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Research paper

In vitro effects of active components of Polygonum Multiflorum Radix on enzymes involved in the lipid metabolism

Wangen Wang, Yanran He, Pei Lin, Yunfei Li, Ruifen Sun, Wen Gu, Jie Yu *,[1], Ronghua Zhao **,[1]

Yunnan University of Traditional Chinese Medicine, Kunming 650500, Yunnan Province, China

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ABSTRACT

Ethnopharmacology relevance: Raw and processed Polygoni Multiflori Radix (PMR and PMRP) are used in the prevention and treatment of non-alcoholic fatty liver disease (NAFLD), hyperlipidemia or related diseases. In our previous research, 2, 3, 5, 4′-tetrahydroxy-stilbene-2-*O*-*β*-D-glucoside (TSG) displayed the most important role in the total cholesterol (TC) lowering effect among all the chemical constituents of *Polygonum multiflorum*. Emodin and physcion displayed more favorable triglyceride (TG) reducing effects than TSG. However, there are few researches focus on the approach and mechanism of how do *Polygonum multiflorum* exhibit good lipid regulation activity. The targeted sites of active substances of *Polygonum multiflorum* are still not clearly elucidated. This research pays close attention to how major chemical components of *Polygonum multiflorum* affect the TC and TG contents in liver cells.

Materials and methods: In this research, a sensitive, accurate and rapid *in vitro* model, steatosis hepatic L02 cell, was used to explore target sites of active chemical substances of *Polygonum multiflorum* for 48 h. Steatosis hepatic L02 cell was exposed to emodin, physcion and TSG, respectively. The contents of four key enzymes in the pathway of synthesis and decomposition of TC and TG were investigated after exposure. Meanwhile, the contents of lipid transfer protein were also tested. The diacylgycerol acyltransferase 1 (DGAT1) controlled the biosynthesis of TG from free fatty acids while 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) limited the biosynthesis of TC. Hepatic triglyceride lipase (HTGL) and cholesterol 7α-hydroxylase (CYP7A) played the key role in the lipolysis procedure of TG and TC.

Results: The synthesis of TC and TG in steatosis L02 cells were apparently increased in the model group compared to the control group. Intracellular contents of HMG-CoA reductase and DGAT1 increased 32.33% and 56.52%, while contents of CYP7A and HTGL decreased 21.61% and 47.37%. Emodin, physcion and TSG all showed down-regulation effects on HMG-CoA reductase, while up-regulation effects on CYP7A. The most remarkable effect on HMG-CoA reductase was found on emodin. Emodin could reduce the DGAT1 content from 438.44 ± 4.51 pg/mL in model group to 192.55 ± 9.85 pg/mL (100 μm). The content of HTGL in 300 μm physcion group was 3.15 ± 0.15 U/mL, which was more significantly effective than the control, lovastatin and fenofibrate group.

Conclusions: TSG could raise the content of CYP7A and then promote the lipolysis of cholesterol. Moreover, TSG also showed the best LDL-reducing effect. Emodin could inhibit HMG-CoA reductase and DGAT1, which were key enzymes in the synthesis of TC and TG. Physcion increased the content of HTGL, and then could boost the lipolysis of triglyceride. At the same time, physcion showed the best VLDL-

Abbreviations: Acetyl-CoA, acetyl coenzyme A; Apo E, apolipoprotein E; CA, cholic acid; CDCA, chenodeoxycholic acid; CYP7A, cholesterol 7-alpha-hydroxylase or cytochrome P450 7A; DAG, diacylglycerol; DGAT 1, diacylgycerol acyltransferase 1; FA, fatty acid; FBS, fetal bovine serum; Glycerol-3P, glycerol-3-phosphate; HDL-C, high density lipoprotein cholesterol; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-CoA reductase; HTGL, hepatic triglyceride lipase; IDL, intermediate density lipoprotein; LDL-C, low density lipoprotein cholesterol; LDLR, low density lipoprotein receptor; LXR, liver X receptor; MAG, monoacylglycerol; MVA, mevalonate; NAFLD, non-alcoholic fatty liver disease; PBS, phosphate buffer saline; PMR, Polygoni Multiflori Radix; PMRP, Polygoni Multiflori Radix Praeparata; RPMI 1640, Roswell Park Memorial Institute medium 1640; SREBP, sterol regulatory element-binding proteins; TC, total cholesterol; TG, triglyceride; TSG, 2, 3, 5, 4′-tetrahydroxy-stilbene-2-*O*-*β*-D-glucoside; VLDL-C, very low density lipoprotein cholesterol

* Corresponding author. Tel.: +86 15887251422.
** Corresponding author. Tel.: +86 13888074508.
E-mail addresses: cz.yujie@gmail.com (J. Yu), kmzhaoronghua@hotmail.com (R. Zhao).
[1] These authors contributed equally to this work.

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reducing effect. In view of the above conclusions, we contributed the lipid regulation activity to an overall synergy of TSG, emodin and physcion.

