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Deoxyandrographolide promotes glucose uptake through glucose transporter-4 translocation to plasma membrane in L6 myotubes and exerts antihyperglycemic effect *in vivo*

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ABSTRACT

Skeletal muscle is the principal site for postprandial glucose utilization and augmenting the rate of glucose utilization in this tissue may help to control hyperglycemia associated with diabetes mellitus. Here, we explored the effect of Deoxyandrographolide (DeoAn) isolated from the *Andrographis paniculata* Nees on glucose utilization in skeletal muscle and investigated its antihyperglycemic effect *in vivo* in streptozotocin-induced diabetic rats and genetically diabetic db/db mice. In L6 myotubes, DeoAn dose-dependently stimulated glucose uptake by enhancing the translocation of glucose transporter 4 (GLUT4) to cell surface, without affecting the total cellular GLUT4 and GLUT1 content. These effects of DeoAn were additive to insulin. Further analysis revealed that DeoAn activated PI-3-K- and AMPK-dependent signaling pathways, account for the augmented glucose transport in L6 myotubes. Furthermore, DeoAn lowered postprandial blood glucose levels in streptozotocin-induced diabetic rats and also suppressed the rises in the fasting blood glucose, serum insulin, triglycerides and LDL-Cholesterol levels of db/db mice. These findings suggest the therapeutic efficacy of the DeoAn for type 2 diabetes mellitus and can be potential phytochemical for its management.

1. Introduction

The increasing prevalence of type 2 diabetes mellitus (T2DM) and associated secondary metabolic complications represent a major threat to public health (Garber, 2012). T2DM is characterized by chronic hyperglycemia resulting from insulin resistance of the peripheral tissues and/or impaired insulin secretion from the pancreas (Saltiel, 2001). Insulin plays a key role in the regulation of glucose and lipid homeostasis in skeletal muscle, adipose tissue and liver. Insulin exerts its metabolic effects to maintain glucose homeostasis by stimulating glucose uptake in skeletal muscle and adipose and suppressing glucose production by the liver (Watson and Pessin, 2006). Insulin resistance in these target tissues results in reduced insulin-stimulated glucose uptake in muscle and adipose tissue, and the impaired suppression of glucose output in liver (Schenk et al., 2008), leading to hyperglycemia, the hallmark feature of diabetes mellitus. Skeletal muscle is the major tissue for insulin-stimulated glucose disposal and is, therefore, the major site for suffering insulin resistance. Insulin resistance in skeletal muscle is characterized by impaired translocation of the insulin sensitive glucose transporter 4 (GLUT4) from an intracellular compartment to the plasma membrane, resulting in consequent defect in the insulin-stimulated glucose uptake, a rate-limiting step for glucose utilization (Petersen and Shulman, 2006).

Glycemic control is the basis for the treatment of T2DM, as it can prevent or slow the progression towards secondary complications (Fonseca, 2003). Alleviation of insulin resistance has been considered as a primary strategy to improve glycemic control in T2DM subjects (Tahrani et al., 2011). Despite considerable progress in the management of T2DM with synthetic drugs, the search for natural antidiabetic agents is ongoing. Therapeutic route through natural agents provide a prolific source for safe, effective and relatively inexpensive new remedies for T2DM and associated

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metabolic complications (Moller, 2001; Tan et al., 2008).

Andrographis paniculata Nees (family: Acanthaceae), a type of herb, well known in Southeastern Asian countries, has been widely used as an immunostimulant (Puri et al., 1993) and for the treatment of the common cold (Melchior et al., 1996) and respiratory tract infections (Coon and Ernst, 2004). Major bioactive diterpenoids of *A. paniculata* Nees include andrographolide, deoxyandrographolide and neoandrographolide (Cheung et al., 2001; Tan et al., 2005). These diterpenoids differ broadly in their pharmacological actions, which might be related to the variations in their chemical structures (Nanduri et al., 2004; Roy et al., 2010). Andrographolide has been reported for the anti-inflammatory (Shen et al., 2002), antidiabetic (Nugroho et al., 2012, 2013), and hepatoprotective (Trivedi and Rawal, 2001) activities. Besides, deoxyandrographolide (DeoAn), an analog of andrographolide with an endocyclic double bond, has been reported for calcium channel blocking activity (Burgos et al., 2003, 2005), cytotoxic (Suriyo et al., 2014), and hepatoprotective effects (Mandal et al., 2013, 2014). However, the pharmacological effect of DeoAn on glucose metabolism remains poorly explored. In present report, we investigated the effect of DeoAn on glucose utilization in L6 myotubes, and validated its antidiabetic potential in *in vivo* models.

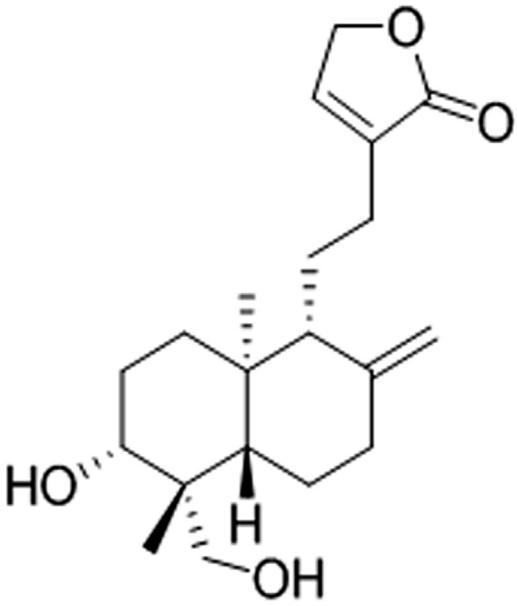

Fig. 1. Chemical structure of deoxyandrographolide (DeoAn) isolated from the *Andrographis paniculata* Nees.

