Anti-atherosclerotic Activity of Triacsin C, an Acyl-CoA Synthetase Inhibitor

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Abstract As previously reported, triacsin C, a selective inhibitor of acyl-CoA synthetase, inhibited the synthesis of cholesteryl ester and triacylglycerol in mouse peritoneal macrophages, leading to a reduction of lipid droplets. Therefore, the *in vivo* efficacy was studied. Low-density lipoprotein receptor-knockout (LDLR^{-/-}) mice were fed a high cholesterol diet (0.15%) for two months to measure the atherogenic areas of the hearts and aortas. When triacsin C was orally administered (10 mg/kg/day), the atherosclerotic areas were significantly reduced by 86% in aorta and 36% in hearts. The results strongly suggested that triacsin C shows anti-atherogenic activity by inhibiting acyl-CoA synthetase activity.

Keywords acyl-CoA synthetase, atherosclerosis, *in vivo*, low-density lipoprotein receptor-knock out mice, triacsin C

Introduction

In the early stage of atherosclerosis, macrophages penetrate into the intima, efficiently take up modified low density lipoprotein (LDL), store cholesterol and fatty acid as the respective forms of cholesteryl ester (CE) and triacylglycerol (TG) in cytosolic lipid droplets, and are converted to foam cells, leading to the development of atherosclerosis in the arterial wall. Therefore, inhibitors of

macrophage lipid droplet formation would be expected to retard the progression of atherosclerosis [1–4].

A cell-based assay was established to observe lipid droplet formation using primary mouse peritoneal macrophages [5] as a model of macrophage-derived foam cell formation in the artery. When macrophages are cultured with phosphatidylserine-containing liposomes, the liposomes are recognized by scavenger receptors and metabolized in a way similar to the natural ligand-modified LDL [6], resulting in the accumulation of lipid droplets in the cytosol. In the morphological assays, lipid droplet formation was observed microscopically after staining lipid droplets with oil red O. In the biochemical assay, the amounts of [¹⁴C]CE and [¹⁴C]TG produced in the lipid droplets were measured by the addition of [¹⁴C]oleic acid to the culture medium.

Taking advantage of this model, we found that triacsins A and C, well-known inhibitors of long chain acyl-CoA synthetase (ACS), inhibited the synthesis of [¹⁴C]CE (IC₅₀ values; 0.18 and 0.19 μM, respectively) and [¹⁴C]TG (IC₅₀ values; 0.16 and 0.10 μM, respectively), and caused the complete inhibition of lipid droplet synthesis in mouse macrophages [7]. Triacsins A to D were isolated from the culture filtrate of *Streptomyces* sp. SK-1894 and consisted of 11-carbon alkenyl chains with a common triazenol moiety at their termini [8, 9]. On the basis of its unique target of inhibition, triacsins have been utilized in many

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studies to investigate the function of ACS in lipid metabolism [5, 10–13]. These findings prompted us to study the *in vivo* efficacy of triacsins in an atherosclerogenic mouse model. In this study, we show anti-atherosclerotic activity of triascin C in LDLR^{-/-} mice.

Materials and Methods

Materials

Triascin C (Fig. 1) was purified from a culture broth of *Streptomyces* sp. SK-1894 by an established method [9]. Oil red O was purchased from SIGMA. Sudan IV was obtained from Wako Pure Chemical Industries (Osaka, Japan). Low-density lipoprotein receptor-knockout (LDLR^{-/-}) mice on a C57BL/6 background were purchased from The Jackson Laboratory (ME, USA).

In Vivo Anti-atherosclerotic Activity

LDLR^{-/-} mice were treated as previously described [14]. Eight-week old mice were fed 0.15% cholesterol-supplemented diets (CLEA Japan, Tokyo, Japan), and 0.05% sodium carboxy methyl cellulose with or without triascin C (10 mg/kg of body weight) was administered orally every day for two months. Blood was collected from the retroorbital venous plexus at zero, one, and two months. The levels of glucose, total cholesterol, TG, and free fatty acid in plasma were measured using an Advantage II (Roche Diagnostics), Determiner TC555 kit (Kyowa Medex, Tokyo, Japan), Triglyceride G-test kit (Wako Pure Chemicals, Osaka, Japan), and NEFA C-test kit (Wako Pure Chemicals), respectively.

