Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.2014C474

Metabolic effects of berberine on liver phosphatidate phosphohydrolase in rats fed on high lipogenic diet: an additional mechanism for the hypolipidemic effects of berberine

Esfandiar Heidarian[1*], Mahmoud Rafieian-Kopaei[2], Abolfazle Khoshdel[3], Morteza Bakhshesh[4]

[1]*Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[2]*Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[3]*Celullar and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[4]*Department of Physiology, Arak University of Medical Sciences, Arak, Iran*

PEER REVIEW

Peer reviewer

Hassan Ahmadvand, Associate Professor of Clinical Biochemistry, Department of Biochemistry, Khoramabad University of Medical Sciences, Khoramabad, Iran.
Tel: +98 913 226 7893
Fax: +98 661 620 0133
E-mail: Hassan_a46@yahoo.com, ahmadvand.h@lums.ac.ir

Comments

This is a good study in which the authors have shown that one of the mechanisms of the hypolipidemic effects of berberine can be through reducing liver phosphatidate phosphohydrolase.
Details on Page S434

ABSTRACT

Objective: To evaluate the effects of berberine (BBR) on the liver phosphatidate phosphohydrolase (PAP) and plasma lipids in rats fed on high lipogenic and normal diet.

Methods: Forty rats were randomly divided into 5 groups. Group I (control) received standard diet. Group II received standard diet plus 90 mg/kg BBR and Groups IV received lipogenic diet (containing sunflower oil, cholesterol and ethanol) without treatment. Groups III and V received lipogenic diet plus 90 mg/kg BBR and 30 mg/kg gemfibrozil, respectively. On Day 60 of the experiment, blood samples were collected and PAP, total cholesterol, triglyceride, low density lipoprotein cholesterol, high density lipoprotein cholesterol, very low density lipoprotein, malondialdehyde, plasma antioxidant, and liver histopathology assessments were conducted.

Results: PAP, plasma triglyceride, total cholesterol, very low density lipoprotein, and malondialdehyde levels decreased significantly ($P<0.05$) in Group III compared to Group IV (24.94%, 36.11%, 21.18%, 36.86% and 19.59%, respectively). The liver triglyceride and cholesterol in Groups III and V had a remarkable decrease ($P<0.001$) compared with Group IV (24.94% and 49.13%, respectively). There was a significant reduction ($P<0.05$) in atherogenic index in Groups III compared with Group IV.

Conclusions: These results clearly suggested that BBR could be effective in reducing liver PAP, lipid abnormality, liver triglyceride and lateral side effects of hyperlipidemia.

KEYWORDS

Atherogenic index, Flavonoid, Fatty liver, Lipid profile, Lipogenic diet, Oxidative stress

1. Introduction

Berberine (BBR), an isoquinoline plant alkaloid, is a natural compound present in many herbs. It has been demonstrated that berberine has multiple pharmacological actions such as being active against hypertension, tumors, bacteria, inflammation, hyperlipidemia, and many other illnesses[1].

Phosphatidate phosphohydrolase (PAP, EC 3.1.3.4) catalyzes the dephosphorylation of phosphatidic acid to form inorganic phosphate and 1,2 diacylglycerol productions[2,3]. In liver tissue, PAP is an important key regulatory enzyme in lipid metabolism pathways especially triacylglycerol and glycerophospholipids. The produced diacylglycerol from phosphatidic acid serves as a precursor for the synthesis of triglyceride (TG) and other phospholipids[3]. In organisms, TG is a critical storage molecule for periods of food deprivation. In human, the

*Corresponding author: Dr. Esfandiar Heidarian, Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.
Tel: +98 381 3346720
Fax: +98 381 3346721
E-mail: heidarian_e@skums.ac.ir, heidarian46@yahoo.com
Foundation Project: Supported by Shahrekord University of Medical Sciences (Grant No. 812), Shahrekord, Iran.

Article history:
Received 23 Jan 2014
Received in revised form 28 Jan, 2nd revised form 5 Feb, 3rd revised form 14 Feb 2014
Accepted 10 Mar 2014
Available online 5 Apr 2014

regulation of TG storage is very important because both excessive and inadequate fat storage are accompanied with dyslipidemia, insulin resistance, and diabetes[4-6]. Therefore, any alteration in PAP activity can influence lipid metabolism in the body.