2. Materials and methods

2.1. Materials

DMEM, FBS, trypsin, and antibiotic/antimycotic solution were from Gibco, USA. Cytochalasin B, O-phenylenediamine dihydrochloride (ODP), 2-deoxyglucose, polyclonal anti-*myc*, monoclonal anti-actinin-1, streptozotocin and all other chemicals unless otherwise noted were from Sigma Chemical (St. Louis, MO). 2-Deoxy-D-[^{3}H]-glucose (2-DG) was from PerkinElmer, USA. Antibodies to phospho-Akt (Ser-473), GLUT4 (IF8), phospho-tyrosine, insulin receptor-β, AMPKα, phospho-AMPKα (Thr-172), and phospho-ACC (Ser-79) were from Cell Signaling Technology (USA). Antibody to GLUT1 was from Santa Cruz Biotechnology, Inc (USA). Biochemical assay kits for the measurement of total triglyceride (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were procured from Dialab, India.

2.2. Isolation of Deoxy-andographolide (DeoAn)

Andrographis paniculata Nees (family: Acanthaceae) was procured from local market and the authentification was done by Dr. K.R. Arya, Botanist, Division of Botany, CSIR—Central Drug Research Institute, Lucknow. A voucher specimen of the plant is preserved in the herbarium of the institute for future reference. Powdered plant material (4 kg) was placed in glass percolator with 95% ethanol (10 L) and allowed to stand for 24 h at room temperature. The percolate was collected and process was repeated four times and the combined percolate of 40 L was evaporated under reduced pressure at 50° C to afford ethanolic extract (300 g). The ethanolic extract was macerated with hexane and the soluble fraction was separated and evaporated under reduced pressure to afford hexane fraction (80 g). The hexane insoluble portion was fractionated with chloroform to afford chloroform fraction (120 g). The water soluble fraction was evaporated under reduced pressure at 60 °C to afford the aqueous fraction (70 g). Chloroform fraction (120 g) was further subjected to column chromatography on a column of silica gel (60–120 mesh) and eluted with hexane-chloroform (20:80) to afford DeoAn (160 mg, Fig. 1). The compound's visualization was done under UV light and by spraying with 10% sulfuric acid in methanol, which also gave positive Liebermann–Burchard test for terpenoids. The structure elucidation of the compound was performed by spectroscopic techniques.

Deoxyandrographolide: IR (KBr) 3435, 3018, 2990, 2937, 1794, 1751, 1644, 1452, 1383, 1345, 1217, 1148, 1078, 1028, 894, 845, 756, 668 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08 (s, 1 H), 4.86 (s, 1 H), 4.76 (s, 2 H), 4.58 (s, 1 H), 3.45 (d, J=9.51 Hz, 1 H), 3.31 (d, J=10.26 Hz, 1 H), 1.22 & 0.62 (each s, 3 H); ^{13}C NMR($CDCl_3$, 75 MHz) 174.77, 147.20, 144.49, 135.00, 107.68, 80.83, 70.51, 64.54, 56.42, 55.66, 43.17, 39.39, 38.54, 37.24, 28.55, 24.88, 24.35, 23.10, 22.30, 15.58; ESI-MS: 335 $[M+H]^+$ (Supporting information).

2.3. Cell culture and treatment

Wild type L6 skeletal muscle cells and L6 cells stably expressing rat GLUT4 with a *myc* epitope (L6-GLUT4*myc*) were a kind gift of Dr. Amira Klip, Program in Cell Biology, The Hospital for Sick Children, Toronto, Canada. Cells were maintained in DMEM and used for experimentation as described previously (Prasad et al., 2013). DeoAn was dissolved in a minimum quantity of DMSO and further diluted in culture medium to achieve the required concentration for *in vitro* experiments. The final DMSO concentration in the cell culture medium was less than 0.1%.

2.4. Glucose uptake assay

Determination of 2-DG uptake in differentiated myotubes was performed as previously described (Tamrakar et al., 2010). Briefly, myotubes were grown in 24 well plates and incubated with indicated concentrations of DeoAn for specified time period. To determine the effect of insulin, cells were incubated for 3 h in serum-deprived medium and stimulated with 100 nM insulin for 20 min. glucose uptake was assessed for 5 min in HEPES-buffered saline containing 10 μM 2-DG (0.5 μCi/ml 2-[^{3}H] DG) at room temperature. Subsequently cells were rinsed with an ice-cold solution containing 0.9% NaCl and 20 mM D-glucose. The amount of radioactivity incorporated was quantified in cells lysate using β-counter. Nonspecific uptake was determined in the presence of cytochalasin B (50 μM) during the assay, and these values were subtracted from all other values. Glucose uptake measured in triplicate and normalized to total protein, was expressed as fold induction relative to unstimulated cells.