stable dose throughout the duration of the study; (4) Currently taking any antipsychotic medications (Abilify® (aripiprazole); Risperdal® (risperidone); Zyprexa® (olanzapine); Seroquel® (quetiapine); Geodon® (ziprazadone); Clozaril® (clozapine)); (5) Taking prescription or over-the-counter appetite suppressants, herbal products or other medications for weight loss within one month of enrollment and did not agree to start such products while participating in the study; (6) Use of prescription, over-the-counter or herbal weight loss products; (7) Using acupuncture for weight loss; (8) Severe eating disorders such as bulimia or binge eating; (9) Obese due to a clinically diagnosed endocrine problem; (10) Pregnant (indicative by positive hCG) or lactating; (11) History of anemia (<10 g/dl) over past 3-months; (12) Significantly abnormal hepatic liver function tests or renal disease; (13) Prior bariatric surgery; (14) History of peptic ulcer disease; (15) Use of another investigational device or agent in the 30 days prior to enrollment; (16) Participation in another clinical study; (17) Life threatening co-morbidity or life expectancy of less than one year; (18) Myocardial Infarction or one or more episodes of unstable angina within six months prior to enrollment; (19) Implanted with a permanent pacemaker, an automatic implantable defibrillator, or other electro-stimulation device; (20) In the opinion of the investigator, this subject will not comply with the protocol or is in some other way not appropriate for this study; or (21) Any laboratory value greater than 3× the upper limit of normal (ULN) at screening.

Subjects were considered to have completed the study when they attended all visits, and all assessments had been performed. Any subject who entered the study but, did not complete the study according to the above definition was considered to have withdrawn.

Study design

 Subjects were enrolled in this study for approximately 13 weeks and were on 1500 mg berberine treatment orally (500 mg three times per day) for 12 weeks. The study was designed to include four visits and the first visit consisted of a screening to determine eligibility through clinical and biochemical measurements. Eligible subjects were required to return in one week for visit two when one bottle of berberine capsules was dispensed to each subject. Visit three occurred at the 6-week interval and any adverse effects were recorded, medication adherence was evaluated, and another bottle of berberine capsules was dispensed. After six more weeks of treatment visit four occurred and all lab measurements were conducted. Subjects were asked to adhere to the visit schedule as closely as possible. Subjects who were not able to comply with the study visits were asked to withdraw from the study based on the investigator’s discretion. All subjects signed informed consent prior to enrollment into the program. Female subjects who suspected they may be pregnant during the study had a serum pregnancy test completed.

Intervention

Berberine hydrochloride hydrate (C23H18CINO4·nH2O, referred to as berberine) was purchased from Shaanxi Jintai Biological Engineering Co., Ltd. (Xi’an, China). A High Performance Liquid Chromatography (HPLC) assay was used to determine the purity of berberine relative to its standard (ChromaDex, Irvine, CA, USA) using methods reported previously (Weber et al. 2003). Avera McKennan Hospital Outpatient Pharmacy compounded the berberine powder into capsules. Subjects received study medication at visit two and visit three. At visit three and visit four, subjects returned the previously dispensed capsules and medication compliance was assessed.
Clinical and biochemical measurements

Measurements of weight, body mass index (BMI), waist/hip ratio, 4-site skinfolds, and vital signs were performed at each visit. Self-reported side effects were recorded at visit three and four. At visit one, the following tests were conducted: Comprehensive Metabolic Panel (CMP), Complete Blood Count (CBC), Lipid panel, Thyroid (TSH, Free T4), and beta HCG. Visit two included the following tests: Cortisol, Adrenocorticotropic hormone (ACTH), Sex Hormone Binding Globulin (SHBG), calcitriol (1,25-dihydroxy vitamin D3), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP). Visit four included CMP, CBC, Cortisol, ACTH, Lipid Panel, TSH, Free T4, calcitriol, and Immune assay (SED, CRP). In addition, blood was collected at visits two and four for use in mRNA expression studies. A CMP includes BUN, Albumin, ALT, AST, Total Bilirubin, Calcium, Carbon Dioxide, Chloride, Creatinine, Glucose, Alkaline Phosphatase, Potassium, Total Protein, and Sodium. A Lipid Panel includes total cholesterol, triglycerides, LDL, HDL, and LDL/HDL ratio. A CBC includes RBC, WBC, Hemoglobin, Hematocrit, Platelet Count, RBC Indices (MCV, MCH, MCHC), and Automated 5-part WBC Differential. Subjects fasted overnight prior to the labs collected at visits two and four. Serum pregnancy tests were performed on all females prior to inclusion in the study. For the duration of the study, in case of suspicion of pregnancy, another serum pregnancy test was performed.