A large body of evidence indicates that the incidence of hyperlipidemia and its complications are growing in the world. Hyperlipidemia develops the risk of many diseases such as coronary heart diseases, atherosclerosis, hypertension, and type 2 diabetes[7]. The antihyperlipidemic drugs such as fibrates, nicotinic acid, and bile acid sequestrants were used for many years. Nevertheless, the side effects of drugs led to the development of new oral antihyperlipidemic drugs including statins (HMG-CoA reductase inhibitors). Although the adverse reactions of statins are relatively low, they can result in rhabdomyolysis condition[8]. Therefore, the research for natural compounds with antihyperlipidemic properties and with less or no adverse reactions, especially herbal medicine, is warranted. These medicinal plants contain biological active substances including antioxidant, hypoglycemic and hypolipidemic compounds. Numerous reports have shown that BBR reduces hyperlipidemia[9-11]. Also, it has been reported that BBR stimulated AMP-activated protein kinase (AMPK) activity and fatty acid oxidation in HepG2 line cells and lowered hyperlipidemia in hamsters fed a high-fat diet[9]. In addition, BBR reduces blood cholesterol by stabilizing hepatic low density lipoprotein receptor in an extracellular signal-regulated kinase manner[12]. In spite of these findings, most of the previous studies on BBR focused less on the enzymes involving in TG metabolism, especially PAP enzyme, in details. Therefore, the aim of this study was to determine the effects of dietary supplementation with BBR on the liver PAP, plasma lipids, liver TG content, plasma antioxidant, and malondialdehyde levels in rats fed on high lipogenic and normal diet.

2. Materials and methods

2.1. Chemicals

Phosphatidic acid (sodium salts), dithiothreitol, phenylmethylsulfonyl fluoride, and 2, 4, 6-Tripyridyl-s-Triazine and berberine chloride were purchased from Sigma (St. Louis, MO). Tris-HCl, sodium acetate, bovine serum albumin, ferrous sulphate ($FeSO_4 \cdot 7H_2O$), ferric chloride ($FeCl_3 \cdot 6H_2O$), ethylenediaminetetraacetic acid (EDTA) and ethylene glycol-bis (beta-aminoethyl ether)-N,N,N′,N′-tetraacetic acid were obtained from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available.

2.2. Experimental animals and diets

Male Wistar rats (150-200 g) were housed in the colony room with a 12-h light and dark cycle at (21±2) °C and had free access to water and food. Rats were randomly assigned to one of 5 diet groups (n=8) as below:

Group I, normal control rats, received standard pellet diet (Pars Dam, Tehran, Iran). This group received 0.5 mL distillated water by gavage to produce injection shock similar to other groups. Group II, animal rats were fed with a standard pellet diet plus 90 mg/kg body weight/day BBR by gavage. Groups III, IV, and V, the rats were fed with a lipogenic diet containing standard pellet diet supplemented with 0.5% (w/w) cholic acid, 20% (w/w) sunflower oil and 2% (w/w) cholesterol for at least two weeks to produce hyperlipidemia. Additionally, these groups drank water containing 3% (v/v) ethanol[13]. After 2 weeks of lipogenic diet feeding, Group III orally received 90 mg/kg body weight/day BBR accompanied with lipogenic diet for 45 days by gavage. The rats in Group IV were maintained on lipogenic diet (hyperlipidemic control group) without treatment throughout the experiment and received 0.5 mL distillated water by gavage to produce equal injection shock similar to other groups. The rats in Group V after 2 weeks were treated by 30 mg/kg body weight/day gemfibrozil through gavage[14]. On Day 60 of the experiment, fasted animals were anesthetized with chloroform. Blood samples were collected into test tubes containing EDTA through cardiac puncture. The plasma samples were separated by low speed centrifugation (2000 r/min) for 10 min and were stored at -80 °C until they were analyzed. All animal procedures were performed with regard to Iranian Animal Ethics Society and local university rules.

2.3. Lipid analysis

Plasma levels of TG, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase were calculated by enzymatic method (Pars Azmun kit, Tehran, Iran) by using autoanalyzer (BT 3000, Biotecnica Instruments, SpA Rome, Italy). Very low density lipoprotein cholesterol (VLDL-C) concentrations were determined with Fridewald formula[15]. Finally, liver TG and cholesterol were extracted from liver tissue by the method of Folch, *et al.*[16] and determined by enzymatic method (Pars Azmun kit, Tehran, Iran).

2.4. Preparation of rat liver homogenate

The rat liver was removed and a piece of the liver was homogenized in 4 volumes of ice-cold homogenate buffer containing 50 mmol/L of Tris-HCl (pH 7.4) buffer, 0.25 mol/L sucrose, 0.1 mmol/L EDTA, and 1 mmol/L Phenylmethanesulfonyl fluoride at 8000 r/min at 4 °C for 6 min[13]. The homogenate was then initially centrifuged at 4500 r/min at 4 °C for 10 min, resulting in a nuclear pellet and then, the supernatant was kept for the enzyme assay.