

THE EFFECTS OF EVODIAMINE ON SERUM TOTAL CHOLESTEROL AND TRIGLYCERIDE LEVELS ARE ASSOCIATED WITH THE ACTIVATION OF THE AMPK SIGNALING PATHWAY IN RATS WITH HYPERLIPEMIA

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Abstract: Evodiamine, a naturally occurring indole alkaloid, has been reported to have numerous biological activities, including antitumor, antimicrobial and anti-inflammatory effects. Previous studies also suggest that evodiamine prevents obesity. In this study, we confirmed that evodiamine lowered the levels of serum total cholesterol (TC) and triglycerides (TG) in rats with hyperlipemia. Furthermore, our findings suggest that the activation of the AMP-activated protein kinase (AMPK) pathway might contribute in part to the effect of evodiamine on the serum levels of TC and TG.

Key words: evodiamine; AMP-activated protein kinase; total cholesterol; triglycerides

INTRODUCTION

Evodiamine is a bioactive compound present in the fruit of *Evodia rutaecarpa* (Juss.) Benth [1]. Initially, it was identified as a vanilloid receptor 1 (TRPV1) agonist, which possesses a property that can be utilized to modulate pain [2]. To date, three proteins are believed to be direct targets of evodiamine, including TRPV1, the aryl hydrocarbon receptor (AhR) and topoisomerases [3-5]. These proteins are apparently important in inflammation, cancer and other diseases. In fact, many other pharmacological properties of evodiamine have been uncovered, such as antitumor, antimicrobial, and anti-inflammatory activities [6].

Previous studies found that evodiamine has anti-obesity effects and possible mechanisms were proposed. Part of the anti-obesity effects of evodiamine was thought to be via enhancement of uncoupling protein-1 (UCP1) thermogenesis through β 3-adrenergic stimulation [7]. It was found that [8] that

evodiamine inhibited neuropeptide Y (NPY) mRNA and peptide levels in the arcuate nucleus (ARC) of the hypothalamus, which might be one of the mechanisms by which evodiamine exerted its anti-obesity effects. It was also reported [9] that the ERK/MAPK signaling pathway might contribute to the fat-loss effects of evodiamine. Moreover, evodiamine increased the plasma concentration of cholecystokinin (CCK), which is involved in the regulation of digestion and appetite [10].

AMP-activated protein kinase (AMPK), a key cellular energy sensor, plays a critical role in the regulation of lipid metabolism [11]. Several upstream kinases have been reported to activate AMPK, including liver kinase B 1 (LKB1) [12]. LKB1 is predominately localized in the nucleus under normal physiological conditions and is translocated to cytosol in response to stimulation, which leads to subsequent AMPK phosphorylation and activation [13]. When activated, AMPK decreases fatty acid levels by phosphorylating

and thus inhibiting acetyl-CoA carboxylase (ACC), a critical enzyme for controlling fatty acid biosynthesis and oxidation [14]. The activation of AMPK also decreases total cholesterol (TC) and triglyceride (TG) levels by inhibiting the activity of glycerol-3-phosphate acyltransferase (GPAT) and HMG-CoA reductase, respectively [15]. AMPK has therefore been proposed as a major therapeutic target for obesity and obesity-linked metabolic disorders such as hyperlipidemia and atherosclerosis [16]. 5-Aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR) is one of the activators of AMPK [17].

It was been reported that evodiamine could activate AMPK [18]. Also, evodiamine has been recognized to improve glucose tolerance and prevent the progress of insulin resistance through AMPK activation followed by inhibition of mTOR-S6K signaling and IRS1 serine phosphorylation in adipocytes [19]. However, the effects of evodiamine-mediated AMPK activation on lipid metabolism remain unclear.

Our previous studies suggested that cordycepin acted as an AMPK agonist to regulate lipid metabolism [20]. Continuing our investigation in this field, the present study investigated the effects of evodiamine on the levels of serum TC and TG in rats with hyperlipemia. We further explored whether the effects of evodiamine on lipid metabolism are mediated, at least in part, by its activation of the AMPK signaling pathway.

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats (weight 120-150 g) were purchased from Vital River Lab Animal Technology Co., Ltd (Beijing, China). All rats were randomly divided into 6 groups: control group, model group, evodiamine high-dose group (HD group, 40 mg/kg), evodiamine middle-dose group (MD group, 20 mg/kg), evodiamine low-dose group (LD group, 10 mg/kg) and AICAR group (n=8 for each group). The control group was fed with a normal diet throughout the experiment.

The model and other 5 drug-treated groups were fed with a high-fat diet (i.e., the normal diet supplemented with 8 % axunge, 1% cholesterol and 0.25% sodium taurocholate) to induce hypercholesterolemia for 4 weeks. For the next 2 weeks, evodiamine was administered to treated animals at the designed dosage. The AICAR dose was 500 mg/kg. Body weights were recorded every 48 h during the experiment. At the end of the experimental period, the rats were fasted overnight. Animal studies were performed under conditions approved by the Local Animal Care and Use Committee.