At visit two (before berberine treatment) and visit four (after berberine treatment); Real-time reverse transcriptase PCR analysis was used to measure mRNA expression of CRP, TNFx, IL-6 and COX-2, using β-actin as an internal control. Briefly, total RNA was isolated from whole blood collected in Paxonge blood RNA tubes and extracted using the Paxgene blood extraction kit (Qia-gen, Valencia, CA, USA) following the manufacturer’s protocol. RNA was quantified using absorption of light at 260 and 280 nm. Total RNA (0.8 μg) from each sample was used in a reverse transcription reaction using the TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA) following the manufacturers’ instructions. Real-time PCR was performed using HT-7900 Fast Real-Time Thermocyclers (Applied Biosystems, Foster City, CA, USA). PCR was carried out using SYBR green Master Mix (Applied Biosystems) using the primer (Integrated DNA Technologies, Coralville, IA, USA) sequences as shown in Table 1 in a 10 μl reaction. Cycling conditions for amplification were as follows: an initial step of denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s. The relative mRNA levels to β-actin were normalized with a control-treated group and calculated by the ΔΔCT method.

Animal study

This animal study protocol was approved by the Institutional Animal Care and Use Committee at University of South Dakota. Female Sprague-Dawley (SD) rats (7 weeks old) were obtained from Charles River Laboratories (Cambridge, MA, USA), housed at 22 °C, on a 12-h light-dark cycle (lights on: 7 am), and allowed ad libitum access to water and rodent chow diet (Open Source Diets #D12450B) throughout the study. Following one week of habituation, rats were randomly assigned to two groups (control group: vehicle treatment and a berberine group: 380 mg/kg/day of berberine in 0.5% methylcellulose) and underwent a teaching period of gavage once per day for one week, followed by two weeks of drug or vehicle treatments via gavage. Rat body weight and food-intake were recorded daily. At the end of treatment, rats were fasted overnight and sedated with isoflurane. Upon sedation, blood was collected via aortic puncture to an EDTA tube and the animals were euthanized. Plasma was extracted within 3 h after blood was drawn. Rat blood glucose levels were checked by the Accutrend Plus instrument and strips (Roche Indianapolis, Indiana, USA). Triglyceride levels were assayed using the EnzyChromTM Triglyceride Assay Kit (BioAssay System, Hayward, CA, USA) and total cholesterol levels were checked using EnzyChromTM AF Cholesterol Assay Kit (BioAssay System, Hayward, CA, USA), according to the manufacturer’s protocols.

Statistical analyses

Significant differences between values prior to and after berberine treatment (or with/without berberine treatment) were determined using Student’s t-test at the p < 0.05 (*)

Results

Patient enrollment and berberine quality control

Sixteen subjects participated in the screening tests at visit one and six people were excluded according to the above mentioned inclusion and exclusion criteria. Of the ten eligible patients, two withdrew after two weeks of medication use due to abdominal pain, one patient did not attend all of the scheduled visits and was excluded, and a total of seven subjects (two males, five females) completed the study with average age at 40. All seven subjects are Caucasians, though the protocol was not intended to enroll people within any specific ethnic group.

Berberine was tested for purity using HPLC. Four different sites were chosen from different locations on the berberine package and each was analyzed for purity. The average berberine purity was 99.6%, which is comparable to standard (97.2% by HPLC).

