Phytomedicine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytomedicine

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

Two triterpeniods from *Cyclocarya paliurus* (Batal) Iljinsk (Juglandaceae) promote glucose uptake in 3T3-L1 adipocytes: The relationship to AMPK activation

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ARTICLE INFO

Article history:
Received 11 December 2014
Revised 17 April 2015
Accepted 19 May 2015
Available online xxx

Chemical compounds:
Cyclocaric acid B (CAS No. 182315-46-4)
Cyclocarioside H (CAS No. 1403937-87-0)

Keywords:
Triterpenoids
Cyclocarya paliurus
Inflammation
Insulin resistance
Glucose uptake
AMPK

ABSTRACT

Purpose: The current study investigated the efficacy of *Cyclocarya paliurus* chloroform extract (CPEC) and its two specific triterpenoids (cyclocaric acid B and cyclocarioside H) on the regulation of glucose disposal and the underlying mechanisms in 3T3-L1 adipocytes.

Methods: Mice and adipocytes were stimulated by macrophages-derived conditioned medium (Mac-CM) to induce insulin resistance. CPEC was evaluated in mice for its ability by oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). To investigate the hypoglycemic mechanisms of CPEC and its two triterpenoids, glucose uptake, AMP-activated protein kinase (AMPK) activation, inhibitor of NF-κB kinase β (IKKβ) phosphorylation and insulin signaling transduction were detected in 3T3-L1 adipocytes using 2-NBDG uptake assay and Western blot analysis.

Results: Mac-CM, an inflammatory stimulus which induced the glucose and insulin intolerance, increased phosphorylation of IKKβ, reduced glucose uptake and impaired insulin sensitivity. CPEC and two triterpenoids improved glucose consumption and increased AMPK phosphorylation under basal and inflammatory conditions. Moreover, CPEC and its two triterpenoids not only enhanced glucose uptake in an insulin-independent manner, but also restored insulin-mediated protein kinase B (Akt) phosphorylation by reducing the activation of IKKβ and regulating insulin receptor substrate-1 (IRS-1) serine/tyrosine phosphorylation. These beneficial effects were attenuated by AMPK inhibitor compound C, implying that the effects may be associated with AMPK activation.

Conclusions: CPEC and its two triterpenoids promoted glucose uptake in the absence of insulin, as well as ameliorated IRS-1/PI3K/Akt pathway by inhibiting inflammation. These effects were related to the regulation of AMPK activity.

1. Introduction

Insulin resistance is a pathological state with reduced sensitivity of insulin targeted tissues relative to the normal levels of insulin, which is a pivotal feature in the pathogenesis of metabolic disturbances including obesity and type 2 diabetes (Reaven 1988). Now it is generally considered that obesity is characterized as a chronic low-grade inflammatory state and causally associated with insulin resistance (Lumeng and Saltiel 2011). Adiposity contributes to the secretion of TNF-α and IL-6, which are pro-inflammatory cytokines, from adipocytes and infiltrated macrophages (Johnson and Olefsky 2013). Pro-inflammatory cytokines activate intracellular kinases IKKβ which increases insulin receptor substrate-1 (IRS-1) serine phosphorylation, then prevents the insulin-induced tyrosine phosphorylation of IRS-1, resulting in insulin resistance (Gual et al. 2005; Hotamisligil 2006). Therefore, inhibition of inflammation in adipose tissue should be a potential therapeutic application for the treatment of obesity-induced insulin resistance.

Abbreviations: Akt, Protein kinase B; AMPK, AMP-activated protein kinase; AUC, Area under the curve; IKKβ, Inhibitor of NF-κB kinase β; IRS-1, Insulin receptor substrate-1; Mac-CM, Macrophages-derived conditioned medium; PI3K, Phosphatidylinositol 3-kinase; TNF-α, Tumor necrosis factor-α.

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http://dx.doi.org/10.1016/j.phymed.2015.05.058
0944-7113/

AMP-activated protein kinase (AMPK), the energy-sensing enzyme, plays an important role in glucolipid metabolism (Friedrichsen et al. 2013). Moreover, it demonstrates anti-inflammatory activity (Bess et al. 2011), which contributes to improving insulin sensitivity (Wang et al. 2013a). AMPK agonists, including AICAR (5-Aminoimidazole-4-carboxamide 1-β-*D*-ribofuranoside) and metformin have been shown to promote glucose disposal in muscle (Sajan et al. 2010). However, they are not ideal that AICAR may probably regulate other AMP-sensitive enzymes, for instance, fructose-1,6-bisphospatase (Vincent et al. 1991) and the disadvantages of metformin are gastrointestinal adverse reaction, lactic acidosis and reduced absorption of vitamin B12 during long-term use (Hermann 1979). Plenty of medicinal plants and their bioactive constituents are cheaper and better at controlling chronic diseases, such as diabetes, and are less likely to drug resistance (Zimmermann et al. 2007). As a potent anti-inflammatory agent, salicylate ameliorates insulin resistance by inhibition of IKKβ activation (Yuan et al. 2001), and a further study indicates its direct action in the positive regulation of AMPK activity (Hawley et al. 2012). These findings raise the possibility that natural products pharmacological activation of AMPK alleviates insulin resistance through inhibition of inflammation.

