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Berberine decreases cholesterol levels in rats through multiple mechanisms, including inhibition of cholesterol absorption

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ABSTRACT

Objective. The objective was to determine the mechanisms of action of berberine (BBR) on cholesterol homeostasis using *in vivo* and *in vitro* models.

Methods. Male Sprague–Dawley rats were fed the AIN-93G diet (normal control) or modified AIN-93G diet containing 28% fat, 2% cholesterol and 0.5% cholic acid with treatment of 0 (atherogenic control), 50, 100, and 150 mg/kg·d of BBR, respectively by gavaging in water for 8 weeks. Cholesterol absorption rate was measured with the dual stable isotope ratio method, and plasma lipids were determined using the enzymatic methods. Gene and protein expressions of Acyl-coenzyme A:cholesterol acyltransferase-2 were analyzed *in vivo* and *in vitro*. Cholesterol micellarization, uptake and permeability were determined *in vitro*.

Results. Rats on the atherogenic diet showed significantly hypercholesterolemic characteristics compared to normal control rats. Treatment with BBR in rats on the atherogenic diet reduced plasma total cholesterol and nonHDL cholesterol levels by 29%–33% and 31%–41%, respectively, with no significant differences being observed among the three doses. The fractional dietary cholesterol absorption rate was decreased by 40%–51%. Rats fed the atherogenic diet showed lower plasma triacylglycerol levels, and no changes were observed after the BBR treatment. BBR interfered with cholesterol micellarization, decreased cholesterol uptake by Caco-2 cells and permeability through Caco-2 monolayer. BBR also inhibited the gene and protein expressions of acyl-coenzyme A cholesterol acyltransferease-2 in the small intestine and Caco-2 cells.

Conclusion. BBR lowered blood cholesterol levels at least in part through inhibiting the intestinal absorption and further by interfering with intraluminal cholesterol micellarization and decreasing enterocyte cholesterol uptake and secretion.

Abbreviations: ABCG, ATP-binding cassette sub-family G; AC, atherogenic control; ACAT, Acyl-coenzyme A cholesterol acyltrasnferase; BBR, berberine; EMEM, Eagle's Minimum Essential Medium; HBSS, Hank's buffered salt solution; IRMS, isotope ratio mass spectrometry; LDL-C, LDL cholesterol; LDLR, LDL receptor; NC, normal control; nonHDL-C, nonHDL cholesterol; NPC1L1, Niemann-pick C1-like 1; RIPA, radioimmunoprecipitation assay; TAG, triacylglycerol; TBST, tris-buffered saline and Tween 20; T-C, total cholesterol.

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1. Introduction

Berberine (BBR) is a plant alkaloid, which is the principal bioactive compound of *Coptis Chinensis* and many other medicinal plants. BBR possesses a wide range of biological and pharmacological functions. It has been used for thousands of years in traditional Chinese medicine to treat various diseases, such as infectious diseases and gastrointestinal disorders, without apparent side-effects being reported from home remedies and clinical uses [1,2]. The cholesterol-lowering effect of BBR was reported back to the 1980s, with an observation that BBR lowered the intracellular cholesterol in cultured human aortic intimal cells [3]. However, this potential health benefit had not been paid serious attention until 2004 when BBR was found to markedly lower blood cholesterol levels *in vivo* in humans and hamsters [4]. Since then, several studies have repeatedly demonstrated the lowering effects of BBR on blood total cholesterol (T-C), LDL cholesterol (LDL-C), or nonHDL cholesterol (nonHDL-C) [5–10], whereas the mechanism of action remains to be further elucidated [4,5].

In 2004, BBR was reported to increase LDL receptor (LDLR) gene expression by stabilizing LDLR mRNA [4]. Following this report, the same groups conducted additional studies predominantly in HepG2 cells, with similar effects being observed [8,11,12,4]. In addition to LDLR-mediated LDL cholesterol clearance in the liver, several other mechanisms are involved in cholesterol homeostasis, including cholesterol absorption, cholesterol biosynthesis, cholesterol secretion, bile acid synthesis and secretion. Nevertheless, the effects of BBR on these processes have not been reported. Amongst these metabolic pathways, cholesterol absorption plays a very important role [13,14]. It is well-known that cholesterol absorption is collectively controlled by multiple factors or processes in the small intestine. These factors include cholesterol micellarization in the intestinal lumen [15], the expression of sterol transporters [5,16,17], cholesterol uptake, and the expression and activity of enzymes that catalyze the esterification of free cholesterol in the enterocytes [18].