Effects of berberine treatment on human body weight and fat composition

Body weight, body mass index (BMI), waist/hip ratio, and 4-site skinfolds for each subject were measured at visit one (screening visit, data not shown), visit two (before berberine treatment), visit three (after six-week berberine treatment, data not shown) and visit four (after 12-week berberine treatment). Body fat percentages were calculated from the 4-site skinfolds measurements. As shown in Table 2, berberine treatment for 12 weeks produced mild weight loss, on average five pounds per subject or a 2.3% mean weight loss. In addition, berberine treatment decreased BMI (2.9%), waist/hip ratio (1.1%), and body fat percentage (3.6%). All changes after 12 weeks of berberine treatment were not statistically significant when compared to the baseline numbers prior to berberine treatment. No altered exercise habits were observed for any of the subjects; however subject 005 and 007 reported a reduced appetite after taking berberine for 12 weeks according to the Dietary and Exercise Questionnaire.

| Table 1 Real-time RT-PCR primers. |  
|---|---|
| CRP forward 5′-TGG TGG ATT TAC CAG CTA CTA CAG GGA-3′  
CRP reverse 5′-TCC TCC ACT TCC ACT GTC GCT TCT-3′  
IL-6 forward 5′-AAA TCC TGC ACA TCC TGC AGC-3′  
IL-6 reverse 5′-AGT GGC ATT TTT CTT CTA CCA CAC-3′  
TNF-α forward 5′-AGG ACG AAC ATG CCT TCC CAA-3′  
TNF-α reverse 5′-TTT GAG CCA GAA GAG GAT GGT GGG-3′  
COX-2 forward 5′-CAA ATC TTT GCT GTT CCC ACC CAT-3′  
COX-2 reverse 5′-GGG TGG CAG TCC CTC CAA-3′  
β-actin forward 5′-AAC TAC TGG GGT CAA CCT CAG AC-3′  
β-actin reverse 5′-TCT GTA CTT CCG AGG AGA GTG-3′  |
Table 2
Berberine effects on body weight, BMI, waist/hip ratio and body fat percentage in human subjects.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Body weight (LB)</th>
<th>BMI</th>
<th>Waist/hip ratio</th>
<th>Body fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>001</td>
<td>313</td>
<td>305</td>
<td>42.4</td>
<td>41.08</td>
</tr>
<tr>
<td>002</td>
<td>180</td>
<td>182</td>
<td>32.92</td>
<td>33.3</td>
</tr>
<tr>
<td>003</td>
<td>181</td>
<td>184</td>
<td>31.1</td>
<td>31.58</td>
</tr>
<tr>
<td>004</td>
<td>252</td>
<td>255</td>
<td>36.2</td>
<td>36.58</td>
</tr>
<tr>
<td>005</td>
<td>193</td>
<td>173</td>
<td>32.1</td>
<td>27.92</td>
</tr>
<tr>
<td>006</td>
<td>217</td>
<td>207</td>
<td>31.6</td>
<td>29.7</td>
</tr>
<tr>
<td>007</td>
<td>200</td>
<td>195</td>
<td>36.6</td>
<td>35.66</td>
</tr>
<tr>
<td>Mean</td>
<td>219.4</td>
<td>214.4</td>
<td>34.7</td>
<td>33.7</td>
</tr>
<tr>
<td>S.E.</td>
<td>18.2</td>
<td>18.2</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Decrease</td>
<td>2.3%</td>
<td></td>
<td>2.9%</td>
<td>1.1%</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.172</td>
<td></td>
<td>0.162</td>
<td>0.524</td>
</tr>
</tbody>
</table>

Table 3
Metabolic effects of berberine treatment on cholesterol, triglycerides and glucose levels in human subjects.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>001</td>
<td>181</td>
<td>151</td>
<td>141</td>
</tr>
<tr>
<td>002</td>
<td>207</td>
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<td>152</td>
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<td>003</td>
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<td>147</td>
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<td>004</td>
<td>162</td>
<td>138</td>
<td>96</td>
</tr>
<tr>
<td>005</td>
<td>203</td>
<td>147</td>
<td>153</td>
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<tr>
<td>006</td>
<td>199</td>
<td>162</td>
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</tr>
<tr>
<td>007</td>
<td>258</td>
<td>271</td>
<td>164</td>
</tr>
<tr>
<td>Mean</td>
<td>197.6</td>
<td>173.4</td>
<td>134.9</td>
</tr>
<tr>
<td>S.E.</td>
<td>11.9</td>
<td>17.9</td>
<td>13.6</td>
</tr>
<tr>
<td>Normal range</td>
<td>50–200</td>
<td>25–150</td>
<td>70–105</td>
</tr>
<tr>
<td>Decrease</td>
<td>12.2%</td>
<td>23%</td>
<td>0.6%</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.026</td>
<td>0.062</td>
<td>0.907</td>
</tr>
</tbody>
</table>

