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Effect of curcumin on the expression of LDL receptor in mouse macrophages

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Abstract

To investigate the molecular mechanisms of lipid-lowering drug, *Rhizoma Curcumae Longae*, we treated the mouse macrophages with curcumin, which was purified from the ethanol extraction of *Rhizoma Curcumae Longae*. The LDL receptors expressed in the macrophages were determined by ELISA, FLISA and assay of LDL uptake. Here for the first time, we found that curcumin obviously up-regulated the expression of LDL receptor in mouse macrophages, and the dose–effect relationship was demonstrated.

Keywords: Curcumin; Low density lipoprotein receptor; Gene expression; Macrophage

1. Introduction

Elevated level of low density lipoprotein-cholesterol (LDL-C) in plasma is one of major causes of atherosclerosis and coronary heart disease. HMG-CoA reductase inhibitors, such as statins, already in clinical use to reduce cholesterol levels, are effective in atherosclerosis patients. They show a good safety profile in patients with high cholesterol levels and/or cardiovascular disease, however, statins may be potentially associated with development of hepatic damage, myalgias, or polyneuropathy (Gaist et al., 2002; Guis et al., 2003). Approximately two-thirds of LDL clearance is normally mediated by the LDL receptor (Brown and Goldstein, 1986). In most cases, high level of plasma LDL-C is due to mutations of LDL receptor, such as familial hypercholesterolemia (FH) or suppression of LDL receptors. This makes us to propose that the expression of LDL receptor meditated by drugs would be an effective way to control LDL-C levels in plasma.

In recent years, studies show that curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, structure displayed in Fig. 1], an ethanol extracted compound of the traditional Chinese medicine-*Rhizoma Curcumae Longae*, plays essential pharmacological roles, such as antioxidant, ani-infammatory agent, anti-thromobotic agent, hepatoprotector (Naika et al., 2004), etc. More and more evidences come to prove that curcumin is an active lipid-lowering compound that can significantly decrease the level of serum lipid peroxides, increase HDL-C, and decrease total serum cholesterol (Soni and Kuttan, 1992; Soudamini et al., 1992; Babu and Srinivasan, 1997). But, all these studies are at the level of plasma lipid (Sree and Rao, 1994), the evidences of molecular mechanisms of this lipid-lowering drug are still poor. We report here that curcumin obviously up-regulated the expression of LDL receptor in mouse macrophages. And we proved that one of the lipid-lowering mechanisms of the Traditional Chinese Medicine, *Rhizoma Curcumae Longae* was by the effect of curcumin on the up-regulation of the expression of LDL receptor.

2. Materials and methods

2.1. Materials

Curcumin (99.0% pure, HPLC) was obtained from National Institute for the Control of Pharmaceutical and Biological Prod-

Abbreviations: LDL, Low density lipoprotein; ELISA, enzyme-linked immunosorbent assay; FLISA, fluorescein-linked immunosorbent assay; FH, familial hypercholesterolemia; LDL-C, low density lipoprotein-cholesterol; SREBP, sterol regulatory element-binding protein; SCAP, SREBP cleavage-activating protein; FBS, fetal bovine serum; PBS, phosphate-buffered saline

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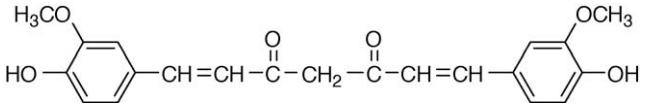

Fig. 1. Chemical structure of curcumin.

ucts. ANA-1 cell lines (mouse macrophages) were provided by Shanghai Cell Bank, Chinese Academy of Science. Cell culture medium was from Gibco. Rabbit anti-bovine LDL receptor was provided by Graz University, Austria. Goat anti-rabbit IgG-HRP was obtained from KPL. DiI was from Biotium Inc. LDL was purified from human plasma by our own laboratory. All other reagents are purchased from Shanghai Sangon.

