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Insulin-sensitizing activities of tanshinones, diterpene compounds of the root of *Salvia miltiorrhiza* Bunge

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Abstract

In this study, the effects of the extract and four tanshinone compounds from the dried root of *Salvia miltiorrhiza* Bunge (Labiatae) on the tyrosine phosphorylation of the insulin receptor (IR) β-subunit and the downstream signaling were examined in Chinese-hamster ovary cells expressing human insulin receptors (CHO/IR cells) as well as in 3T3-L1 adipocytes. In addition the translocation of the glucose transporter 4 was investigated in 3T3-L1 adipocytes. Total extract of Danshen (1–10 μg/ml) and the four tanshinones (10 μM) did not show any activity, but the total extract and the tanshinone I, IIA and 15, 16-dihydrotanshinone I except cryptotanshinone enhanced the activity of insulin (1 nM) on the tyrosine phosphorylation of the IR as well as the activation of the downstream kinases Akt, ERK1/2, and GSK3β. In the adipocytes the same IR-downstream signaling and the translocation of glucose transporter 4 were demonstrated by the three tanshinones in the presence of insulin. These insulin-sensitizing activities of tanshinones may be useful for developing a new class of specific IR activators as anti-diabetic agents.

Keywords: Tanshinones; Salvia miltiorrhiza; Insulin receptor tyrosine phosphorylation; Insulin signal transduction; Glucose transporter; 3T3-L1-adipocytes

Introduction

Danshen, the dried root of *Salvia miltiorrhiza* Bunge (Lamiaceae) is a commonly used traditional Chinese medicine for promoting blood circulation. It has been used for the treatment of cardiovascular diseases (CVD) such as coronary heart disease, hyperlipidemic, and cerebral vascular disease (Zhou et al., 2005). Various pharmacological studies *in vitro* and *in vivo* have concentrated on Danshen components such as hydrophilic phenolic compounds, danshensu, salvianolic acid B, and lipophilic diterpene compounds, tanshinones. These studies suggested that Danshen could improve microcirculations, dilate the coronary arteries, increase blood flow, and prevent myocardial ischemia (Wang et al. 2007). However, their clinical relevance needs to be established further.

Further the mode of action of Danshen needs to be established. We hypothesized that its common use in CVD may be related to the modulation of risk factors of CVD. Elevated blood glucose and/or blood pressure as well as dyslipidemia promote the process of atherosclerosis and are independent risk factors for CVD. We

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investigated whether Danshen has the potential to influence blood glucose levels through insulin receptor (IR) activators with insulin-mimetic and/or insulin-sensitizing activity by analyzing the effects of Danshen on IR signaling.

The IR is a tetrameric protein consisting of two identical extracellular α-subunits and two identical transmembrane β-subunits that have intracellular tyrosine kinase activity (Goldfine 1987; Moller and Flier 1991). Binding of insulin to the α-subunits of the IR leads to a conformational change and stimulation of the receptor kinase activity via auto-phosphorylation of tyrosine residues in the β-subunits (Goldfine 1987; Kahn 1994). This triggers two major kinase-signaling cascades: the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways (Hajduch et al. 1998; Shepherd et al. 1998). Activation of PI3K, one of the earliest steps in the insulin-signaling pathway, relays the signal to several kinases including the serine/threonine kinase, Akt and glycogen synthase kinase (GSK) 3β and plays a major role in many insulin-regulated translocation of glucose transporter 4 (GLUT4) followed by glucose uptake (Cong et al. 1997; Wang et al. 1999; Bryant et al. 2002).

