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變異脂蛋白解脂酵素表現之調控(1/2)

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摘要

脂蛋白解酯酵素能催化低密度脂蛋白及乳糜微粒內的三酸甘油脂的水解，如此酵素有缺陷時則會導致第一型高脂蛋白血症。在我們以前對第一型高脂蛋白血症病人之研究中，發現有 L252V 及 L252R 之兩種變異。本研究想來評估薑黃素及馬栗樹皮素對變異。以分別帶有野生型及變異型脂蛋白解酯酵素的質體轉染人類 293 細胞株後，再分別以薑黃素及馬栗樹皮素處理之。結果發現兩者皆不會促進該酵素 mRNA 的表現，然而薑黃素對 L252R 能增加 7 倍酵素活性及 5.7 倍的酵素濃度的分泌，對 L252V 而言，在細胞內則分別上升了 2.5 倍及 2.3 倍。而對野生型及兩種變異型其細胞內不管是酵素活性或濃度皆下降。至於馬栗樹皮素對於野生型或兩種變異型在培養液或細胞中的效應，類似薑黃素的效應。可見此兩種物質對於脂蛋白解酯酵素分泌之作用機制是一樣的，至於其臨床上的應用則有待繼續去探討。

關鍵詞：薑黃素、馬栗樹皮素、脂蛋白解酯酵素、變異

Abstract

Lipoprotein lipase (LPL) catalyzed the hydrolysis of triglycerides in very low-density lipoprotein and chylomicrons. The defect in LPL causes type I hyperlipoproteinemia. In our previous study, L252V and L252R mutants were found in type I hyperlipoproteinemic patients. The aim of this study was to evaluate the effect of curcumin and esculetin on these mutated LPL. HEK293 cells were transfected with wild type and mutated LPL, respectively. These transfected cells were then treated with curcumin and esculetin, respectively. Neither curcumin nor esculetin increased the mRNA expression of wild type and mutated LPL. However, curcumin increased 7-fold L252R LPL activity and 5.7-fold in mass in culture medium, and LPL activity and mass of L252V transfected cells increased 2.5-fold and 2.3-fold, respectively. Contrary to the culture medium, both activity and mass were decreased in wild type and mutants in cell lysate. The effect of esculetin on wild type and mutated LPL activity and mass both in culture medium and cell lysate was the same as that of curcumin. This suggests that the mechanism of both curcumin and esculetin for these effects is the same, and the clinical application remain to be determined.

Key Words

Curcumin, Esculetin, Lipoprotein lipase, Mutation.

Introduction

Lipoprotein lipase (LPL) is synthesized by parenchymal cells in many tissues. After secretion, LPL is transported to the lumen surface of the vascular endothelium, where it is bound to the surface. In the presence of its cofactor, apolipoprotein (apo) CII , LPL hydrolyzes triglycerides in both of chylomicrons and very low density lipoproteins (VLDL) to mono- and diglycerides and free fatty acids. LPL is important in the metabolism of chylomicrons, VLDL, and HDL. When LPL activity is high, the VLDL concentration is low and the HDL level is high, and vice versa. Persons lacking LPL will present with severe hypertriglyceridemia and hyperchylomicronemia. Patients who are heterozygous for some mutations have moderate reductions in LPL activity and concomitant modest increases in serum triglyceride levels, along with low levels of HDL and associated with increased risk of atherosclerosis.

We found a single base C → G substitution in codon 252 of the *LPL* gene, encoding in a change of a leucine to a valine residue in the mature protein and leading to an inactive enzyme, in three women who had hypertriglyceridemia and recurrent pancreatitis. Another one is T → G substitution in codon 252 of the *LPL* gene, encoding in a change of a leucine to an arginine residue in the mature protein and leading to an inactive enzyme.

Dietary curcuminoids have been associated with antioxidative, anticarcinogenic activities and the apoptotic action. In recent years, much attention has been focused on the hypolipidemic action of curcumin. Esculetin is a coumarin derivative present in many plants. It inhibits tumor cell proliferation and shows antioxidant activity in biological systems. Because esculetin has similar functions as curcumin, the aims of this study were to investigate the *in vitro* effects of curcumin and esculetin on the expression and secretion of these mutated LPL.

Material and Methods

Expression plasmids and Transfection

Expression of the wild type and mutant *LPL* plasmids were constructed. These plasmids were designated as L252L, L252V and L252R for wild type, Leu252Valine and Leu252Arg mutant, respectively. Human embryonal kidney 293 (HEK 293) cells were cultured for transfection. After electroporation, the cells were incubated for 10 minutes at 25 then cultured in 10-cm cell culture dishes in DMEM/HAM's F12 containing 10% fetal calf serum. All cells were examined 48 h after transfection.

