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Research paper

Baicalin and its metabolites suppresses gluconeogenesis through activation of AMPK or AKT in insulin resistant HepG-2 cells

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ABSTRACT

Scutellaria baicalensis Georgi (*S. baicalensis*), as a traditional Chinese herbal medicine, is an important component of several famous Chinese medicinal formulas for treating patients with diabetes mellitus. Baicalin (BG), a main bioactive component of *S. baicalensis*, has been reported to have antidiabetic effects. However, pharmacokinetic studies have indicated that BG has poor oral bioavailability. Therefore, it is hard to explain the pharmacological effects of BG *in vivo*. Interestingly, several reports show that BG is extensively metabolized in rats and humans. Therefore, we speculate that the BG metabolites might be responsible for the pharmacological effects. In this study, BG and its three metabolites (M1-M3) were examined their effects on glucose consumption in insulin resistant HepG-2 cells with a commercial glucose assay kit. Real-time PCR and western blot assay were used to confirm genes and proteins of interest, respectively. The results demonstrate that BG and its metabolites (except for M3) enhanced the glucose consumption which might be associated with inhibiting the expression of the key gluconeogenic genes, including glucose-6-phosphatase (G6Pase), phosphoenolypyruvate carboxykinase (PEPCK) and glucose transporter 2 (GLUT2). Further study found that BG and M1 could suppress hepatic gluconeogenesis *via* activation of the AMPK pathway, while M2 could suppress hepatic gluconeogenesis *via* activation of the PI3K/AKT signaling pathway. Taken together, our findings suggest that both BG and its metabolites have antihyperglycemic activities, and might be the active forms of oral doses of BG *in vivo*.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by deregulation of glucose and lipid metabolism [1]. Type 2 diabetes accounts for about 90% the cases of diabetes [2]. The global incidence of type 2 diabetes is projected to double to 350 million cases by the year 2030 [3]. Type 2 diabetes mellitus is characterized by the impairment of insulin secretion from pancreatic beta cells and insulin resistance in peripheral tissues such as skeletal muscle, adipose tissue and liver [4]. The liver plays a major role in substrate metabolism and is a primary target of insulin action. Indeed, the liver regulates glucose homeostasis by maintaining equilibrium between glucose storage in the form of glycogen (glycogenesis) and glucose production through glycogen breakdown (glycogenolysis) or de novo synthesis of glucose (gluconeogenesis). Gluconeogenesis is under the control of two main enzymes: glucose-6-phosphatase (G6Pase) and phosphoenolypyruvate carboxykinase (PEPCK). However, dysregulation of gluconeogenesis is critically responsible for fasting hyperglycemia in type 2 diabetes [5,6]. In addition, regulated expression of specific GLUTs (glucose transporters) play a major role in glucose homeostasis in a tissue-specific manner. GLUT2 is the main isoform of glucose transporters in liver [7].

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Scutellaria baicalensis Georgi (*S. baicalensis*) is widely used as an ethnobotanical herb for the treatment of various ailments ranging from inflammation and nervous disorders [8,9]. It is also an important component of several famous Chinese medicinal formulas for treating patients with diabetes mellitus, for example, Huang-Lian-Jie-Du Decoction and San-Huang-Jiang-Tang Recipe [10,11]. Furthermore, *S. baicalensis* also shows promise antidiabetic effects, both alone and in combination with other herbs [12]. Baicalin (5,6,7-trihydroxyflavone -7-β-D-glucuronide, BG), the main bioactive component of *S. baicalensis*, showed anti-oxidative, anti-inflammatory, anti-HIV, anti-tumor and neuro-protective activities [13–16]. In addition, BG has been reported to improve the IL-6-mediated hepatic insulin resistance in Hepa-1c1c7 cells [17]. And it also has protective effect on the TNF-α-mediated development of insulin resistance in differentiated 3T3-L1 cells [18]. Furthermore, BG has significant antihyperglycemic effects in diabetic rats and the possible mechanisms include increasing the hepatic glycogen content and glycolysis, and reducing the serum levels of TNF-α [19]. However, pharmacokinetic studies have confirmed that BG had low oral bioavailability [20]. Apart from the intact BG in the systemic circulation, extensive levels of baicalein (M1) were observed [21,22]. M1, the aglycone form of BG, was produced by β-glucuronidase in intestinal microbiota [23]. It has also been demonstrated that the Phase 2 conjugates of M1 (both glucuronides and sulfate conjugates) were extensively circulating in the plasma after orally administrated BG. Therefore, we speculated that the metabolites of BG might also be the main existing forms in the human body which might account for the pharmacological effects *in vivo*.

In the present study, we prepared BG and its metabolites (M1-M3), and investigated the glucose-lowering activity of BG and its metabolites in insulin-resistant human hepatoma HepG-2 cells and clarified the molecular mechanism. The results of our investigations should be helpful in providing a better understanding of the antidiabetic properties of BG *in vivo*.