2.2. Cell culture and determination of number of LDL rceptors

ELISA: ANA-1 cell lines were cultured in two 24-well plates in RPMI1640 medium containing 10% bovine calf serum with six groups by seven repeated wells (each well has a total volume of 2.5 ml, density 1.0×10^6 cells ml^{-1}). Of the all six groups, five groups were treated with the medium containing 10, 20, 30, 40 and 50 μM of curcumin, respectively. The control group was treated with the medium containing no curcumin. After 24 h, 1.5 ml of the cell solution was transferred from each of all the wells to each of 1.5 ml Eppendorf tubes (which were treated with 1% FBS-PBS, pH 7.4 to block non-specific antibody binding before use) respectively and the tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded, and 1 ml of 4% formaldehyde was added to each of the tubes to fix the cells for 10 min. Then all the tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded. PBS (pH 7.4) was added to the tubes to regulate the cell concentration to 1.0×10^6 cells $ml^{-1}$of each tube. The tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded. 0.5 ml of diluted LDL receptor antibody (rabbit anti-bovine LDL receptor, 1:1000 in PBS) was added to each of the tubes. The tubes were shaken to re-suspend the cells and the cells were incubated at 37 °C for 1.5 h. The tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded. About 1 ml PBS was added to each of the tubes to wash the cells for 2 min, and then the tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded. The wash and centrifugation were repeated for three times. About 0.5 ml of diluted secondary antibody (goat anti-rabbit IgG-HRP, 1:1000 in PBS) was added to each of the tubes. The tubes were shaken to re-suspend the cells and the cells were incubated at 37 °C for 1.5 h. The tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded. About 1 ml of PBS was added to wash the cells for 2 min. The tubes were centrifuged at $900 \times g$ for 5min. The supernatant was discarded. The wash and centrifugation were repeated for three times. The supernatant was discarded. About 0.1 ml of OPD reagent was added into each of the tubes and incubated at RT for 15 min in the dark. The reaction was stopped by adding 0.1 ml of 20% sulfuric acid in each of the tubes. 0.1 ml of reaction mixture was taken from each of the tubes and measured at 492 nm by Multiscan MK3.

FLISA: The procedure was the same as that of ELISA until "...0.5 ml of diluted secondary antibody was added to each of the tubes". But here, goat anti-rabbit IgG-FITC, 1:1000 in PBS, were used as the secondary antibody and 6 groups without repeated well were set. After incubated with the secondary antibody and washed by PBS, 10,000 cells of each group were measured by FACSan (BD FACSort).

2.3. LDL uptake assays

The cell culture was similar as above, but no repeat wells and one more groups were set for negative control. Thus, five groups were treated with the medium containing 10, 20, 30, 40 and 50 μM of curcumin, respectively. The control group was treated with the medium containing no curcumin. The negative control group was treated with the medium containing 30 μM of curcumin. After 24 h, 1.5 ml of the cell solution from each of the groups was transferred to each of 1.5 ml Eppendorf tubes, respectively, and they were centrifuged at $900 \times g$ for 5 min, and the supernatant was discarded. About 1 ml of serum-free RPMI1640 medium was added to each tube to wash the cells for 2 min, and then the tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded. For the negative control group, 1 ml of diluted LDL receptor antibody (1:1000 in serum-free RPMI1640 medium) was added and the cells were incubated at 37 °C for 1.5 h to block the function of LDL receptor. The other groups were treated with 1 ml of diluted mouse IgG (non-LDL receptor antibody, 1:1000 in serum-free RPMI1640 medium). The tubes were centrifuged and the supernatant was discarded. About 1 ml (15 μg ml^{-1}) of DiI-LDL [labeled as the method of Barak (Barak and Webb, 1981)] in serum-free RPMI1640 medium was added to each of the tubes. The medium in the negative control group still contained the receptor antibody (1:1000 diluted) for blocking the interaction between the receptor and DiI-LDL, and that in the other groups contained mouse IgG (non-LDL receptor antibody, 1:1000 diluted). The cells were incubated at 37 °C for 4.5 h and then at 4 °C for 0.5 h. The tubes were centrifuged at $900 \times g$ for 5 min. The supernatant was discarded. The cells were fixed by 4% formaldehyde, and then washed by PBS for three times. The uptake of DiI-LDL was detected by LSCM (Zeiss LSM 510) and measured by FACSan (BD FACSort).

3. Results

3.1. Curcumin increases the number of LDL receptors

To study effect of curumin on the expression of LDL receptor, we chose a stable mouse macrophage cell line, ANA-1. Quantitative analysis of LDL receptor numbers was performed by ELISA and FLISA.

In Tables 1 and 2, we found that the expressed LDL receptors in ANA-1 were remarkably increased by curcumin. Comparing to the control, it increased them to 107% (by ELISA) or 121% (by FLISA) at 10 μM, 186% (by ELISA) or 204% (by FLISA) at 20 μM, 284% (by ELISA) or 292% (by FLISA) at 30 μM, 227%