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Danshen extract 15,16-dihydrotanshinone I functions as a potential modulator against metabolic syndrome through multi-target pathways

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ABSTRACT

Hypertension is a common complication of type 2 diabetes mellitus (T2DM), and is the main cause for T2DM-associated mortality. Although the stringent control of blood pressure is known to be beneficial in reducing the cardiovascular mortality of T2DM patients, drugs with both anti-hypertensive and anti-hyperglycemic effects are seldom reported. The traditional Chinese medicine *danshen* has long been used for lowering both blood pressure and blood glucose in T2DM patients, shedding lights on the development of such medication. However, the molecular mechanism and active component remain unclear. Here, we report that the lipophilic component, 15,16-dihydrotanshinone I (DHTH) from *danshen* potently antagonized both mineralocorticoid and glucocorticoid receptors, and efficiently inhibited the expression of their target genes like Na^+/K^+ ATPase, glucose 6-phosphatase (G6Pase), and phosphoenolpyruvate carboxykinase (PEPCK). In addition, DHTH increased AMPKα phosphorylation and regulated its downstream pathways, including increasing acetyl-CoA carboxylase (ACC) phosphorylation, inhibiting transducer of regulated CREB activity 2 (TORC2) translocation and promoting glucose uptake. Such discovered multi-target effects of DHTH are expected to have provided additional understandings on the molecular basis of the therapeutic effects of *danshen* against the metabolic syndrome.

1. Introduction

Cardiovascular disease is an extremely common morbidity and the main cause for the mortality of type 2 diabetes mellitus (T2DM). Notably up to 75% of cardiovascular disease in diabetes may be attributable to hypertension [1]. The mechanisms for T2DM-associated hypertension are complicated. It is believed that the enhanced sodium re-absorption in the renal tubules and the resultant increased sodium retention in the body caused by hyperinsulinaemia mainly account for the T2DM-induced hypertension [2]. Numerous clinical data suggest that rigorous control of blood pressure (BP) could reduce the incidence of cardiovascular complications and effectively decrease fatality of T2DM patients [3–5]. Therefore, captopril, atenolol, ramipril or other BP lowering drugs are frequently employed in the treatment of T2DM in combination with anti-hyperglycemic reagents or insulin sensitizers. However, due to the risk of drug–drug interactions in the combination therapy, drugs with both BP lowering and anti-hyperglycemic effects integrated into a single chemical entity are needed.

Danshen, the dried root of *Salvia miltiorrhiza*, is a traditional Chinese medicine that has been used for hundreds of years, with the conventional application in the treatment of cardiovascular disease including hypertension [6]. Notably, this herb was recently found to efficiently improve insulin sensitivity, lower blood glucose level and alleviate diabetic nephropathy in T2DM patients [7,8]. In order to clarify the potential molecular therapeutic mechanism for *danshen*, its components and biological activities were extensively studied. Generally, the chemical constituents of *danshen* include lipophilic and hydrophilic components, of which cryptotanshinone, tanshinone I and tanshinone IIA are the major lipophilic components, while salvianic acid A, protocatechuic aldehyde and salvianolic acid B are the major hydrophilic components [9]. Recently, lithospermic acid B, the hydrophilic component of *danshen*, was found to inhibit angiotensin converting enzyme (ACE), and markedly attenuated angiotensin I-induced vasoconstriction [10–12], which might partially explain the anti-hypertension effect of *danshen*. However, the contribution of the lipophilic components to *danshen*'s therapeutic effect was poorly understood.

In the current work, we discovered that 15,16-dihydrotanshinone I (DHTH, Fig. 1a), a lipophilic component of *danshen*, functioned as antagonists of both mineralocorticoid and glucocorticoid receptors, and inhibited the expression of their target genes, such as glucose 6-phosphatase (*G6Pase*), phosphoenolpyruvate carboxykinase (*PEPCK*) and *Na^+/K^+ ATPase*.

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Besides, DHTH was also identified as an AMPK pathway activator, which could increase AMPKα phosphorylation and activate its downstream pathways, including increasing acetyl-CoA carboxylase (ACC) phosphorylation, inhibiting transducer of regulated CREB activity 2 (TORC2) translocation and promoting glucose uptake. Our findings have provided one possible understanding for the anti-hypertension and anti-T2DM effects of *danshen*, while DHTH could serve as a promising lead compound for further development in the treatment of T2DM associated with hypertension.

2. Materials and methods

2.1. Reagents

15,16-Dihydrotanshinone I was isolated from *Salvia miltiorrhiza* as described in supplementary method. Cytochalasin B, 2-deoxy-D-glucose, biotin, 3-isobutyl-1-methylxanthine, dexamethasone, ionomycin, *p*-nitrophenyl-galactopyranoside and forskolin were purchased from Sigma–Aldrich. Pantothenic acid calcium salt was purchased from Eastman. 2-[^{3}H]-Deoxy-D-glucose was purchased from PerkinElmer. Cell culture reagents were all bought from GIBCO. Hoechst 33342 was obtained from Invitrogen. All antibodies were purchased from Cell Signaling Technology except anti-GAPDH antibody (KangChen, China). Dual Luciferase Assay System was purchased from Promega. ATP Analysis Kit was obtained from Beyotime (Shanghai, China). TRIzol reagent was from Generay Biotech (Shanghai, China). PrimeScriptTM RT reagent Kit was obtained from TaKaRa (Japan). SYBR Green Real-time PCR master mix was from TOYOBO (Japan).