Biochemical analysis

Blood samples were collected from the marginal ear vein of each rat. The serum was separated for the estimation of TC and TG levels using commercially available kits (Biosino Biotechnology Company Ltd, Beijing, China) as described previously [21].

Histological analyses

The animals in each group were sacrificed by intraperitoneal pentobarbital overdose followed by immediate exsanguination. The livers were removed, immersed in 10% (v/v) formaldehyde in neutral buffer solution, dehydrated, cleared and embedded in paraffin. Paraffin sections (4 μ m thick) underwent hematoxylin and eosin (H&E) staining. All observations were done with a fluorescence microscope (Olympus, Tokyo, Japan).

Western blot

The expression levels of AMPK, ACC and LKB1 were determined by Western blot analysis. The protein samples were subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE), and the separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Sangon Biotech, Co., Ltd., Shanghai, China). The relative amounts of proteins and the extent of phosphorylation were estimated using the following primary antibodies: anti-phospho-AMPK, anti-AMPK, anti-phospho-ACC, anti-ACC, anti-phospho-LKB1, anti-LKB1, and anti-

β -actin (1:1000 dilution, Cell Signaling Technology, Danvers, MA, USA), following incubation with horseradish peroxidase-conjugated secondary antibodies (Sangon Biotech, Co., Ltd., Shanghai, China). Blots were visualized using enhanced chemiluminescence substrate (ECL, Sangon Biotech, Co., Ltd., China)

Statistical analysis

Statistical analysis was performed using SPSS15.0. Multiple groups were compared using one-way ANOVA, followed by the least significant difference test. A *P* value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effects of evodiamine on body weights of rats

Evodiamine is a known bioactive compound with anti-obesity effects. In the present study, we assessed whether evodiamine does indeed contribute to the fat-loss effects in rats with hyperlipemia. As shown in Fig. 1A, the body weights of rats in the model group were significantly higher than those of the control group ($P < 0.05$). The body weights in rats administrated with evodiamine at dosages of 20 and 40 mg/kg (MD and HD groups) were significantly decreased compared to the model rats, demonstrating that evodiamine is an alkaloidal compound with anti-obesity effects. This was consistent with previous results [7-10]. As a positive control, AICAR treatment also significantly decreased the body weights in rats with hyperlipemia ($P < 0.05$). However, there were no significant differences in body weight between the LD and model groups ($P > 0.05$).

Effects of evodiamine on the levels of serum TC and TG

We further investigated whether evodiamine affected the levels of serum TC and TG in rats with hyperlipemia. The levels of TG and TC were measured *in vivo* using AICAR as a positive control. The lipid profiles

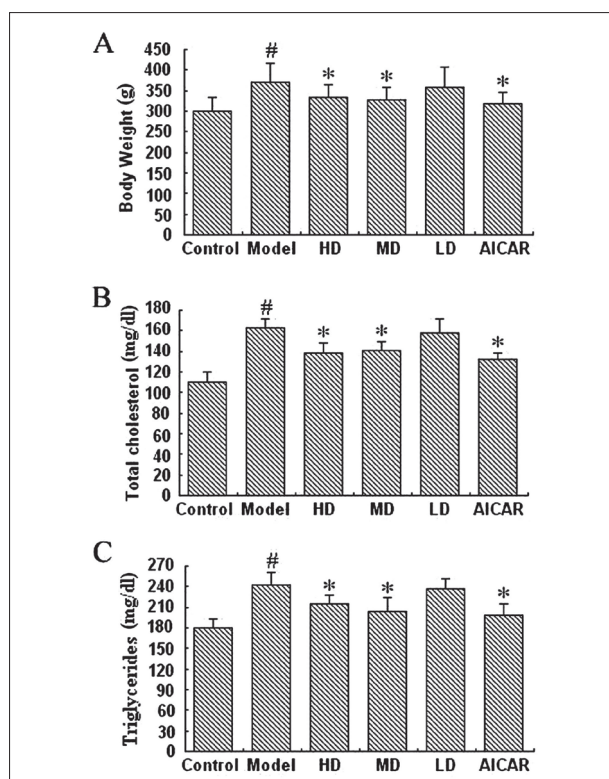


Fig. 1. Effects of evodiamine on the body weight (A) and levels of serum TC (B) and TG (C) in rats with hyperlipemia. * $p < 0.05$ vs control, [#] $p < 0.05$ vs model.

of the serum (TG, TC) of rats from all groups are summarized in Fig. 1B and 1C. The rats in the model group showed markedly higher serum TG and TC levels than those in the control group ($P < 0.05$). Evodiamine administered in doses of 20 and 40 mg/kg to rats with hyperlipemia showed a reduction in the levels of TC and TG, demonstrating that evodiamine significantly decreased the contents of serum TC and TG in rats with hyperlipemia. Similar effects were observed after treatment with AICAR, as shown in Fig. 1B and 1C. However, there is no statistically significant in the levels of TC and TG between the LD and model groups ($P > 0.05$).