RNA extraction and Reverse transcriptase-polymerase chain reaction (RT-PCR) assays

For gene expression studies, the transfected HEK 293 cells were treated with either curcumin or esculetin and incubated. The mRNA levels of LPL were determined by using a RT-PCR assay. The reaction that yielded specific amplification of LPL was performed using forward primer 5'-AGGACTTGGAGATGTGGACC- 3' and reverse primer 5'-CACTCTCA GTCCCAGAAAAATG-3' and Klen Taq polymerase. The control PCR was performed with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward primer 5'-ACCACAGTCCATGCCATCAC-3' and reverse primer 5'- TCCACCACCCTGTTGCTGTA-3'. The PCR products were evident following agarose electrophoresis on a 2% agarose gel. After the ethidium bromide staining, the intensity of bands was analyzed by using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Morphological evaluation

Before and after 24-hour treatment of curcumin or esculetin, transfected cells were observed under the light microscope to evaluate their integrity.

Statistical analysis

The data are expressed as mean ± SD. P values less than 0.05 were considered statistically significant (ANOVA and Student's t test).

Result

Dose-dependent effect of chemical compound on LPL secretion from transfected cells

As shown in Fig. 1, esculetin increased extracellular LPL activity and mass in a dose-dependent manner. The levels of LPL activity and mass in response to increasing concentrations of esculetin (1 – 9 ì mol/l) are illustrated in Fig. 1A and 1B. While the esculetin stimulates in a dose-dependent manner the secretion of LPL activity and mass into the culture medium in all transfected cells, the maximal stimulatory effect was observed in the wild type LPL. Maximal effect of curcumin on LPL activity and mass occurred at 3.5 ì mol/l. Therefore, those concentrations were used in the following experiments.

Effect of curcumin and esculetin on transfected cells LPL mRNA expression

Our results did not demonstrate any significant change in LPL mRNA expression following treatment of transfected cell for 36 hours with both curcumin and esculetin (Fig. 2). The effect of curcumin and esculetin on LPL gene expression was specific as reflected by the lack of modulation of the mRNA expression of the housekeeping

gene GAPDH (Fig. 2A). LPL mRNA levels, normalized to the levels of GAPDH mRNA are presented in Fig. 2B and 2C.

Effect of curcumin and esculetin on LPL secretion by transfected cells

Levels of transfected cells LPL activity and mass in the culture medium in response to curcumin and esculetin are illustrated in Fig. 3 and 4, respectively. Significant stimulation of LPL secretion into the medium was observed after a 12-hour incubation period, especially from the cells transfected with the wild type plasmid. While LPL activity of L252R transfected cells increased 7.4-fold at the end of 60 hours incubation with curcumin, the LPL mass increased from 0.72 to 4.07 ng/ml, which is 5.7-fold increase. LPL activity and mass of L252V transfected cells increased 2.5-fold (2.92 to 7.42 mU/ml) and 2.3-fold (0.9 to 2.07 ng/ml), respectively. At the end of 60 hours incubation with esculetin, LPL activity and mass of L252R transfected cells increased 4-fold and 2.3-fold, respectively. However, LPL activity and mass of L252V transfected cells increased only 3.6-fold and 2.0-fold, respectively.

A time-dependent decrease in LPL activity and mass in the cell lysate after incubation of all transfected cells with curcumin or esculetin is shown in Fig. 5 and 6. Significant decrease of LPL in cells was observed after an 18-hour incubation period. LPL activity of L252R transfected cells decreased 52% in the cell lysate at the end of 60 hours incubation with curcumin, the LPL mass decreased 59%. However, LPL activity and mass of L252V transfected cells decreased 48% and 70%, respectively. After a 60-hour incubation with esculetin, LPL activity and mass of L252V transfected cells decreased 31% and 33% in the cell lysate, respectively. While LPL activity of L252R transfected cells decreased 39% in the cell lysate, the LPL mass decreased 60%.

Morphological evaluation

To assess the effect of curcumin or esculetin on the integrity of transfected cells, the transfected cells were observed under the light microscope. The morphology of the cells did not change after 24-hour treatment of curcumin or esculetin.

Discussion

The present study demonstrates that curcumin and esculetin increase transfected cells LPL secretion. Our data which show that induction of LPL protein secretion by curcumin and esculetin requires 12 hours of treatment. Neither curcumin nor esculetin upregulates transfected cells LPL mRNA expression suggested that the induction of LPL secretion was due to its release from the cell, which was reflected in the decrease

in LPL activity and mass in the cell lysate after incubation of all transfected cells with curcumin or esculetin. In contrast to the previous results showing that both curcumin and esculetin exhibited antiproliferative effects on vascular smooth muscle cells and curcumin inhibited cell proliferation, arrested the cell cycle progression and induced cell apoptosis in vascular smooth muscle cells, these two chemical did not arrest transfected cells and lead to cell lyses. Differences in the concentration used in the two studies may provide a better explanation for these discrepancies.

