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Inhibitory Activity of Diacylglycerol Acyltransferase by Tanshinones from the Root of *Salvia miltiorrhiza*

Jeong Suk Ko[1], Shi Young Ryu[2], Young Sup Kim[2], Mi Yeon Chung[1], Jong Seong Kang[3], Mun-Chual Rho[1], Hyun Sun Lee[1], and Young Kook Kim[1]

[1]Cardiovascular Research Laboratory, Korea Research Institute of Bioscience and Biotechnology, P. O. Box 115, Yusong, Taejon 305-600, Korea, [2]Korea Research Institute of Chemical Technology, Yusong P. O. Box 107, Taejon 305-600, Korea, and [3]College of Pharmacy, Chungnam National University, Taejon 305-764, Korea

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The inhibitory activity of tanshinones from *Salvia miltiorrhiza* was tested on rat liver diacylglycerol acyltransferase (DGAT). Cryptotanshinone (**1**) and 15,16-dihydrotanshinone I (**3**) exhibited potent DGAT inhibitory activities dose-dependently with IC_{50} values of 10.5 μg/ml and 11.1 μg/ml. However, tanshinone IIA (**2**) and tanshinone I (**4**) showed very weak inhibition (IC_{50} value: > 250 μg/ml). A dihydrofuran moiety was seemed to be responsible for the stronger inhibitory activity

Key words: *Salvia miltiorrhiza*, Labiatae, Tanshinones, Diacylglycerol acyltransferase

INTRODUCTION

Triacylglycerols are the most important storage form of energy for eukaryotic cells, and triacylglycerol synthesis plays an important metabolic role in the intestine, liver, mammary gland, and adipose tissue. But high triglyceride levels is known to be a major risk factor for coronary heart disease, obesity and hypertriglyceridemia

Acyl CoA:diacylglycerol acyltransferase (DGAT) is a microsomal enzyme that plays a central role in the metabolism of cellular glycerolipids and has been recently cloned (Cases *et al.*, 1998; Lehner *et al.*,1996). DGAT catalyses the reaction of acyl residue transfer from acyl-CoA to diacylglycerol to form triacylglycerol in the final step of the glycerol-phosphate pathway of triglyceride synthesis. The enzyme is also believed to catalyze the final step of the monoacylglycerol pathway, which is important in intestinal fat absorption, found predominantly in enterocytes of the small intestine (Lehner *et al.*, 1996). Therefore, DGAT inhibition may be worthwhile strategy for the treatment of triglyceride metabolism disorders, such as obesity or hypertriglyceridemia (Smith *et al.*, 2000; Gray *et al.*, 2000). From this point of view, this paper deals with DGAT inhibitory effects of tanshinones isolated from the roots of *Salvia miltiorrhiza*.

Correspondence to: Hyun Sun Lee, Cardiovascular Research Laboratory Korea Research Institute of Bioscience and Biotechnology P. O. Box 115, Yusong ,Taejon 305-600, Korea
E-mail: leehs@kribb.re.kr

MATERIALS AND METHODS

Materials

Tanshen, the roots of *Salvia miltiorrhiza* B. was obtained from Chien Yuan Medicinal Co., Taipei, Taiwan and was identified by the Herbarium of Natural Products Research Institute of Seoul National University, where a voucher specimen (SNU-9-412) is deposited. Bovine serum albumin and *sn*-1,2-dioleoylglycerol were obtained from Sigma Chemical Co. [^{14}C]palmitoyl CoA was purchased from Amersham.

Extraction and isolation

Four tanshinones were isolated from the dried roots of *S. miltiorrhiza* as reported previously (Ryu *et al.*, 1997). Their structures were determined by physicochemical and spectral data and identified as cryptotanshinone (**1**), tanshinone IIA (**2**), 15,16-dihydrotanshinone I (**3**) and tanshinone I (**4**) (Fig. 1).

Cryptotanshinone (**1**): $C_{19}H_{20}O_3$ (Mw: 296) orange needles in hexane; m.p. 191°C. Tanshinone IIA (**2**): $C_{19}H_{18}O_3$ (Mw: 294) orange needles in EtOAc; m.p. 205°C. 15,16-Dihydrotanshinone I (**3**): $C_{18}H_{14}O_3$ (Mw: 278) reddish brown needles in MeOH; m.p. 201°C.

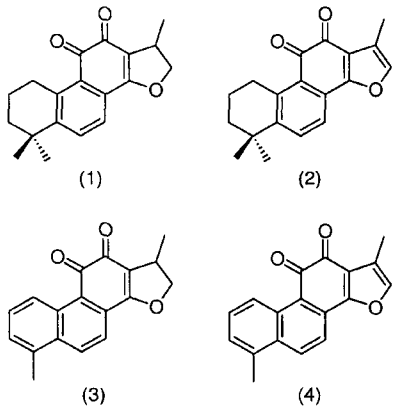

Fig. 1 Structures of tanshinones isolated from the root of *S. miltiorrhiza*

Tanshinone I (**4**): $C_{18}H_{12}O_3$ (Mw: 276) reddish brown needles in MeOH; m.p. 230°C.

Preparation of microsome from rat liver (Coleman *et al.*, 1992)

Rat livers (Male Sprague-Dawley rat, 250-300 g) were minced and then homogenized in 9 volumns of STE buffer (0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 1.0 mM EDTA) with a Teflon-glass homogenizer by 10 up-and-down strokes at medium speed. The homogenate was centrifuged at 14,000 g for 20 min at 4°C. The supernatant was centrifuged at 100,000 g for 1 hr at 4°C to obtain microsomal pellet. The pellet was suspended in STE buffer without EDTA and centrifuged at 100,000 g for 1 hr at 4°C. The final pellet was resuspended in STE buffer without EDTA. The microsomal fractions of rat livers were prepared and aliquots were stored at -70 °C.