For atherosclerotic lesion analyses, whole aortas were stained with Sudan IV, and cross sections of the proximal aorta were prepared and stained with oil red O. The hearts were perfused with phosphate-buffered saline containing 3.0% formalin, were embedded in OCT compound (Sakura Tissue-Tek, Tokyo, Japan), and 6- μ m-thick serial sections were cut by using a Cryostat (Leica, Tokyo, Japan). Four sections, each separated by 60 μ m, were counter-stained with oil red O and hematoxylin and used to evaluate the lesions. Images of the sections were captured with a digital camera (CoolSnap Photometrics, Olympus, Tokyo, Japan) mounted on a light microscope (Vanox-S model, Olympus). Image analysis was performed by using Photoshop 6.0 software (Adobe).

Fig. 1 Structure of triascin C.

Results and Discussion

After two-month feeding with a high cholesterol diet, LDLR^{-/-} mice developed atherosclerotic lesions in the aortas and the hearts (Fig. 2A and 2C, respectively). The atherosclerotic area was calculated to be 6.27% in the aortas and 10.2 μ m² in the hearts. When triascin C was administered orally (10 mg/kg/day) for two months, the atherosclerotic areas decreased to 0.90% ($P < 0.05$) in the aortas (Fig. 2B) and to 6.5 μ m² ($P < 0.05$) in the hearts (Fig. 2D), indicating 86% (Fig. 2E) and 36% reduction (Fig. 2F), respectively. The administration concentration of triascin C

Fig. 2 Effect of triascin C on aortic atherosclerosis of LDLR^{-/-} mice.

LDLR^{-/-} mice were fed 0.15% cholesterol-supplemented diets with or without triascin C (10 mg/kg/day) for two months ($n=10$, each group). (A and B) Pinned-out aortas showing Sudan-IV-stained lesions from LDLR^{-/-} mice that received 0.05% sodium CM-cellulose containing triascin C (10 mg/kg/day orally for two months) (B) and only 0.05% sodium CM-cellulose (control) (A). (C and D) Cross sections of proximal aortic roots of hearts showing oil-red-O-staining lesions in LDLR^{-/-} mice treated with triascin C (D) and control (C). Comparison of the size of whole aorta surface (E) for A and B, and cross-sectional (F) lesions for C and D between the control and triascin C-treated groups. Arrows indicate the atherosclerotic lesion. Bars indicate mean and error bars represent $\pm$ SD; *, $P < 0.05$.

Fig. 3 Effect of triacsin C on plasma TG, plasma cholesterol, plasma free fatty acid, and plasma glucose concentration.

Plasma concentration of TG(A), cholesterol (B), free fatty acid (C), or glucose (D) of LDLR^{-/-} mice treated with triacsin C (●) or vehicle alone (■) for two months. Each concentration was measured as shown in the "Materials and Methods". Bars indicate mean and error bars represent $\pm$ SD.

(10 mg/kg/day) was set up by referring to the case of beauveriolides I and III. They inhibited the accumulation of CE in mouse peritoneal macrophages with IC₅₀ values of 0.78 and 0.41 μ M, respectively, and exerted anti-atherosclerotic activity by administering 25~50 mg/kg/day under the same conditions [14].

There were no significant differences in the levels of glucose, total cholesterol and free fatty acid in the plasma during the two-month experiments, while the TG levels decreased to 70% of the control on day 60 (Fig. 3). Nestel *et al.* reported that *cis*-9,*trans*-11 isomer of conjugated linoleic acid reduced plasma TG but did not suppress aortic atherosclerosis [16]. Furthermore, triacsin C showed no toxic effects on various tissues such as adrenal tissues and livers, showed no diarrhea in mice, and only a slight weight loss was observed (Fig. 4).

It might be that triacsin C, an ACS inhibitor, directly affects macrophage-derived foam cell formation in the aortas and hearts of LDLR^{-/-} mice by inhibition of CE and TG synthesis in macrophages. Furthermore, triacsin C was reported to show anti-atherogenic activity to affect the several atherogenic factors. Triacsin C was reported to inhibit the generation of nitric oxide and superoxide anion [13, 18], to induce the downregulation of leptin [19, 20] and the hydrolysis of TG [21], and to inhibit the secretion

Fig. 4 Effect of triacsin C on the body weight of LDLR^{-/-} mice.

Body weight of LDLR^{-/-} mice treated with triacsin C (●) or vehicle alone (■) for two months. Bars indicate mean and error bars represent $\pm$ SD.

of apolipoprotein B [22, 23]. We considered that the accumulation of these several effect induced the suppression of atherosclerosis.

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