Effects of berberine treatment on human blood glucose and lipid levels

Metabolic effects of berberine treatment were identified by testing fasted blood glucose, total cholesterol, and triglyceride levels prior to and after 12 weeks of berberine treatment in obese human subjects. As shown in Table 3, the average total cholesterol level for all subjects decreased significantly (p = 0.026) when compared to pre-treatment levels (197.6 vs. 173.4 mg/dl). Two subjects with an above normal baseline cholesterol level, were in the normal range after 12 weeks of treatment with berberine. 12 weeks of berberine treatment also decreased blood triglyceride levels by an average of 23% and was marginally statistically significant (134.9 vs. 103.9 mg/dl, p = 0.062). Three of four subjects with an abnormally high triglyceride level at baseline improved within normal limits after berberine treatment. Interestingly, the triglyceride levels of all subjects decreased with berberine treatment (Table 3). Blood glucose levels were not significantly changed by berberine treatment (90.6 vs. 90 mg/dl, p = 0.907), six subjects with a normal glucose level remained normal, and one subject with a slight abnormally high glucose level remained in the abnormal range after berberine treatment.

Effects of berberine treatment on human blood hormone levels

Levels of five hormones and one hormone related protein in each of the subject’s blood were measured; with the aim of exploring the possible secondary effects of berberine and also to identify possible mechanisms of berberine action. As shown in Table 4, treatment with berberine did not significantly change the blood levels of calcitriol (33.86 vs. 54 pg/ml, p = 0.11), cortisol (15.03 vs. 12.91 µg/dl, p = 0.325), ACTH (12 vs. 15.29 pg/ml, p = 0.19), TSH (1.9 vs. 2.22 µIU/ml, p = 0.117), FT4 (1.11 vs. 1.11 ng/dl, p = 0.929), and SHBG (37.29 vs. 41.71 nmol/l, p = 0.24). Blood calcitriol, ACTH, TSH, FT4, and SHBG levels were increased by 59.5%, 27.4%, 17.1%, 0.4%, and 11.9% respectively after 12 weeks of berberine treatment. In addition, blood cortisol level was decreased by 14.1% after 12 weeks of berberine treatment. We did observe an increase of blood calcium levels in all human subjects (data not shown). However, all
hormone levels remained within the clinically normal range after 12 weeks of berberine treatment.

Effects of berberine treatment on expression of human blood inflammatory factors

In order to examine any anti-inflammatory effects associated with berberine treatment; the blood CRP level, erythrocyte sedimentation rate (ESR), and peripheral blood mononuclear cell (PBMC) gene expression of several inflammatory factors were determined. As shown in Table 5, a 12-week berberine treatment did not change the blood CRP level and ESR significantly (p-value = 0.711 and 0.100 respectively) and all subjects remained within the clinically normal range. The real-time RT-PCR results (Fig. 1) demonstrate that berberine did not significantly change the mRNA expression of any of the four inflammatory factor genes studied when corrected for multiple tests (alpha of 0.0125 after Bonferroni corrections). Berberine treatment only slightly enhanced the mRNA expression of CRP (0.1-fold), TNF-α (0.3-fold), and COX-2 (0.3-fold); and decreased the expression of IL-6 (0.1-fold).

Effects of berberine treatment on electrolyte balance, gastrointestinal, hematological, cardiovascular, liver, and kidney function

Safety for berberine treatment was determined through measurements of complete blood count (CBC), blood pressure (BP), electrocardiograph (ECG), complete metabolic panel (CMP), and collection of self reported adverse effects. Among seven subjects completing 12 weeks of berberine treatment, two subjects reported temporary abdomen cramping but subsiding in less than one week. The measurement data (not shown) demonstrated that 12 weeks of berberine treatment did not significantly change values in the CBC, blood electrolytes, blood pressure, and ECG; all which remained in the normal range after berberine treatment.