In type 2 diabetes, the decreased ability of insulin to stimulate translocation of intracellular vesicles that store GLUT4 to the plasma membrane and the reduced glucose uptake into muscle or adipose tissues in response to insulin, results in a condition called insulin resistance (Kahn 1994). Although the molecular basis of type 2 diabetes is poorly understood, it is well established that insulin signaling, including activation of IR tyrosine kinase activity, is impaired in most type 2 diabetics (Thies et al. 1990; Goldfine 1999). Recently, small non-peptide molecules known as IR activators have been developed that restore IR auto-phosphorylation in insulin-resistant cells (Zhang et al. 1999; Manchem et al. 2001; Ding et al. 2002; Pender et al. 2002; Jung et al. 2007). Such pharmacological agents that enhance IR β-subunit tyrosine kinase activity could be useful for treating type 2 diabetes (Zhang and Moller 2000; Salituro et al. 2001).

Our search for IR activators from medicinal herbs has identified that diterpenoid components of medicinal plants greatly stimulate the effect of insulin on IR signaling (Jung et al., unpublished data). In the present study, we employed CHO/IR cells (Chinese-hamster ovary cells expressing modest amounts of human IR) (Frattali et al. 1991), as well as 3T3-L1 primary adipocytes, to evaluate whether tanshinone compounds work as IR activators with insulin-mimetic and/or insulin-sensitizing activity. Among various diterpenoid tanshinones that possess a variety of pharamacological activities such as antibacterial, antioxidant, anti-inflammatory, and antineoplastic (Wang et al. 2007), we selected tanshinone I (T-I), tanshinone IIA (T-IIA), 15, 16-dihydrotanshinone I (DHT-I), and cryptotanshinone (CT). These four tanshinones are the major constituents of the plant, and the biological studies have mainly focused on these relatively abundant tanshinones: activities as coronary artery dilators and protective effects against myocardial ischemia are known (Yagi et al. 1994; Wang et al. 2007).

Materials and methods

Antibodies (Abs) and reagents

Dulbecco's modified Eagle's medium (DMEM), α-minimal Eagle's medium (αMEM)+, fetal bovine serum (FBS), bovine calf serum, L-glutamine, penicillin, and streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). Trypsin-EDTA, insulin, dexamethasone, 3-isobutyl-1-methylxanthine, and *o*-phenylenediamine dihydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Blotting antibodies (Abs) against the IR β chain (IRβ, C-19), GLUT4 (H-61), and extracellular signal-regulated kinase (ERK) were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Abs against phospho-Akt (pAkt, Ser 473), phospho-GSK3β (pGSK3β, Ser 9), and phospho-ERK1/2 (pERK, Thr202/Tyr204) were from Cell Signaling Technology Inc. (Beverly, MA, USA). Blotting Ab against phosphotyrosine (pTyr, clone 4G10) and biotin-conjugated Ab 4G10 were from Upstate Biotechnology Inc. (Lake Placid, NY, USA). Monoclonal anti-human IR β-subunit Ab for coating, and streptavidin conjugated to horseradish peroxidase (HRP), were obtained from Biosource International (Camarillo, CA, USA). HRP-conjugated second Abs, affinity purified mouse anti-rabbit IgG and rabbit anti-mouse IgG were purchased from Bio-Rad Laboratories (Hercules, CA, USA), and the enhanced chemiluminesence (ECL) kit was from Amersham Biosciences Ltd. (Piscataway, NJ, USA).

Plant compounds

The dried roots of *Salvia miltiorrhiza* Bunge (Lamiaceae) were purchased from the Sung-Lim Company, Korea and authenticated by Professor J.H. Lee (College of Pharmacy, Kyung Hee University, Seoul, Korea). The dried roots (17.45 kg) were percolated with 70% ethanol (EtOH) three times and the EtOH extract concentrated in vacuo (2.4 kg) was suspended in H_2O, partitioned successively with hexane, CH_2Cl_2, EtOAc, and butanol (BuOH). A portion of the hexane and CH_2Cl_2 fractions (76.7 g) were chromatographed on a silica gel column (hexane-CH_2Cl_2, 80:20–40:60) to yield eleven subfractions (HC1–HC11). Tanshinone