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Original Article

Ghrelin inhibits foam cell formation via simultaneously down-regulating the expression of acyl-coenzyme A:cholesterol acyltransferase 1 and up-regulating adenosine triphosphate-binding cassette transporter A1

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Abstract

Background: Ghrelin, an endogenous ligand of the growth hormone secretagogue receptor (GHS-R), revealed cardioprotective effects in both experimental models and human. There is far less information on the mechanisms that produce antiatherogenic effects. We assessed the expression of acyl-coenzyme A:cholesterol acyltransferase 1 (ACAT-1) and adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1), which have been implicated in regulating cellular cholesterol homeostasis and therefore play critical roles in foam cell formation, in THP-1-derived foam cells in the presence of various concentration of ghrelin. **Methods:** After 48 h of culture in the presence of phorbol myristate acetate, THP-1 monocytes differentiated to macrophages. After another 24 h of culture with ox-LDL, the differentiated cells transformed to foam cells. Different concentrations of ghrelin and other intervention factors were added, respectively. The expression of ACAT-1 and ABCA1 was detected by a technique in molecular biology. The content of cellular cholesterol was measured by zymochemistry via a fluorospectrophotometer. **Results:** Ghrelin could down-regulate the expression of ACAT-1 and up-regulate the expression of ABCA1 in a dose-dependent manner simultaneously. Ghrelin also decreased cellular cholesterol content and increased cholesterol efflux. These effects could be abolished by the specific antagonist of GHS-R and a peroxisome proliferator-activated receptor γ (PPARγ)-specific inhibitor, respectively. **Conclusions:** The results suggest that ghrelin inhibited foam cell formation via simultaneously down-regulating the expression of ACAT-1 and up-regulating ABCA1. Those effects may be achieved via pathways involving GHS-R and PPARγ.

Keywords: Ghrelin; Foam cell; Acyl coenzyme A:cholesterol acyltransferase 1; ATP-binding cassette transporter A1; Growth hormone secretagogue receptor; Peroxisome proliferator-activated receptor γ

1. Introduction

The accumulation of cholesteryl esters in macrophages contributes to foam cell formation, a hallmark of the early stage of atherosclerosis [1]. Monocytes/macrophages were the major cellular component of acyl coenzyme A: cholesterol acyltransferase 1 (ACAT-1)-expressing cells in atherosclerotic lesions [2]. ACAT-1 catalyzes the formation of cholesteryl ester from free cholesterol and long-chain fatty acid [3]. A major concern for using the ACAT-1 inhibitor is cellular toxicity induced by free cholesterol loading [4,5]. The intracellular cholesterol homeostasis is regulated by cholesterol synthesis, influx, and efflux. Accordingly, it is useful to reduce lipidoses by inhibiting ACAT-1 and to raise free cholesterol efflux simultaneously. Adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) was found recently to be a critical gatekeeper in many human tissues. It is the critical channel of cholesterol efflux in macrophages and scavenges redundant lipid, which protects against the

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formation of foam cells [6–8]. Thus, how to reduce lipid by down-regulating ACAT-1 and increase cholesterol efflux by up-regulating ABCA1 is a problem that needs to be solved.

Ghrelin, an endocrine peptide newly identified mainly in stomach epithelium, stimulates food intake in humans [9]. The biological actions of ghrelin, including food intake and energy homeostasis, are mainly mediated by its receptor, the growth hormone secretagogue receptor (GHS-R) [10]. In addition, ghrelin might also regulate the peripheral effects through activation of the downstream cascades involving cAMP/PKA, ERK1/2, and PI3K/Akt signaling [11]. Furthermore, cardioprotective effects of ghrelin have been suggested. The main cardiovascular actions are possible inotropic effects, vasodilation, and cardioprotective effects against ischemia [12]. Recently, results suggest a beneficial role of this hormone in the development of atherosclerosis [13,14]. Therefore, we hypothesize that ghrelin can inhibit foam cell formation by regulating the expression of ACAT-1 and ABCA1 via the signaling pathways of GHS-R and nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ).