As shown in Table 6, berberine treatment for 12 weeks significantly decreased subjects blood total protein (7.54 vs. 7.16 g/dl, p = 0.009), globulin (2.97 vs. 2.66 g/dl, p = 0.012), AST (22.86 vs. 18.57 U/l, p = 0.025) and significantly increased A/G ratio (1.54 vs. 1.73, p = 0.011). However, all the changes were minor (decreased total protein, globulin, and AST level by 5%, 10.4%, 18.4% respectively; and increased A/G ratio by 12.3%); and all of which remained in the clinically normal range. Berberine treatment did not significantly change other liver function parameters including albumin, ALT, Alkaline Phosphatase, and total Bilirubin levels. As shown in Table 7, a 12-week berberine treatment regime significantly increased blood BUN levels by 15.6% and BUN/Creatinine Ratio by 19.55. However, all items (BUN, Creatinine, BUN/Creatinine, and GFR) reflecting kidney function remained in the clinically normal range.

Effects of berberine treatment in rats

The rat experiment (n=8 per group) was carried out to confirm the effects of berberine treatment on body weight and lipid metabolism. As shown in Table 8, two weeks of berberine treatment resulted in a slight decrease in weight gain (mean 21.5 g weight gain per rat in control group vs. a mean 18.75 g weight gain in the experimental group, 12.8% less, p = 0.528). Taking berberine for 12 weeks did not change the rat’s food-intake (14.51 g vs. 14.63 g, p = 0.648) and blood glucose levels (204.1 mg/dl vs. 214.9 mg/dl, p = 0.251). However, berberine treatment did significantly reduce rat blood triglyceride levels (95.2 vs. 62.2 mg/dl, p = 0.037) by 34.7%, and there was also a marginally significant decrease in total cholesterol levels (66.4 vs. 60.4 mg/dl, p = 0.084) by 9%.

Discussion

Obesity is a well-recognized risk factor for hyperlipidemia, type II diabetes, hypertension, coronary heart disease, stroke, obstructive sleep apnea, asthma, osteoarthritis, and also post-menopausal breast cancer (Batch and Baur 2005). Current strategies for obesity management include diet, exercise, drug therapy, and bariatric surgery; either alone or in combination. There is a growing demand for safe and effective anti-obesity drugs (Elangbam 2009). Currently, pharmaceutical therapies produce either undesirable efficacy or side effects (Seo et al. 2011). Derived from a variety of plants, berberine has demonstrated promising therapeutic potential both in vitro and in vivo for diabetes and obesity (Xie et al. 2011).

We have previously reported that berberine prevents fat accumulation in cultured mouse adipocytes (Hu and Davies 2009) and in subsequent experiments, we extended this study into human cell lines showing very similar results (Hu et al. 2010). We have also replicated the in vitro findings in a high-fat diet induced-obesity mouse model (Hu and Davies 2010). As a natural progression of these previous studies, we sought to examine the safety and efficacy of berberine treatment in human obese subjects.

In our human pilot study, berberine treatment for 12 weeks produced an average 5 lb/subject weight loss (Caucasian). This data is completely consistent with the results from a previous clinical trial showing that three months of berberine treatment resulted in 5.1 lb/subject weight loss in a Han Chinese population, a significantly greater weight loss than that in the placebo group (p = 0.034) (Zhang et al. 2008). Furthermore, the results from the rat experiment demonstrated similar results, a 12.8% decrease of weight gain in the berberine treatment group when compared to the control.
Table 6
Tests of liver function affected by berberine treatment.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G ratio</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
<th>Alk Phos (U/l)</th>
<th>Total Bili (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>6.0–8.3</td>
<td>3.5–5.0</td>
<td>1.5–4.5</td>
<td>0.9–1.5</td>
<td>0–37</td>
<td>0–41</td>
<td>40–129</td>
<td>0–1.0</td>
</tr>
<tr>
<td>Before</td>
<td>7.54 ± 0.09</td>
<td>4.57 ± 0.1</td>
<td>2.97 ± 0.14</td>
<td>1.54 ± 0.1</td>
<td>22.86 ± 3.36</td>
<td>29.71 ± 9.18</td>
<td>76.71 ± 9.25</td>
<td>0.74 ± 0.25</td>
</tr>
<tr>
<td>After</td>
<td>7.16 ± 0.15</td>
<td>4.5 ± 0.09</td>
<td>2.66 ± 0.15</td>
<td>1.73 ± 0.1</td>
<td>18.57 ± 2.3</td>
<td>24 ± 6.74</td>
<td>80.86 ± 9.36</td>
<td>0.67 ± 0.16</td>
</tr>
<tr>
<td>Decrease</td>
<td>5%</td>
<td>1.5%</td>
<td>10.4%</td>
<td>−12.3%</td>
<td>18.8%</td>
<td>19.2%</td>
<td>−5.4%</td>
<td>9.5%</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.009</td>
<td>0.31</td>
<td>0.012</td>
<td>0.011</td>
<td>0.025</td>
<td>0.104</td>
<td>0.125</td>
<td>0.642</td>
</tr>
</tbody>
</table>

