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Differential regulation of baicalin and scutellarin on AMPK and Akt in promoting adipose cell glucose disposal

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ABSTRACT

Baicalin and scutellarin, two flavonoid glucuronic acids isolated from *Scutellaria baicalensis*, exhibit beneficial effects on glucose homeostasis. Baicalin and scutellarin are similar in structure except scutellarin has an additional hydroxyl at composition *C*-4′. In this work, we observed that baicalin and scutellarin promoted glucose disposal in mice and in adipocytes. Baicalin selectively increased phosphorylation of AMP-activated kinase (AMPK), while scutellarin selectively enhanced Akt phosphorylation. Both of them increased AS160 phosphorylation and glucose uptake in basal condition. AMPK inhibitor or knockdown of AMPK by siRNA blocked baicalin-induced AS160 phosphorylation and glucose uptake, but showed no effects on scutellarin. In contrast, Akt inhibitor and knockdown of Akt with siRNA decreased scutellarin-stimulated glucose uptake but had no effects on baicalin. The molecular dynamic simulations analysis showed that the binding energy of baicalin to AMPK (−34.30 kcal/mol) was more favorable than scutellarin (−21.27 kcal/mol), while the binding energy of scutellarin (−29.81 kcal/mol) to Akt was much more favorable than baicalin (4.04 kcal/mol). Interestingly, a combined treatment with baicalin and scutellarin acted synergistically to enhance glucose uptake in adipocytes (combination index: 0.94–0.046). In conclusion, baicalin and scutellarin, though structurally similar, promoted glucose disposal in adipocytes by differential regulation on AMPK and Akt activity. Our data provide insight that multicomponent herbal medicines may act synergistically on multiple targets.

1. Introduction

The regulation of glucose transport into fat tissue, skeletal muscle and liver is critical for the maintenance of whole-body glucose homeostasis [1]. Accumulating evidence has shown that adipose dysfunction is tightly associated with insulin resistance [2]. Insulin resistance is characterized by the delayed glucose disposal due to the damage of glucose uptake by insulin sensitive tissues, leading to the disturbance of glucose homeostasis responsible for the complications in diabetes [3]. Therefore, promotion of glucose disposal is essential for the maintenance of glucose homeostasis in the management of insulin resistance and diabetes.

Herbal medicines have played an important role in treating diabetes and insulin resistance. It has been well established that herbs are multi-component mixtures and take effects *via* a multi-target additive, synergistic, and/or competitive mode [4]. Multi-target drugs that impact different signaling pathways simultaneously are better at combating complex and multi-gene diseases, and are less prone to drug resistance [5].

Radix Scutellariae, derived from the roots of *Scutellaria bacicalensis*, is used for the improvement of blood glucose homeostasis [6]. Baicalin and scutellarin (Fig. 1), two flavonoid glucuronic acids, are the major bioactive constituents of Radix Scutellariae [7] with inhibitory effects on oxidative stress [8] and inflammation [9], and recently, its antidiabetic action is under investigation [10]. Although several mechanisms have been investigated, such as inhibiting adipocyte differentiation [11], suppressing systemic inflammation [12] and preventing hepatic lipid disorder [13], it remains unknown how baicalin and scutellarin modulate glucose homeostasis and whether they have potential synergistic glucose-lowering effects in adipocytes.

In this study, we observed that baicalin and scutellarin improved glucose tolerance in mice. We further showed that baicalin and scutellarin regulated glucose disposal in different mechanisms. Baicalin promoted glucose uptake in adipocytes *via* regulation of AMP-activated kinase (AMPK) activation, while scutellarin acted in a manner dependent of Akt activation. Interestingly, baicalin in combination with scutellarin synergistically enhanced glucose uptake in adipocytes. The new findings provide novel insights into the possible mechanism of baicalin and scutellarin for their glucose uptake-promoting effects.

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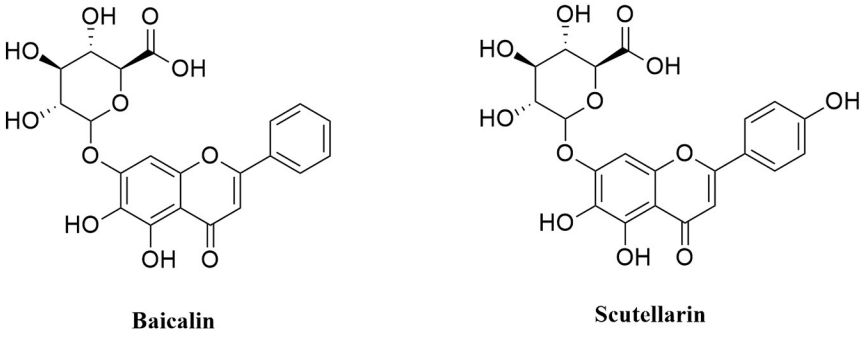

Fig. 1. Chemical structures of baicalin and scutellarin.