Determination of the levels of LPL activity and mass in the culture medium of transfected cells demonstrates that curcumin and esculetin lead to the production of increased amount of catalytically active LPL. Although not very dramatic, increased LPL secretion was observed with each of the chemicals used in the present study. The increased LPL secretion was reflected in the decreased cellular LPL. Because the transfected cells were still intact after 24-hour treatment with curcumin or esculetin, this increasing secretion is not due to the cell lyses. The mechanism of the increased LPL secretion from the transfected cells by curcumin or esculetin is unclear. Kuwajima et al. reported that infusion of heparin into fed rats caused a rapid accumulation in plasma of heparin releasable lipoprotein lipase. Friedman et al. reported that treatment of cultured rat heart cells with dibutyryl cAMP or isoproterenol for 3 minutes resulted in a 3-fold increase in heparin-releasable lipoprotein lipase and a concomitant decrease in residual cellular enzyme activity. This indicates that translocation of LPL from intracellular pools to the cell surface appears to be stimulated by â–adrenergic stimulation. Whether curcumin or esculetin increasing transfected cells LPL secretion follows the same path way need further elucidation.

Based on the ability of releasing LPL from cells with curcumin or esculetin, the results of this study may raise the possibility of using curcumin or esculetin to correct the hypertriglyceridemia due to LPL mutation.

Evaluation

The goal of this project is achieved and the results obtained could submit for publication and provide information for further investigation of their clinical applications.

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Fig. 1 Dose-dependent effect esculetin on transfected HEK 293 LPL mass and activity. Transfected HEK 293 cells were treated for 24 hours with increasing concentrations of esculetin. At the end of the incubation period, LPL mass (A) and activity (B) were determined in the culture medium. Data represent the mean ±SD of five experiments, $*p < 0.05$.

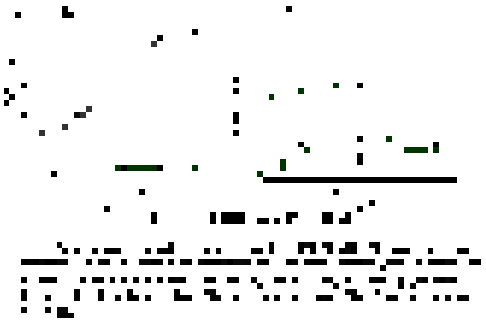

Fig. 2. Effect of curcumin (A and B) and esculetin (C) on transfected cell LPL mRNA levels. Transfected cell were cultured for 0 to 36 hours in presence of curcumin (3.5 μmmol/l), or esculetin (9 μmol/l). At the end of the incubation periods, transfected cells were lysed. LPL and GAPDH mRNA expressions were analyzed by RT-PCR. Graphs represent the levels of LPL mRNA normalized to the levels of GAPDH mRNA. Data represent the mean ±SD of five experiments.

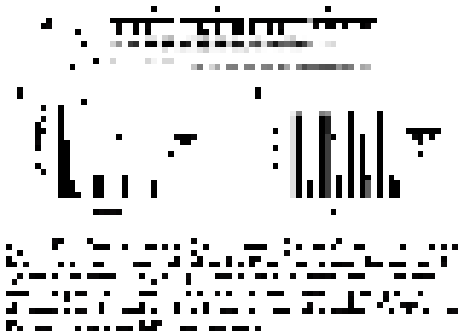

Fig. 3. Effect of curcumin on LPL mass and activity in transfected cells. Transfected cells were cultured for 12 to 60 hours in presence of curcumin (3.5 μmol/l). At the end of the incubation periods, LPL activity (A) and mass (B) were determined in the culture medium. Data represent the mean ±SD of five experiments, *$p < 0.05$.

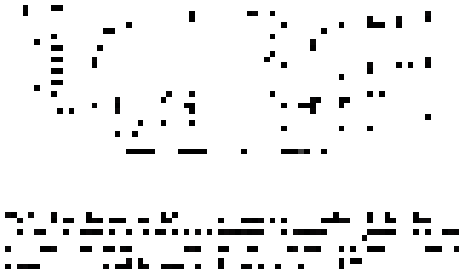

Fig. 4. Effect of esculetin on LPL mass and activity in transfected cells. Transfected

cells were cultured for 12 to 60 hours in presence of esculetin (9 μmol/l). At the end of the incubation periods, LPL activity (A) and mass (B) were determined in the culture medium. Data represent the mean ±SD of five experiments, *$p < 0.05$.

Fig. 5. Effect of curcumin on LPL mass and activity in transfected cells. Transfected cells were cultured for 12 to 60 hours in presence of curcumin (3.5 μmol/l). At the end of the incubation periods, LPL activity (A) and mass (B) were determined in the cell lysate. Data represent the mean ±SD of five experiments, *$p < 0.05$.

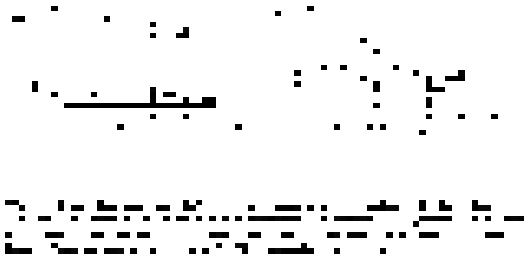

Fig. 6. Effect of esculetin on LPL mass and activity in transfected cells. Transfected cells were cultured for 12 to 60 hours in presence of esculetin (9 μmol/l). At the end of the incubation periods, LPL activity (A) and mass (B) were determined in the cell lysate. Data represent the mean ±SD of five experiments, *$p < 0.05$.