Table 7

<table>
<thead>
<tr>
<th>Tests</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>BUN/creatinine ratio</th>
<th>GFR (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>6–20</td>
<td>0.7–1.2</td>
<td>10–20</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Before</td>
<td>11.86 ± 1.22</td>
<td>0.83 ± 0.06</td>
<td>14.93 ± 2.08</td>
<td>&gt;60</td>
</tr>
<tr>
<td>After</td>
<td>13.71 ± 1.41</td>
<td>0.81 ± 0.08</td>
<td>17.84 ± 2.57</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Increase</td>
<td>15.6%</td>
<td>−2.4%</td>
<td>19.5%</td>
<td>0.041</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.021</td>
<td>0.604</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

group. We understand that a limitation of this pilot study is the small sample size as the obese human study contained only seven individuals and there were only eight rats in each experimental group to test berberine’s effect on body weight. However ongoing experiments with a greater sample size will give us added power to detect significant differences in body weight change in response to berberine treatment.

When examining the weight loss individually, four human subjects lost weight (largest individual weight loss was 20 lb) and three others had no weight loss at all. In addition, two of the four people with weight loss also reported a decrease in overall appetite. Sero-tonin has been shown to have an important role in appetite control (Blundell 1984) and we have previously reported that genetic variations in the promoter region of the Serotonin Transporter (SERT) gene play a critical role in the pharmacologic effects of berberine in vitro (Hu et al. 2011). Since obesity is a complex genetic disorder, individual genetic variations in a variety of different genes may be instrumental to the differential effects of berberine treatment on weight.

Although, berberine did not significantly affect weight loss in our study, it did have a potent effect on lipid metabolism. Hyperlipidemia is one of the most common co-morbidities associated with obesity, and is also a significant risk factor for cardiovascular disease. Berberine has been reported to decrease lipid levels in vitro and in vivo (Affuso et al. 2010). Here we also show that berberine treatment can significantly decrease berberine total cholesterol levels (12.2%) and lower blood triglyceride levels (23%) in a Caucasian population. Remarkably, three individuals began the study with a clinically high cholesterol level which was subsequently lowered to normal levels following 12-weeks of berberine treatment. In addition, berberine was able to change the triglyceride level in human subjects from a clinically high level to a normal level in three of four subjects. The lipid lowering effect of berberine treatment in human subjects is consistent with previous reports (Cicero et al. 2007; Kong et al. 2008). The decrease in lipid levels (cholesterol and triglycerides) was also replicated in our rat study. Taken together, the evidence for berberine as a potent lipid-lowering compound in both human subjects and rats appears in this study to be very convincing.

Berberine treatment has been reported to decrease blood glucose levels in a number of clinical trials (Yin et al. 2008; Zhang et al. 2008, 2010). However, we did not observe a significant change in blood glucose levels in human subjects. The majority of subjects in the experimental studies had normal blood glucose levels before berberine treatment and as a result, we would not expect to see a significant decrease in glucose levels.

The secondary effects and possible mechanisms of berberine’s action were examined by comparing hormone levels and inflammation factor expression prior to and after treatment. Calcitriol (1, 25-dihydroxy vitamin D3), the hormonally active form of vitamin D, is commonly prescribed for treatment and prevention of osteoporosis.