1. Introduction

Lipid metabolism plays important role in the life activities, which is closely related to the occurrence of coronary heart disease, cerebral apoplexy, obesity and other major cardiovascular and metabolic diseases. With the rapid improvement of people's living standards and huge changes of lifestyle, lipid metabolism abnormality related diseases have extremely high prevalence all around the world. On account of the effectiveness and more acceptable prices, the prevention and treatment of NAFLD and hyperlipidemia by traditional Chinese medicine attract more and more attention worldwide (Chen et al., 2011; Guo et al., 2011).

Polygoni Multiflori Radix (PMR, Heshouwu in Chinese) and Polygoni Multiflori Radix Praeparata (PMRP, Zhiheshouwu in Chinese) (shown in Fig. 1), originated from the root of *Polygonum multiflorum* Thunb., are applied in the treatment of NAFLD and hyperlipidemia in oriental counties for centuries (Commission of Chinese Pharmacopoeia, 2010). *Polygonum multiflorum* ranks the fifth most frequently used crude drugs in prevention and treatment of hyperlipidemia and NAFLD (Zhang and Chen, 2007). Extract from *Polygonum multiflorum* Thunb (Wang et al., 2006) and 2,3,5,4′-tetrahydroxy-stilbene-2-*O*-*β*-D-glucoside (TSG) could inhibit cholesterol synthesis enzyme and elevate low density lipoprotein receptor (LDLR) expression. Water extract and the total glycosides of *Polygonum multiflorum* (Fang et al., 2005) show good lipid-lowering activity in several animal experiments. They could not only reduce the contents of the TG and TC in hyperlipidemia rats caused by high fat diet but also had good effects in hyperlipidemia rats induced by the intraperitoneal injection of Triton and hyperlipidemia rats caused by lack of Apo E gene.

Our research group has focused on the lipid regulation effects of *Polygonum multiflorum* for decades. In our previous research, we have confirmed the profound lipid reducing effects of *Polygonum multiflorum* by both *in vitro* (Wang et al., 2012) and *in vivo* (Li et al., 2012) assays. Moreover, the differences between lipid regulation effects of PMR and PMRP have also been clarified in these researches. PMR showed more pronounced effects on lipid regulation in liver samples for the treatment of early-stage NAFLD. PMRP, however, displayed better effects in regulating lipids in circulating blood for the treatment of hyperlipidemia.

However, the lipid regulation mechanisms of *Polygonum multiflorum* are still not clearly elucidated. In addition, the activities comparison between TSG and anthraquinones have not been detailed researched, although both stilbene (mainly 2,3,5,4-tetrahydroxystilbene-2-*O*-*β*-D-glucoside, TSG) (Gao et al., 2007; Luo et al., 2008) and anthraquinones (mainly emodin and physcion) (Dong et al., 2005; Li et al., 2008; Zhao et al., 2009) show some lipid metabolism regulation activity. So we chose the following key enzymes and transporters as the investigation objectives.

In this research, a sensitive, accurate and rapid *in vitro* model was used to explore the target sites of major chemical substances of *Polygonum multiflorum*. L02 hepatocytes, normal human hepatic parenchymal cell, were exposed to medical fat emulsion to achieve intracellular lipid accumulation model. This cell line could maintain characteristics and ultrastructure of normal liver cells after continuous passage (Ye et al., 1980; Li et al., 2007). L02 cell line had been shown to express many specific liver cell functions and could be used in many fields such as activity (Liu et al., 2006; Jin et al., 2009; Liu and Zhang, 2009; Chen et al., 2010; Ye et al., 2011), toxicity (Wang et al., 2008; Yuan et al., 2009) and artificial liver research (Bao-san et al., 2008). The steatosis L02 cells were widely used in the lipid metabolism regulation researches (Gomez-Lechon et al., 2007; Shen et al., 2008; Pan et al., 2010; Zhang et al., 2011), especially for traditional Chinese medicine and their active compounds, such as saponin (Zhang et al., 2011), ursolic acid (Shen et al., 2008) and curcumin (Pan et al., 2010). This steatosis L02 cells were also used to investigate the activities of PMR and PMRP extractions in our previous researches (Wang et al., 2012).

We focus on the effects of TSG, emodin and physcion on the lipogenesis and lipolysis procedure in this research. Lipogenesis is the process by which acetyl-CoA is converted to fatty acids. Lipolysis is the breakdown of lipids and involves hydrolysis of triglycerides into glycerol and free fatty acids.

3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), cholesterol 7-alpha-monooxygenase (CYP7A), diacylgycerol acyltransferase 1(DGAT 1), hepatic triglyceride lipase (HTGL) and three lipoproteins are our research objectives.

HMG-CoA reductase is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. Competitive inhibitors of this reductase induce the expression of low density lipoprotein (LDL) receptors in the liver, which in turn increases the catabolism of plasma LDL and lowers the plasma concentration of cholesterol, an important determinant of atherosclerosis. This enzyme is thus

PMR **PMRP**

Fig. 1. Polygoni Multiflori Radix (PMR) and Polygoni Multiflori Radix Praeparata (PMRP).