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Berberine metabolites could induce low density lipoprotein receptor up-regulation to exert lipid-lowering effects in human hepatoma cells

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ABSTRACT

Berberine (BBR) is an isoquinoline alkaloid isolated from several Chinese herbal medicines, such as *Coptis chinensis*, *Berberis aristata*, and *Coptis japonica*. It exhibits a lipid-lowering effect by up-regulating the hepatic low density lipoprotein receptor (LDLR) expression. However, the plasma concentration of BBR is very low after oral administration for the reason that BBR is poorly absorbed and rapidly metabolized. Therefore, it is hard to explain the pharmacological effects of BBR in vivo. Here, RT-PCR, Western blotting and Oil Red O staining were used to investigate the effects of four BBR metabolites on LDLR expression and lipid accumulation in human hepatoma Hep G2 cells. Our results suggested that BBR increased the LDLR mRNA and protein levels in a time- and dose-dependent manner. Four metabolites of BBR, jatrorrhizine, columbamine, berberrubine and demethyleneberberine, were found to be able to up-regulate LDLR mRNA and protein expression. Moreover, almost all the metabolites had potent effects on inhibiting cellular lipid accumulation. These results suggest that both BBR and its metabolites exhibit lipid-lowering effects by up-regulating LDLR expression, and BBR and its metabolites might be the in vivo active forms of BBR produced after oral administration. This study provides information to help us understand the mechanisms underlying the hypolipidemic effects of BBR in vivo.

1. Introduction

Despite considerable improvements in medical care over the past 25 years, cardiovascular disease remains a major public health challenge. It is the leading cause of death in Europe and USA and, in China, heart and cerebrovascular diseases are responsible for nearly 40% of all deaths [1–3]. Hypercholesterolaemia is characterized by high low density lipoprotein (LDL) plasma concentrations. The relationship between LDL-cholesterol and the risk of cardiovascular disease is well established and a reduction in LDL-cholesterol levels is a treatment goal and an indicator of the success of lipid-lowering therapies [4,5]. LDLR is a cell surface transmembrane protein that mediates the uptake of LDL and its degradation in lysosomes, which provides cells with cholesterol [6]. Some drugs are prescribed to lower LDL-cholesterol concentrations, and the most efficacious group are the statins [7]. Therefore, increased hepatic LDLR expression results in improved clearance of plasma LDL-cholesterol, which is

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strongly associated with a decreased risk of cardiovascular diseases.

Berberine (BBR) is an isoquinoline alkaloid isolated from several Chinese herbal medicines, such as *Coptis chinensis*, *Berberis aristata*, and *Coptis japonica*. It has been used in traditional oriental medicine for the treatment of gastroenteritis and secretory diarrhea [8]. Multiple pharmacological effects have been attributed to BBR and its related derivatives, such as antidiarrheic, antimicrobial, anticancer, antiinflammatory, and antiarrhythmic actions [9–13]. Recently, BBR was identified as a promising lipid-lowering drug, able to effectively up-regulate hepatic LDLR expression in liver cells and vascular cells, and it has been shown to decrease both serum triglycerides and cholesterol [14–16].

However, more and more investigations have revealed the limitations of BBR, including its low bioavailability and poor intestinal absorption [17,18]. The plasma concentration of BBR is very low after oral administration because BBR is poorly absorbed [19,20]. Intriguingly, BBR still exhibits a lipid-lowering effect even though it is rapidly transferred from the blood to the liver and bile and eliminated quickly. Zuo et al. [21] found that BBR was extensively metabolized in the body, and its metabolites maintained high plasma levels. Therefore, we speculated that the metabolites of BBR might be the main existing forms in the human body which would account for the pharmacological effects in vivo. In our previous work, we identified nine urinary metabolites of BBR in rats and humans [22]. Here, we examined four phase I metabolites of BBR involving their effects on LDLR up-regulation and lipid-lowering in human hepatoma Hep G2 cells which are considered suitable and convenient models for studying the regulation of hepatic LDL-cholesterol metabolism [23–25]. The results of our investigations should be helpful in providing a better understanding of the mechanisms underlying the lipid-lowering properties of BBR in vivo.

2. Materials and methods

2.1. Chemicals

Berberine chloride (purity > 99.5%) was supplied by the Northeast General Pharmaceutical Factory (Shenyang, China). Jatrorrhizine (M1) and columbamine (M2) were isolated from *C. chinensis* Franch [26]. Berberrubine (M3) was obtained by hydrolysis of 3,10-demethylpalmatine-10-*O*-sulfate with β-glucuronidase, followed by extraction with chloroform [22]. Demethyleneberberine (M4) was prepared by structural modification of BBR [21]. The chemical structures of the four BBR metabolites are shown in Fig. 1. BBR and its metabolites were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution, and the DMSO concentration was kept below 0.05% in all the cell cultures so that it had no detectable effect on cell growth.

2.2. Reagents

Fetal bovine serum (FBS) was obtained from TBD Biotechnology Development (Tianjin, China) and RPMI-1640 medium was obtained from Gibco/BRL (Gaithersburg, MD, USA). Antibodies against LDLR, β-actin and horseradish peroxidase-conjugated secondary antibodies (goat-anti-rabbit and goat-anti-mouse) were purchased from Santa Cruz Biotechnology

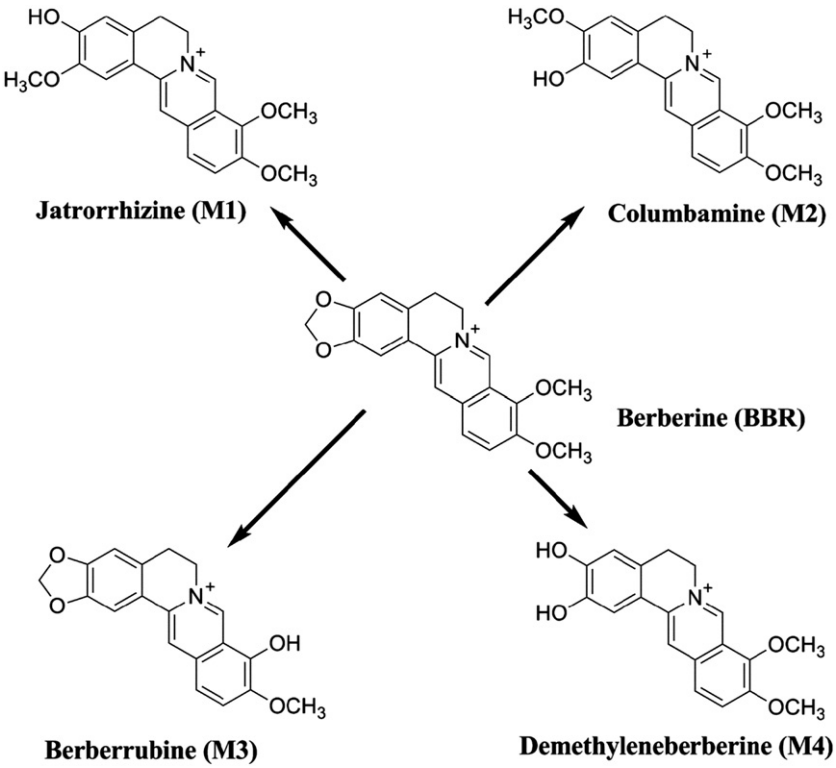

Fig. 1. Chemical structures of BBR and its metabolites (M1–M4).