In this human pilot study, we observed increases in calcitriol concentrations in all subjects. Calcitriol has been commonly used to treat or prevent osteoporosis. Several investigators have suggested a potential application of berberine to treat/prevent osteoporosis in vitro and in vivo (Li et al. 1999; Xu et al. 2010; Lamb et al. 2011), this is the first evidence in human subjects which strongly supports a potential pharmacologic application of berberine for osteoporosis treatment and/or prevention. We did not observe a significant change in the hormone levels of SHBG, cortisol, ACTH, FT4, and TSH in the blood of subjects after berberine use, which implies the lipid-lowering and mild weight loss effects of berberine treatment do not result from the dysregulation of these metabolic hormones.

Recent research efforts have confirmed that inflammation is closely associated with obesity (Browning et al. 2008; Dalmas et al. 2011), and circulating inflammatory factors are subtly but significantly elevated in obese human subjects (Pickup 2004). Berberine treatment has been reported to inhibit inflammation in cultured macrophages (Chen et al. 2008; Zha et al. 2010), 3T3-L1 adipocytes (Choi et al. 2006), and mouse fat tissue (Jeong et al. 2009). We hypothesized that berberine would inhibit inflammation in human subjects. However, in this study we did not observe any anti-inflammatory effects of berberine treatment as reflected by the serum CRP level or the erythrocyte sedimentation rate (ESR). It is also noteworthy, that all subjects had normal CRP and ESR levels prior to berberine treatment. Expression studies of genes involved in inflammation; CRP, IL-6, TNF-α, and COX-2 all show no

Table 8
Effects of berberine treatment on rat weight gain, food-intake, blood glucose and lipid levels.

<table>
<thead>
<tr>
<th>Records/tests</th>
<th>Weight gain (g)</th>
<th>Food-intake (g)</th>
<th>Glucose (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.5 ± 3.23</td>
<td>14.51 ± 1.27</td>
<td>204.1 ± 6.6</td>
<td>95.2 ± 14.3</td>
<td>66.4 ± 3.1</td>
</tr>
<tr>
<td>Berberine</td>
<td>18.75 ± 2.74</td>
<td>14.63 ± 2.2</td>
<td>214.9 ± 13.9</td>
<td>62.2 ± 8.6</td>
<td>60.4 ± 2.7</td>
</tr>
<tr>
<td>t-Test, p-value</td>
<td>0.528</td>
<td>0.648</td>
<td>0.251</td>
<td>0.037</td>
<td>0.084</td>
</tr>
<tr>
<td>Decrease</td>
<td>0.128</td>
<td>−0.008</td>
<td>−0.053</td>
<td>0.347</td>
<td>0.09</td>
</tr>
</tbody>
</table>
significant differences in expression after treatment with berberine. Since research has shown only subtle differences in circulating inflammatory factors associated with obesity, we may not have the adequate power to detect minor changes in circulating factors with berberine. It is also possible berberine has no effect on inflammation in human subjects.

Gastrointestinal symptoms (such as diarrhea, constipation, flatulence, and abdominal pain) were reported previously as the main side effects of berberine treatment in human subjects (Yin et al. 2008; Zhang et al. 2008), and the incidence reached to 34.5% even though everybody completed the trial following a temporary decrease of berberine dose (Yin et al. 2008). In our pilot clinical trial, two subjects dropped out due to abdominal pain and two other subjects experienced temporary abdominal pain but completed the trial. The associated abdominal pain with berberine use could possibly be explained by the higher dose we used (0.5 g t.i.d.) when compared to other berberine trials (Kong et al. 2008; Yin et al. 2008; Zhang et al. 2008). Future modified methods would include a dose titration or a small pharmacokinetic study of the bioavailability of oral berberine administration. Consistent with previous excellent safety reports of berberine treatment in human subjects (Cicero et al. 2007; Kong et al. 2008; Yin et al. 2008; Zhang et al. 2008), our results illustrate that berberine treatment did not produce any side effects on electrolyte metabolism, hematological, cardiovascular, liver, and/or kidney function.

Collectively, our study demonstrated that a 12-week berberine treatment approach significantly decreased blood lipid levels both in obese human subjects and in SD rats. Berberine also produced mild weight loss and increased blood calcitriol levels. Taken together, berberine is a potent lipid-lowering compound and has potential for additional investigation into its treatment application. Additional studies may also be valuable to study possible applications for berberine as a treatment or prevention approach to osteoporosis.

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References


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