Fitoterapia 93 (2014) 239–244

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Alkaloids from *Coptis chinensis* root promote glucose uptake in C2C12 myotubes

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ARTICLE INFO

Article history:
Received 23 October 2013
Accepted in revised form 3 January 2014
Available online 18 January 2014

Keywords:
Coptis chinensis
Alkaloid
C2C12 cells
Skeletal muscle
Glucose uptake

Chemical compounds studied in this article:
8-Oxyberberine (PubChem CID 11066)
Berberine (PubChem CID 2353)
Noroxyhydrastinine (PubChem CID 89047)
Octadecyl caffeate (PubChem CID 5320237)
Corydaldine (PubChem CID 610097)
β-Sitosterol (PubChem CID: 222284)
[^{14}C]-2-deoxy-glucose (PubChem CID: 6439681)
Ethanol (PubChem CID: 702)
KBr (PubChem CID: 253877)
Bicinchoninic acid (PubChem CID: 71068)

ABSTRACT

The root of *Coptis chinensis* Franch. (COCH) is regularly used for medicinal purposes, and has been prescribed alone or in combination with other traditional herbs for the treatment of diabetes. To investigate the effects of COCH on glucose utilization by skeletal muscles, we prepared an ethanol extract of COCH root (COCH-Et) partitioned with dichloromethane, *n*-butanol, and water and tested its effects on glucose uptake in differentiated C2C12 myotubes. We found that dichloromethane and *n*-butanol sub-fractions of COCH-Et promoted glucose uptake in differentiated C2C12 cells at 50 μg/mL. Further fractionation of these preparations by using column chromatography, analysis of their effects on glucose uptake and characterization using nuclear magnetic resonance, mass spectrometry, and thin layer chromatography helped identify two new alkaloids, 8,13-dioxocoptisine hydroxide (**1**) and coptisonine (**2**), together with eleven known compounds. These were isolated from the dichloromethane layer of COCH-Et. In particular, exposure of C2C12 cells to berberine (**6**) at 12.5 and 6.25 μg/mL for 24 h resulted in significant promotion of glucose uptake. Coptisonine (**2**) and octadecyl caffeate (**9**) also stimulated glucose uptake at 25 and 50 μg/mL. These findings indicate that active constituents of COCH root may help alleviate hyperglycemia in diabetes by promoting glucose uptake by skeletal muscles.

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1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose level (hyperglycemia) owing to failure of the pancreatic beta cells to produce enough insulin, or loss of an effective target tissue response to insulin. DM is estimated to affect 347 million people worldwide and to have caused 3.4 million deaths in 2010 [1]. The World Health Organization (WHO) predicts that DM will be the 7th leading cause of death in 2030 [2]. Therefore, DM is an important global

Abbreviations: COCH, *Coptis chinensis* Franch.; 2-DG, [^{14}C]-2-deoxy-glucose; DM, diabetes mellitus; ESI, electrospray ionization; NMR, nuclear magnetic resonance; MS, mass spectrometry; TLC, thin layer chromatography; HR ESI MS, high-resolution electrospray ionization mass spectrometry; BCA, bicinchoninic acid; HMBC, heteronuclear multiple bond correlations; NOE, nuclear Overhauser effect; COSY, correlation spectroscopy; HSQC, heteronuclear single quantum coherence; NOESY, nuclear Overhauser enhancement spectroscopy.

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0367-326X/$ – see front matter © 2014 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.fitote.2014.01.008

and regional issue, and is likely to become more prevalent in the coming decades [3].

Type 1 and type 2 sub-classifications of DM have been established by WHO, depending on the cause of the disease [4]. Type 2 DM (also called non-insulin-dependent DM) results from insulin resistance and beta-cell dysfunction [5] and accounts for 90% of the global prevalence of DM. Several studies have suggested that insulin resistance is the primary pathogenic factor in type 2 DM [6]. Insulin resistance is characterized by diminished response of insulin-sensitive tissues, including the liver, adipose tissue, and skeletal muscle, and marked decrease in glucose uptake and utilization. This leads to hyperglycemia and increases the risk for hypertension, obesity, coronary heart disease, and kidney disease [7].

Roots of *Coptis chinensis* Franch. (COCH) (also called Huang Lian) have been used in traditional Chinese medicine in the treatment of fever, diarrhea, dysentery, jaundice, acute febrile and suppurative infections, seasonal febrile diseases, carbuncle, and sore throat [8,9]. The anti-diabetic activity of COCH roots was recorded for the first time in "Note of Elite Physicians" by Hongjing Tao in 500 AD, and confirmed more recently [10]. It has been prescribed alone or in combination with other traditional herbs for the treatment of diabetes [11]. In addition, pharmacological research has identified antibacterial [12], antiprotozoal [12], anti-inflammatory [13], antihypertensive [14], anticholinergic [15,16], antiarrhythmic [15,16], anticancer [17], anti-angiogenic [18], cholesterol-lowering [19,20], and anti-adipogenic [21] effects of isoquinoline alkaloids extracted from the COCH root. Phytochemical investigations of this plant have revealed the presence of alkaloids such as berberine, coptisine, palmatine, jatrorrhizine, and magnoflorine [22], although a range of compounds including lignans, flavonoids, benzenoids [14], and polysaccharides [23] are also present.