Cholesterol micellarization determines the amount of cholesterol that can be transported across the 'unstirred' water layer to the apical side of enterocytes for absorption [15]. Cholesterol is then taken up by the enterocytes. Apart from the passive penetration, cholesterol uptake by enterocyte also involves the active transport through sterol transporters such as Niemann-Pick C1 Like 1 (NPC1L1) [19] and ATP-binding cassette, subfamily G, member 5 (ABCG5) and 8 (ABCG8) [20,21]. The expression of these transporters, together with passive penetration, mediates the dynamic flux of cholesterol at the apical side membrane of the enterocytes and thus the net amount of cholesterol that is taken up by the enterocytes. Prior to absorption, cholesterol esters of dietary and biliary sources are hydrolyzed to free cholesterol, which is the predominant form being absorbed into the enterocytes. Nevertheless, after entering the enterocytes free cholesterol needs to be converted back to esters because cholesterol is secreted out predominantly as ester form from the basolateral side membrane of enterocytes into the lymphatics and then the general circulation, and ultimately to be delivered to the liver. This step is catalyzed by acetyl-coenzyme A cholesterol acyltransferase (ACAT), primarily the ACAT2 isoform [18,22]. Our previous study showed that BBR lowered blood cholesterol through a mechanism that is independent of the expression of sterol transporters NPC1L1, ABCG5, and ABCG8 [5]. Therefore, in the present study we investigated the mechanism of action of BBR on cholesterol metabolism in diet-induced hypercholesterolemic rats with a primary focus on the intestinal absorption and further on cholesterol micellization prior to absorption and the apical side uptake and basolateral side secretion of the enterocytes during the absorption.

2. Materials and Methods

2.1. Animals and diets

Sixty male Sprague–Dawley rats (Charles River Laboratories, Montreal, QC, Canada) were housed one per cage with a 12 h light:dark cycle and fed a regular rodent chow. After 1 week of adaptation, rats were randomly divided into 5 groups (n = 12/group). One group was fed a semi-purified cornstarch-casein-sucrose-based AIN-93G diet and used as the normal control (NC). The other four groups were fed an atherogenic diet, which was the NC diet modified to contain 28% fat in a form of mixture of beef tallow and sunflower oil (4:1, w:w), 2% cholesterol and 0.5% cholic acid to induce hyperlipidemia [23]. One group was used as a hypercholesterolemic/atherogenic control (AC) and the other three were treated, twice a day, by gavage feeding with 50, 100, and 150 mg/kg·d of BBR in water, respectively. The control rats were gavaged with water only at the same volume as for the treatment groups. The purity of BBR (chloride salt) was > 98% (Sigma-Aldrich, ON, Canada). The animal use and experimental procedures were approved by the Joint Animal Care and Research Ethics Committee of the National Research Council Canada in Charlottetown and the University of Prince Edward Island. The study was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Rats were treated for 8 weeks to obtain the stable treatment effect. Seventy-two hours prior to sacrifice, they were injected through tail vein with 0.4 mg ^{13}C-cholesterol (99.5 atom% ^{13}C, CDN Isotopes, Quebec) in 1.0 mL Intralipid (20%, Baxter International, Deerfield, IL), followed immediately by intragastric administration of 1.5 mg ^{18}O-cholesterol (80.0 atom% ^{18}O, CDN Isotopes, Quebec) suspended in 1.0 mL of equal amounts of coconut, olive and sunflower oil-mix as reported previously [5]. At the end, rats were anesthetized with isoflurane inhalation. Blood was collected from cardiac puncture into EDTA-tubes and placed on ice. Plasma and red blood cells were separated by centrifugation and stored at −80 °C for later analysis of plasma lipids and the determination of cholesterol absorption. Liver and small intestine were dissected, weighed and immediately frozen in liquid nitrogen and stored at −80 °C for the measurement of protein expression.

2.2. Analysis of plasma lipids

After 2 weeks of storage at −80 °C, plasma T-C, HDL cholesterol (HDL-C) and triacylglycerol (TAG) were measured in