Diacylglycerol acyltransferase assay

DGAT activity was measured as reported previously (Coleman *et al.*, 1992) with some modification. In brief, the reaction mixture, containing 175 mM Tris-HCl (pH 8.0 , 8.0 mM $MgCl_2$, 0.2 mM *sn*-1,2-diacylglycerol, 0.25 mg of fatty acid free bovine serum albumin, 30 mM [1-^{14}C]palmitoyl-CoA (0.02 μCi) in total volumn of 200 μl, was initiated by the addition of rat river microsomal fraction, followed by vortexing gently and briefly. After incubation for 10 min at 25°C the reaction was stopped by the addition of 1.5 ml of 2-propanol-heptane-water (80: 20: 2, v/v) and one milliliter of heptane and 0.5 ml of water to extract lipid. After vortexing 1.2 ml of the organic phase was transferred to a glass tube and washed once with 2.0 ml of alkaline ethanol solution [ethanol-0.5 N NaOH-water (50 10: 40, v/v)] and then the amount of radioactivity was determined in a liquid scintillation counter.

Table I. Inhibitory effects of tanshinones from the root of *Salvia miltiorrhiza* on rat liver diacylglycerol acyltransferase activities

Compounds	IC_{50} (μg/ml)
Cryptotanshinone (**1**)	10.5
Tanshinone IIA (**2**)	>250
15,16-Dihydrotanshinone I (**3**)	11.1
Tanshinone I (**4**)	>250

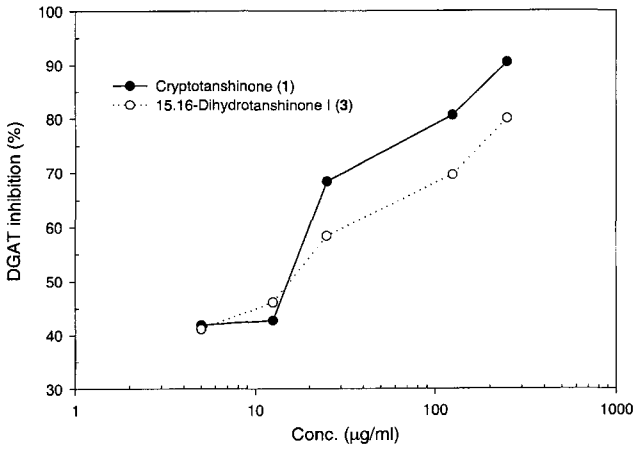

Fig. 2. DGAT inhibition by tanshinones from the root of *S. miltiorrhiza* in rat liver microsomes

RESULTS AND DISCUSSION

In the course of our search for DGAT inhibitors from natural products, it was observed that the methanol extract of the root of *S. mitiorrhiza* showed potent inhibition of DGAT prepared from the liver membrane of male Sprague-Dawley (SD) rats. Dried root of *S. miltiorrhiza* Bunge (Labiatae, Tan-shen in Chinese) has been commonly used in traditional Chinese medicine for the treatment of coronary heart disease, particularly angina pectoris and myocardial heart infarction. It has also been applied to circulatory disorders such as amenorrhea, dismenorrhea, and insomnia (Chang *et al.*, 1986). In previous study, we isolated eighteen abietane diterpenes (tanshinones) from the root of this plant, which showed a significant cytotoxicity against cultured human tumor cell lines (IC_{50} values ranged from 0.2 to 8.1 μg/ml).

Recently, the generation of DGAT-deficient mice has provided a better understanding of triglyceride synthesis and its relationship to obesity. Further, the obesity resistance, increased energy expenditure, and apparently improved glucose metabolism associated with DGAT deficiency suggest that DGAT inhibition may be worthwhile strategy for the treatment of triglyceride metabolism disorders, such as obesity or hypertriglyceridemia (Smith *et al.*, 2000). Although there are currently no known synthetic

inhibitors of DGAT, several naturally occurring compounds have been reported to inhibit DGAT activity. Amidepsin A-D (*Humicola* sp. FO-2942, IC_{50}; 10.2-51.6 μM), xanthohumol and xanthohumbol B (*Humulus lupulus*, IC_{50}; 50.3 μM and 194 μM), roselipins (*Gliocladium roseum* KF-1040, IC_{50}; 15.0-22.0 μM) have been reported as DGAT inhibitors (Tomoda, *et al.*, 1999; Tabata *et al.*, 1997; Tomoda *et al.*, 1995). Therefore, the inhibitory effects of four tanshinones (**1-4**) were tested on rat liver DGAT activity. As shown in Table 1, the cryptotanshinone (**1**) and 15,16-dihydrotanshinone I (**3**) exhibited potent DGAT inhibitory activities dose-dependently with IC_{50} values of 10.5 μg/ml and 11.1 μg/ml (Fig. 2). However, tanshinone IIA (**2**) and tanshinone I (**4**) showed very weak inhibition (IC_{50} value: > 250 μg/ml). Structure-activity relationships were observed in that the compounds with a dihydrofuran moiety were found to be more potent than the corresponding compounds with a furan moiety and a dihydrofuran moiety was seemed to be responsible for the stronger inhibitory activity.

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