The skeletal muscle is the largest contributor to whole-body glucose utilization and is the primary site of glucose uptake. Insulin resistance in the skeletal muscle is a major contributor to the development of type 2 DM [24]. Therefore, promotion of the glucose uptake in muscle cells could improve whole-body glucose metabolism, and represents a potential route for the management of DM.

The present study used differentiated C2C12 muscle cells as a model to evaluate the effects of an ethanol extract of COCH root on glucose uptake. Because the COCH ethanol extract promoted glucose uptake in this cell line, we investigated its bioactive constituents and identified two new alkaloids, along with eleven known compounds. The present paper describes the isolation and structural elucidation of these compounds, and their effects on glucose uptake in differentiated C2C12 cells.

2. Material and methods

2.1. General experimental procedures

Proton nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 500 (500 MHz) spectrometers. Chemical shifts (ppm) were measured using tetramethylsilane as the internal standard and deuterated chloroform ($CDCl_3$) as the solvent. Mass spectrometry (MS) was performed in the electrospray ionization (ESI) mode on a Finnigan LCQ spectrometer. High-resolution electrospray ionization mass spectrometry (HR ESI MS) was performed in the electrospray ionization (ESI) mode on a Finnigan Mat 95S Mass spectrometer. Merck silica gel 60 (Merck 70–230, 230–400 mesh) was used for column chromatography. Glass sheets of silica gel $60F_{254}$ (Merck, 0.2-mm thick) were used for thin-layer chromatography (TLC). Melting point (mp) was measured on a Fischer-Johns apparatus and data were uncorrected. Ultra-violet (UV) spectra were recorded on a Shimadzu UV-160 spectrophotometer, and infrared (IR) spectra were determined as potassium bromide (KBr) disks on a Shimadzu FT-IR8700 spectrophotometer. The protein concentration was measured by bicinchoninic acid (BCA) protein assay and using the Spectra Max190 microplate reader. Radioactivity was measured using a liquid scintillation counter (TopCount NXT, Perkin Elmer).

2.2. Plant material

COCH root was supplied by Chien-Yuan Co., Taipei, Taiwan, in September 2010. The plant was authenticated by Hang-Ching Lin from a voucher specimen (NDMCP no. 990901).

2.3. Preparation of the crude plant extracts

COCH root (2.1 kg) was powdered and extracted with 90% ethanol (10 L × 4) at room temperature for 24 h. Evaporation of the solvent under reduced pressure yielded 473.8 g of crude extract (COCH-Et), which was dissolved in water and partitioned using dichloromethane (CH_2Cl_2–H_2O) and *n*-butanol (*n*-BuOH–H_2O) successively to yield fractions referred to as COCH-D (45.0 g), COCH-B (145.6 g), and COCH-W (282.4 g), respectively.

In addition, 7.7 kg of COCH root was powdered and extracted with acetone (50 L × 4) at room temperature for 24 h. Evaporation of the solvent under reduced pressure yielded 145.0 g of crude extract, which was dissolved in water and partitioned as described above to yield COCH-D$^{\#}$ (63.4 g), COCH-B$^{\#}$ (45.5 g), and COCH-W$^{\#}$ (27.9 g) fractions.

2.4. Isolation of compounds

COCH-D (29.0 g) was chromatographed on silica gel (70–230 mesh) by using increasingly polar mixtures of CH_2Cl_2–MeOH (CH_2Cl_2 only; CH_2Cl_2:MeOH, 95:5; and MeOH only) to obtain three fractions: CO–D1 (6.69 g), CO–D2 (2.93 g), and CO–D3 (12.2 g), respectively. The CO–D2 fraction was then chromatographed on silica gel by using increasingly polar mixtures of CH_2Cl_2 and acetone to give 8-oxyberberine (**3**, 21.9 mg) (structures in Supplementary information), 8-*oxo*-epiberberine (**4**, 5.4 mg), and 8-oxocoptisine (**5**, 10.4 mg). The COCH–B fraction was crystallized to obtain berberine (**6**, 26.9 g).

The combined fractions COCH-D$^{\#}$ (58.4 g) and COCH-D (9.0 g) were chromatographed on silica gel (70–230 mesh) by using increasingly polar mixtures of CH_2Cl_2 and MeOH to obtain five fractions: CO-D$^{\#}$1 (26.0 g, CH_2Cl_2 only), CO-D$^{\#}$2 (561.1 mg, CH_2Cl_2 only), CO-D$^{\#}$3 (1.6 g, CH_2Cl_2 only), CO-D$^{\#}$4 (15.6 g, CH_2Cl_2:MeOH, 95:5), and CO-D$^{\#}$5 (20.3 g, MeOH only). CO-D$^{\#}$2 was crystallized to yield β-sitosterol (**13**, 1.4 mg). CO-D$^{\#}$3 was chromatographed on silica gel (230–400 mesh) by using *n*-hexane:acetone (5:1) to give 8,13-dioxocoptisine hydroxide

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