In the present study, we examined the expression of ACAT-1 and ABCA1 in THP-1-derived foam cells with various concentrations of ghrelin. We also investigated the relationship between cellular cholesterol content and ACAT-1/ABCA1.

2. Materials and methods

2.1. Materials

Human ghrelin and [D-Lys3]-GHRP-6 (DG), a specific antagonist of GHS-R, were purchased from Anaspec Company. ACAT-1 polyclonal antibody was from Cayman Chemical. Mouse antihuman ABCA1 polyclonal antibody was from Santa Cruz Biotechnology. GW9662, a specific inhibitor of PPARγ, was from Sigma-Aldrich. Enhanced chemiluminescence reagent kit was from Pierce Biotechnology. Reverse transcription-polymerase chain reaction (RT-PCR) reagent kit was from Takara Bio. All other chemicals were of the highest purity available from Sigma-Aldrich.

2.2. LDL isolation and oxidization

LDL was purified from human plasma obtained from healthy volunteers according to published standard protocols [15]. The preparation was performed in a Beckman L8-M ultracentrifuge at 4°C, and densities were adjusted with solid NaBr. Lipoprotein fractions were dialyzed repeatedly in phosphate-buffered saline (PBS) containing 5 mmol/l EDTA. After the final dialysis step, LDL lipoproteins were sterilized using a 0.45-μm sterile filter. The isolated LDL was oxidized with $CuCl_2$ 10 μmol/l for 18 h at 37°C. Oxidation of LDL was measured by the thiobarbituric acid-reactive substance assay.

2.3. Cell culture

THP-1 cells, a human monocytic leukemia cell line obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences, were cultured in RPMI 1640 medium (Gibco) containing 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. To induce monocyte-to-macrophage differentiation, THP-1 cells were cultured in the presence of 160 nmol/l phorbol 12-myristate 13-acetate (Sigma) for 48 h. THP-1-derived macrophages were preincubated with different concentrations of human ghrelin (0, 10^{-7}, 10^{-6}, 10^{-5} mol/l) in RPMI 1640 without FBS for 2 h at 37°C. To induce foam cell formation of differentiated cells, ox-LDL (100 mg/l) was then added for 24 h. Then the cells were washed once with PBS and incubated in serum-free medium containing apolipoprotein A-I (apoA-I, 10 μg/ml) for another 12 h. Cells were collected for subsequent analyses.

Cultured in RPMI 1640 without FBS, THP-1-derived macrophages were preincubated with different concentrations of DG (0, 10^{-5}, 5×10^{-5}, 10^{-4} mol/l) or GW9662 (0, 10, 20, 50 μmol/l) in serum-free medium for 2 h at 37°C. Then, human ghrelin (10^{-5} mol/l) was added for another 2 h. To induce foam cell formation of differentiated cells, ox-LDL (100 mg/l) was then added for 24 h. Then the cells were washed once with PBS, incubated in serum-free medium containing apolipoprotein A-I (10 μg/ml) for 12 h, and harvested for the subsequent analyses.

2.4. Oil Red O staining

The formation of lipid droplets in cells was analyzed by Oil Red O staining as follows according to published standard protocols [16]. After removal of the culture medium, cells were washed once with PBS, then fixed for 15 min with prechilled 4% paraformaldehyde in PBS. Cells were stained with Oil Red O solution (a mixture of three parts of 0.5% Oil Red O in isopropanol and two parts of water) for 10 min at room temperature. Then the cells were stained with hematoxylin for 1 min followed by washing with PBS twice, ethanol once, and then PBS twice. Cells were kept in PBS and photographed.

2.5. Reverse transcription-polymerase chain reaction

Cells were collected and washed with PBS. Total RNA was extracted from cells using Trizol reagent according to the protocol provided by the manufacturer. The RNA was treated with DNase before analysis by RT-PCR. Oligonucleotide primers were designed using Primer premier and were synthesized by Invitrogen Biotechnology (Shanghai). Sequences of primers for ACAT-1 were 5′-TCC CAG GAA TCC CAC TGT AA-3′ and 5′-ACG AAG AGC ACG GGA TAG AA-3′ (amplification product, 495 bp). The cycling