Human atherosclerotic lesions have been found to contain tumor necrosis factor-α (TNF-α) mRNA [1]. The accumulation of cholesteryl esters in macrophages exposed to LDL is associated with the increased synthesis and release of TNF-α [2]. TNF-α released from macrophages promotes a variety of inflammatory reactions including the induction of vascular adhesion molecules and the recruitment and proliferation of monocytes/macrophages [3, 4], which is essential in initiation of atherosclerosis. TNF-α also alters plasma lipid metabolism by decreasing the activity of adipocyte-derived lipoprotein lipase [5] and increasing the production of hepatic very low density lipoproteins [6] in response to acute endotoxin exposure. TNF-α decreases collagen synthesis and increases matrix metalloproteinase activity in vitro, perhaps leading to plaque rupture [7]. These activities are considered to promote atherosclerosis.

Acylocoenzyme A: cholesteryl acyltransferase (ACAT) catalyzes the formation of cholesteryl esters from cholesterol and long chain fatty acyl coenzyme A. ACAT is believed to play significant roles in lipoprotein assembly and in dietary cholesterol absorption [8]. ACAT-1 and ACAT-2 are two subtypes of ACAT gene family. ACAT-1 protein is present at high levels in macrophages and steroid hormone-producing cells, and ACAT-2 mRNA can be detected in intestine and liver [9]. ACAT-1 gene expression is up-regulated in human monocytes during differentiation and foam cell formation [10]. Under pathological conditions, accumulation of cholesteryl esters produced by ACAT-1 can promote foam cell formation in atherosclerotic lesions [11]. TNF-α can stimulate the synthesis of cholesteryl esters via ACAT in human fibroblasts [12]. But the relationship between TNF-α and ACAT-1 in atherogenesis is poorly understood. In this study, the effect of TNF-α on ACAT-1 activity in macrophages and the possible mechanisms were investigated.

1 MATERIALS AND METHODS

1.1 Materials

The human monocyte cell line THP-1 was purchased from China Cell Type Culture Collection (CCTCC, China). RPMI 1640 medium, Trizol reagent and fetal calf serum were from Gibco (USA). Recombinant human TNF-α was from Pepro Tech (USA). [1-14C] oleoyl CoA, 60 mCi/mmol was from Dupont Amersham (UK). RT-PCR reagents were from Takara (Japan). The primers were synthesized by Saihaisheng (China). Other chemicals were purchased from China.

1.2 Cell Culture and Treatment

THP-1 monocytes were routinely cultured in suspension in RPMI 1640 medium supplemented with 2 mmol/L L-Glutamine, 1.5 g/L NaHCO₃, 4.5 g/L glucose,
10 mmol/L HEPES, 1.0 mmol/L sodium pyruvate, 0.05 mmol/L 2-mercaptoethanol and 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO₂. Phorbol 12-myristate 13-acetate (PMA) was used for the differentiation of THP-1 monocytes into macrophages for 48 h. TNF-α (60 ng/mL) was added at different time points into the macrophages-containing medium. Cells were harvested and tested for ACAT activity. After treatment with TNF-α (60 ng/mL) for 24 h, the expression of ACAT-1 protein and mRNA was detected in the THP-1 macrophages.

1.3 Determination of ACAT Activity

ACAT activity was determined by the incorporation of oleoyl CoA into cholesteryl esters as described by Billheimer et al. Cells were harvested by hypotonic shock method according to Chang et al. Protein was measured by Bradford method. The standard assay in a final volume of 200 µL contained 100 µg protein, 1 mg fatty acid-free bovine serum albumin in 0.1 mol/L potassium phosphate buffer (pH 7.4), 1 mmol/L DTT and 100 µmol/L [1-14C] oleoyl-CoA (166 Bq/nmol). Exogenous cholesterol (20 µg) was added as an aqueous dispersion in Triton WR-1339 (600 µg). All components except oleoyl-CoA were preincubated for 30 min. The reaction was initiated by the addition of [14C] labeled and unlabeled oleoyl-CoA and incubated for 10 min. The reaction was stopped by the addition of 4 mL chloroform: methanol (2:1, v/v). After separation into two layers by the addition of 0.8 mL water, the chloroform layer was separated by chloroform and isopropanol. The spots corresponding to cholesteryl esters were cut out and placed directly into scintillation vials for counting.

1.4 Detection of ACAT-1 Protein Expression by Western Blot

Cells (10⁷) were washed by 5 mL PBS at 4°C and harvested with 10% SDS in 50 mmol/L Tris, 1 mmol/L EDTA (pH=7.5) with 25 mmol/L dithiothreitol, and incubated at 37°C for 20 min, then sheared with a syringe fitted with an 18-gauge needle. Protein concentration of the cell extract was determined by Bradford method. 50 µg of total cellular protein was subjected to SDS-PAGE in 10% gels. Western blot analysis for ACAT-1 was performed by using commercially synthesized goat antibody against human. The second antibody was rabbit body against goat (Vector, USA). Luminol ECL reagents were used to detect the immunocomplex.

1.5 Detection of ACAT-1 mRNA Expression by RT-PCR

THP-1 cells were extracted with Trizol and RNA was separated by chloroform and isopropyl alcohol. The RT-PCR reaction was conducted according to the instruction of reaction kit. Primers for ACAT-1 were as follows: forward, 5’-TATTGCCCTATTCTC-3’; reverse, 5’-TTTACGACATTACCA-3’. The product was 544 bp. Primers for GAPDH were as follows: forward, 5’-TCCCTCAAGATTTCTCAGCA-3’; reverse, 5’-AGATCCACAAAGGATACATT-3’. The product was 309 bp. The cycling conditions in the GeneAmp 9600 system for the amplification of the target cDNA were 38 cycles and 28 cycles of 94°C, 55°C, 72°C for 30 s each.

PCR products were analyzed by 1.5% agarose gel electrophoresis.

2 RESULTS

2.1 Effects of TNF-α Treatment with Different Durations on ACAT Activity in THP-1 Macrophages

TNF-α could increase the activity of ACAT in THP-1 macrophages in a time-dependent manner. The activity of ACAT in THP-1 macrophages incubated with TNF-α for 48 h was almost increased by 4 fold compared to that immediately (0 h) after TNF-α incubation (table 1).

Table 1 Effect of TNF-α treatment with different durations on ACAT activity [nmol/(min·mg), X±s]

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>ACAT activity</th>
<th>Time (h)</th>
<th>ACAT activity</th>
</tr>
</thead>
<tbody>
<tr>
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<td>6</td>
<td>8.68±1.33</td>
</tr>
<tr>
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<td>11.21±2.43</td>
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<tr>
<td>2</td>
<td>8.42±1.52</td>
<td>48</td>
<td>20.41±2.50</td>
</tr>
</tbody>
</table>

2.2 Up-regulation of ACAT-1 Protein Expression by TNF-α

Western analysis revealed that few ACAT-1 protein was expressed in THP-1 monocytes. PMA-differentiated THP-1 macrophages expressed ACAT-1 protein. When TNF-α was added, the expression of ACAT-1 protein was increased in THP-1 macrophages (P<0.05, fig. 1).

![Fig. 1](image)

Fig. 1 Effect of TNF-α treatment on the ACAT1 protein expression (MC: Monocytes; MP: Macrophages)

2.3 Effect of TNF-α on ACAT-1 mRNA Expression

RT-PCR analysis revealed an increase of ACAT-1 mRNA expression in response to TNF-α in THP-1 macrophages. There was significant difference in the ACAT-1 mRNA expression between cells treated with or without TNF-α (P<0.05, fig. 2).