2. Results

2.1. Effects of BG and its metabolites on HepG-2 cells viability

To determine the cytotoxic effect, the viabilities of HepG-2 cells treated with increasing concentrations of BG were measured with MTT assay. As shown in Fig. 1A, the viabilities of the cells treated with 3.125–50 μM of BG for 24 h were not significantly different from that of the control group. However, BG at 25 and 50 μM reduced the viabilities to 85%. These results demonstrated that BG up to 12.5 μM could be used to treat HepG-2 cells without the concern of cytotoxic effects in the current experimental settings. To investigate cellular toxicity of the other test compounds, 12.5 μM of them were treated on HepG-2 cells for 24 h as described. The metabolites of BG did not show any cellular toxicity up to 12.5 μM concentration (Fig. 1B). Thus, we conducted the subsequent experiments with 12.5 μM of the test compounds including BG.

2.2. Palmitate (PA) induces insulin resistance on HepG-2 cells

As there is evidence that free fatty acid (FFA) can cause insulin resistant [24], HepG-2 cells were incubated with PA (0.2 mM) for 24 h and then were stimulated with insulin for 4 h. As shown in Fig. 2, insulin significantly increased the glucose consumption compared with the normal control group ($P < 0.05$), while there was no significant difference between PA treated group and PA & insulin treated group. Compared with the PA-untreated group, insulin could not increase the glucose consumption of HepG-2 cells treated with PA ($P < 0.01$). Taken together, these results suggested that the insulin resistant HepG-2 cells model were obtained by inducing with PA.

2.3. Effect of BG and its metabolites on glucose consumption of insulin resistant HepG-2 cells

First, cell viability was assayed by the MTT assay. As shown in Fig. 3A, the results showed that cells treated with the combination of the test compounds and PA did not show significant cellular toxicity. Next, we investigated the effect of BG and its metabolites on glucose consumption of insulin resistant HepG-2 cells. As shown in Fig. 3B, the inclusion of PA (0.2 mM) in the culture medium resulted in a 10% decrease ($P < 0.001$) on glucose consumption compared with the normal control group after 24 h of treatment. By contrast, the addition of BG and its metabolites (except for M3) at a dose of 12.5 μM reversed glucose consumption of the PA-induced insulin resistant cells.

Here, rosiglitazone was used as a positive control. The glucose consumption in rosiglitazone-treated cells was 7% higher ($P < 0.01$) than that in the PA group. The cells treated with M1 showed similar glucose consumption with the rosiglitazone group, but higher glucose consumption ($P < 0.01$) than the PA group, while M3 did not affect the glucose consumption in insulin resistant HepG-2 cells. In addition, BG and M2 showed similar glucose consumptions with the normal cells, but higher consumptions ($P < 0.001$) than the PA-induced insulin resistant cells, respectively. In a nutshell, our experiments showed that BG and its metabolites significantly increased the glucose consumption of the insulin resistant HepG-2 cells.

2.4. AMPK was activated by BG and its metabolites in insulin resistance HepG-2 cells

AMPK activation is thought to be a key proximal event in glucose metabolism and AMPK phosphorylation levels in threonine 172 are currently accepted as a marker of AMPK activity [25]. Therefore, the phosphorylation of AMPK by BG in PA-induced insulin resistance HepG-2 cells was determined at first. As illustrated in Fig. 4A and B, treating PA-induced insulin resistance HepG-2 cells with BG led to concentration- and time-dependent increases in phospho-AMPK levels. Next, we found that BG, M1 and M2 at 12.5 μM significantly increased AMPK phosphorylation and showed similar effects with rosiglitazone (Fig. 4C).

2.5. AKT was activated by BG and its metabolites in insulin resistance HepG-2 cells

The activation of AKT is necessary for insulin-stimulated glucose transport [26]. Therefore, we first determined the phosphorylation of AKT by BG in PA-induced insulin resistance HepG-2 cells. As illustrated in Fig. 5A and B, treating PA-induced insulin resistance HepG-2 cells with BG led to concentration- and time-dependent increases in phospho-AKT levels. Then, the effects on AKT phosphorylation by BG and its metabolites were examined. As shown in Fig. 5C, BG, M1 and M2 induced the up-regulation of AKT phosphorylation but less effective than rosiglitazone.

2.6. BG and M1 induced the down-regulation of gluconeogenic genes and GLUT2 expression in insulin resistance cells via AMPK signal pathway

Since impaired insulin-mediated suppression of gluconeogenesis and unregulated hepatic glucose production contributes to the fasting hyperglycemia observed in patients with diabetes [27], the effects of BG and its metabolites on gluconeogenic gene expression and GLUT2 gene expression were studied in our