Cyclocarya paliurus (Batal.) Iljinsk (Juglandaceae) (CP) is a Chinese endemic plant. Numerous studies have shown that CP has multiple bioactivities, including anti-hyperglycemia, anti-hyperlipidemia and anti-inflammation (Jiang et al. 2014; Kurihara et al. 2003a, 2003b). Recent studies revealed that chloroform extract of CP (CPEC) exhibits blood glucose-lowering action (Li et al. 2011), while polysaccharides and flavonoids seems not to be its active anti-diabetic constituents (Wang et al. 2013b). However, it is reported that triterpenoids derived from CP such as cyclocarioside A, cyclocarioside II and cyclocarioside III showed an insulin-like activity in adipocytes (Kurihara et al. 2003a), some secodammarane and epoxydammarane triterpenoids such as cyclocarioside-H also exhibited inhibitory activities against α-glucosidase and aldose reductase (Li et al. 2012). What's more, ursolic acid and oleanolic acid also exist in CP demonstrated a variety of bioactivities including hypoglycemia and anti-inflammation (Lee et al. 2010a; Li et al. 2014; Takada et al. 2010). Our previous results demonstrated CP could reduce blood sugar and inflammatory lesions similar to metformin (Jiang et al. 2014), meanwhile natural phytochemicals such as curcumin and quercetin also inhibit inflammatory response by targeting AMPK (Leiherer et al. 2013). These findings suggested that triterpenoids may be contributed to CP's hypoglycemic action by regulating AMPK activation, but its principles and underlying mechanisms are still not fully clear. Therefore, the current study was designed to investigate the effects of CPEC and its two specific triterpenoids (cyclocaric acid B (CA-B) and cyclocarioside H (CS-H)) (Fig. 1) on the regulation of glucose disposal, and investigate the underlying mechanisms under inflammatory conditions.

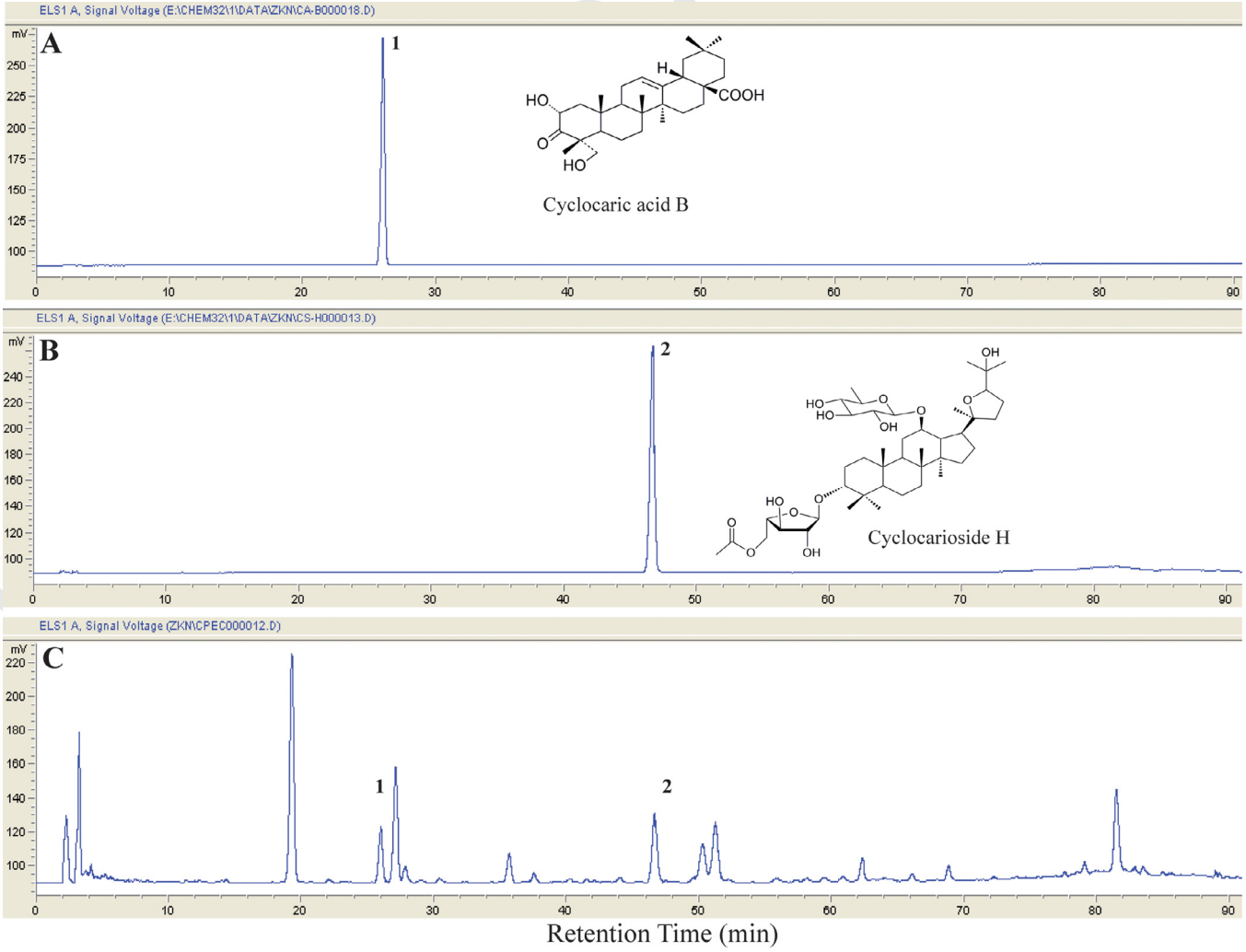

Fig. 1. HPLC-ELSD chromatograms of Cyclocaric acid B (A), Cyclocarioside H (B) and CPEC (C).

Please cite this article as: K.N. Zhu et al., Two triterpeniods from *Cyclocarya paliurus* (Batal) Iljinsk (Juglandaceae) promote glucose uptake in 3T3-L1 adipocytes: The relationship to AMPK activation, Phytomedicine (2015), http://dx.doi.org/10.1016/j.phymed.2015.05.058