2.2. Plasmids

pGL3-GRE/MRE-Luc was constructed by inserting 2× GRE/MRE sequence (TGTACAGGATGTTCTctctgcctctgcTGTACAGGATGTTCT) into pGL3-promoter vector. pSuper.neo.gfp-LKB1 plasmid was constructed by inserting the LKB1-siRNA sequence (cgaagagaagcagaaaatg) [13] into BglII-HidIII sites.

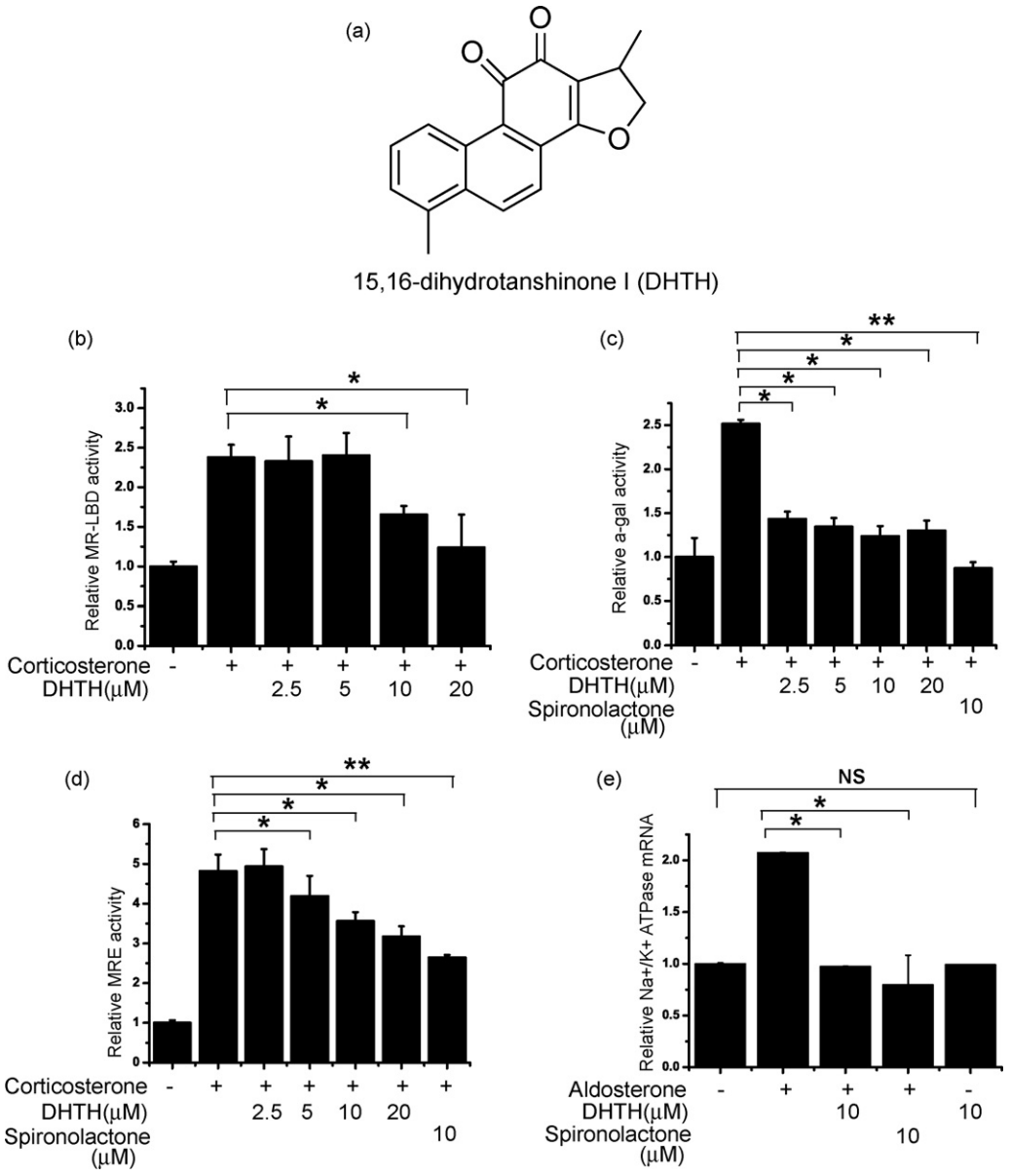

Fig. 1. DHTH is an MR antagonist. (a) Chemical structure of 15,16-dihydrotanshinone I (DHTH). (b) DHTH inhibited MR-LBD activation. (c) DHTH blocked MR-SRC1 interaction. (d) DHTH inhibited MRE transactivation. (e) DHTH inhibited *Na$^+$/K$^+$ ATPase* expression. Significant difference at $^*P < 0.05$, $^{**}P < 0.01$, NS: no significant difference, $n = 3$.