Effects of evodiamine on the adipose tissue deposition in the liver of rats

The liver is thought to be the primary organ responsible for lipid metabolism. In the present study, stain-

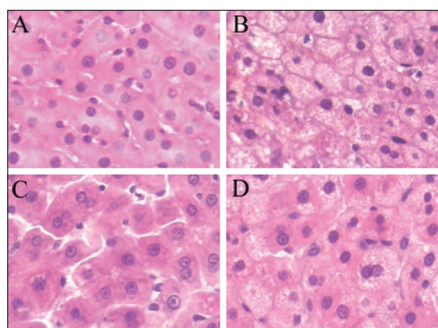


Fig. 2. Hepatic lipid deposition in the four experimental groups. **A** – control; **B** – Model; **C** – HD; **D** – AICAR. All the photomicrographs show H&E staining. Original magnification $\times 400$.

ing liver sections with H&E showed apparent lipid deposition in the livers of rats in the model group. The marked accumulation of lipid vesicles was observed in the hepatocytes in liver sections (Fig. 2B). Treatment with 20 mg/kg of evodiamine substantially decreased lipid accumulation in the liver of rats with hyperlipemia, as evidenced by H&E evaluation (Fig. 2C). Histologic analyses suggest that evodiamine helped maintain normal fat deposition in the liver when rats were fed with a high-fat diet.

Effects of evodiamine on the phosphorylation of AMPK and ACC

It is widely accepted that the overall function of the AMPK cascade is to control metabolism in response to variations in the energy status of the cell [22]. As a downstream target for AMPK, ACC is phosphorylated by AMPK, which leads to the inhibition of fatty acid synthesis. To gain insight into the mechanism by which evodiamine regulates the contents of serum TC and TG, phosphorylation of AMPK and ACC in evodiamine-treated animals was measured. As shown in Fig. 3A, the phosphorylation of AMPK was significantly stimulated by evodiamine at the dosage of 20 mg/kg, but the expression of the total AMPK was not changed. We also observed that phosphorylation of ACC was significantly stimulated, but the expression of endogenous ACC protein was not changed (Fig. 3B). Previous studies showed that evodiamine could influence lipid metabolism through the regulation of the expressions of its key genes, as well as reduce body

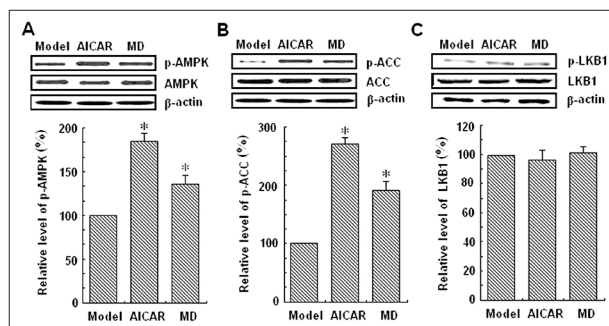


Fig. 3. The effects of evodiamine on the AMPK pathway. Evodiamine increases the phosphorylation levels of AMPK (**A**) and ACC (**B**), and does not significantly affect the dephosphorylation level of LKB1 (**C**). $^*p < 0.05$ vs the model.

heat and body weight [23]. In this study, our results further suggest that the AMPK pathway is involved in evodiamine-mediated TC and TG metabolism of rats with hyperlipemia.

Effects of evodiamine on the dephosphorylation of LKB1

LKB1 is an upstream regulator of the AMPK pathway, and the activation of AMPK leads to the phosphorylation of downstream targets, such as ACC [24]. Recently, Sid et al. [25] reported that folic acid supplementation may improve cholesterol and glucose metabolism by restoring AMPK activation, which was mediated through an elevation of its allosteric activator AMP and activation of its upstream kinase LKB1 in the liver. To determine whether LKB1 is required for the activation of AMPK by evodiamine, the dephosphorylation of LKB1 was monitored in rats treated with 20 mg/kg of evodiamine. The results indicated that evodiamine treatment had no effect on LKB1 dephosphorylation (Fig. 3C), suggesting that evodiamine treatment increased the phosphorylation of ACC and AMPK in a LKB1-independent manner.

In conclusion, our results confirm that evodiamine does indeed influence lipid metabolism *in vivo*. Our present study also provides the first evidence that the AMPK pathway may mediate, at least in part, the effects of evodiamine on lipid metabolism in a LKB1-independent manner.

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Conflict of interest disclosure: The authors declare no conflicts of interest.

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