2. Materials and methods

2.1. Reagents

Baicalin and scutellarin (purity ≥98%) were purchased from Chengmust Biological Technology Co., Ltd. (Sichuan, China). For cell experiments, compounds were dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO did not exceed 0.1%. For animal experiments, the flavonoids and metformin were suspended in 0.5% sodium carboxymethyl cellulose. IBMX, dexamethasone and insulin were obtained from Sigma (St. Louis, MO, USA). Antibodies against phospho-AMPK-Thr172, AMPK, phospho-Akt-Ser473, Akt, phospho-AS160-Thr642, AS160 and goat anti-rabbit IgG HRP were obtained from Cell Signaling Technology (Bosten, MA, USA). Compound C, MK-2206 were obtained from Selleck (Stratech Scientific Ltd., Suffolk, UK). 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose) (N13195, lot: 873337) was purchased from Invitrogen (Eugene, Oregon, USA).

2.2. Culture and differentiation of 3T3-L1 cells

3T3-L1 preadipocytes were purchased from American Type Culture Collection (Manassas, VA) and were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, until the cells grew to confluence. Confluent monolayers of 3T3-L1 cells were induced to differentiate into adipocytes by incubation DMEM containing 0.5 mM IBMX, 1 mM dexamethasone, 10 $g \cdot mL^{-1}$ insulin for 2 days, then for 48 h in DMEM (10% FBS) containing 10 $g \cdot mL^{-1}$ insulin alone. Adipocytes viability was measured using a cell counting kit-8 (CCK-8 kit, Dojido, Japan) according to the manufacturer's instruction.

2.3. Animals

Male ICR mice (5 weeks) were supplied by the Laboratory Animal Center of Nanjing Qinglongshan. Animals were housed in a room with a constant temperature (22 ± 1 °C) and a 12-h light-dark cycle, and allowed free access to a standard diet and water *ad libitum*, housed in groups of ten until testing. The care and treatment of these mice were conducted in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation. This study was approved by the Science and Technology Department of Jiangsu Province (license number: SYXK (Su) 2012-0005).

2.4. Oral glucose tolerance test and insulin sensitivity assay in mice

Mice deprived of food overnight were orally administered with baicalin (50 $mg \cdot kg^{-1}$), scutellarin (50 $mg \cdot kg^{-1}$) or metformin (200 $mg \cdot kg^{-1}$), respectively. After 1.0 h, the mice were given glucose (2 $g \cdot kg^{-1}$) by gavage. Blood was collected from the orbital sinus at regular intervals after glucose load, and blood glucose and insulin levels were measured with commercial kit. Area under the curve for glucose (AUC-G) and insulin sensitivity index (ISI) was calculated, as described [14].

Diabetic mice model was induced by macrophages-derived conditioned medium (Mac–CM) according to a previous method the authors established [15]. Mice were administrated with drugs, and 30 min later, were intraperitoneally (i.p.) injected with Mac–CM (0.1 mL 10 g^{-1}, diluted with saline, 1:1, $v \cdot v^{-1}$). After another 0.5 h, mice were given glucose, and glucose intolerance and insulin sensitivity were tested.

2.5. Glucose consumption

Differentiated 3T3-L1 cells were cultured in plates (1×10^5 cells/well) and starved in Krebs–Ringer phosphate–HEPES buffer (KRHB, containing 118-mM NaCl, 5-mM KCl, 1.3-mM $CaCl_2$, 1.2-mM $MgSO_4$, 1.2-mM KH_2PO_4, and 30-mM HEPES, containing 0.5% BSA, pH 7.4) for 4 h, then treated with baicalin and scutellarin in KRH buffer containing 11 mM glucose. After 4 h, glucose content in the culture supernatant was examined with a glucose kit. The amount of glucose consumption was calculated by subtracting the glucose from the blank well.

2.6. Detection of glucose uptake with fluorescence microscopy

Adipocytes were seeded in 48-well plates (1×10^5 cells/well) in KRHB and pretreated with agents at given concentrations for 0.5 h. Cells were then incubated at 37 °C for 70 min with 2-NBDG probe (1 mM) in the dark. After washed with KRHB, the cells were observed by inverted fluorescence microscope (Nikon ECLIPSE Ti-s).

2.7. Measurement of 2-deoxyglucose (2DG) uptake and assessment of synergism and dose–response relationship

Cells were seeded in 6-well plates, and treated with baicalin (2.5, 5, 10, 20, 40, 80 μM), or scutellarin (2.5, 5, 10, 20, 40, 80 μM), or their combination at a ratio of 1:1. Glucose uptake was determined by 2DG uptake with an enzymatic photometric assay by using 2DG uptake measurement kit (Cosmo, Tokyo, Japan). The Chou–Talalay approach [16] was employed to evaluate synergistic effects between baicalin and scutellarin for the promotion of glucose uptake in adipocytes. To calculate combination index (CI) values, the CalcuSyn software (ComboSyn Inc., USA) was used. Interaction was quantified based on a CI to assess synergism ($CI < 1$), additive effect ($CI = 1$), and antagonism ($CI > 1$).

2.8. Measurement of cellular PI3K

Adipocytes were treated with baicalin or scutellarin at concentration of 10 μM for 0.5 h, and then lysed in ice-cold cell lysis buffer, and centrifugated at 13,000*g* for 15 min at 4 °C. The level of PI3K in the supernatant was detected by PI3K Assay Kit (Beyotime, Nanjing, China).