TO901317 regulating apolipoprotein M expression mediates via the farnesoid X receptor pathway in Caco-2 cells

Zhu, Chunhua; Di, Dongmei; Zhang, Xiaoying; Luo, Guanghua; Wang, Zongchun; Wei, Jiang; Shi, Yuanping; Berggren Söderlund, Maria; Nilsson-Ehle, Peter; Xu, Ning

Published in:
Lipids in Health and Disease

DOI:
10.1186/1476-511X-10-199

2011

Link to publication

Citation for published version (APA):
Zhu, C., Di, D., Zhang, X., Luo, G., Wang, Z., Wei, J., Shi, Y., Berggren Söderlund, M., Nilsson-Ehle, P., & Xu, N. (2011). TO901317 regulating apolipoprotein M expression mediates via the farnesoid X receptor pathway in Caco-2 cells. Lipids in Health and Disease, 10. https://doi.org/10.1186/1476-511X-10-199

Total number of authors:
10

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal
Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
TO901317 regulating apolipoprotein M expression mediates via the farnesoid X receptor pathway in Caco-2 cells

Chunhua Zhu1, Dongmei Di1, Xiaoying Zhang1*, Guanghua Luo2, Zongchun Wang1, Jiang Wei2, Yuanping Shi2, Maria Berggren-Söderlund3, Peter Nilsson-Ehle3 and Ning Xu3*

Abstract

Background: Apolipoprotein M (apoM) may have potential antiatherosclerotic properties. It has been reported that apoM expression could be regulated by many intracellular and extracellular factors. In the present study we further investigated regulation of apoM expression in Caco-2 cells stimulated by a liver X receptor (LXR) agonist, TO901317.

Materials and methods: Caco-2 cells were cultured in the presence of either TO901317, farnesoid X receptor (FXR) antagonist guggulsterone or TO901317 together with guggulsterone at different concentrations for 24 hrs. The mRNA levels of ATP-binding cassette transporter A1 (ABCA1), apoA1, apoM, liver receptor homologue-1 (LRH-1) and short heterodimer partner 1 (SHP1) were determined by real-time RT-PCR.

Results: When Caco-2 cell cultured with TO901317 alone, the mRNA levels of ABCA1, apoA1, apoM, LRH-1 and SHP1 were significantly increased with dose-dependent manners (p < 0.05), whereas when the cells cultured with guggulsterone alone, the mRNA levels of apoM, SHP1 and LRH-1 (p < 0.05) were strongly inhibited. Moreover, guggulsterone could abolish the TO901317 enhanced mRNA levels of apoA1 apoM, SHP1 and LRH-1.

Conclusion: The present study demonstrated that LXR agonist TO901317 induced apoM expression in Caco-2 cells might be mediated via the LXR/FXR pathway.

Keywords: Liver X Receptor, Farnesoid X Receptor, Caco-2 cell line, Apolipoprotein M

Introduction

With the aging population and changing lifestyles, the incidence of cardiovascular diseases (CVD) has gradually increased [1]. Abnormal lipid metabolism has been considered as one of the major risk factors of CVD [2]. Previously studies have demonstrated that serum concentrations of apolipoprotein (apo) A1 and apoB have significantly correlated with the occurrences of CVD [3,4], and other apolipoproteins may also involve in the initiation and progression of the diseases [5]. ApoM is one of the latest discovered apolipoproteins that is mainly synthesized in the liver, and to a smaller amounts, in the kidney [6].

In human plasma, most apoM are located in the high-density lipoproteins (HDL) and small proportion present also in apoB-containing lipoproteins, i.e. chylomicrons, very low-, and low-density lipoproteins (VLDL and LDL) [6,7]. Recent investigations have suggested that apoM may participate in the HDL-related biological activities as an important component of HDL particle on the protection of endothelial cells [8]. Wolfrum, et al., [9] reported that apoM is required for preβ-HDL formation and cholesterol efflux to HDL particles, which is an initial and crucial stage of reverse cholesterol transport, and subsequently protects against atherosclerosis. In addition, the physiological and patho-physiological roles of apoM may also involve in the inflammatory activities and the potential immuno- and inflammm-reactive properties, and apoM may therefore contribute to the anti-inflammatory function of HDL, being as
generally acknowledged as a significant antiatherogenic mechanism [10,11].

ApoM could be regulated by many factors including leptin, insulin, hyperglycemia and many cytokines in vivo and in vitro [12]. It has been demonstrated that apoM gene expression could be also affected by some nuclear receptors, such as hepatocyte nuclear factor-1α (HNF-1α) [13], hepatocyte nuclear factor-4α (HNF-4α) [12] and liver receptor homolog-1 (LRH-1) [12]. Liver X receptor (LXR) is a nuclear receptor, as a lipid sensor, protects cells from lipid overload and directly or indirectly controls apolipoprotein-mediated cholesterol efflux [14]. Our previous studies demonstrated that the synthetic LXR agonist TO901317 could down-regulate hepatic apoM expression in vivo and in vitro [15]. Whereas Calayır., et al. [16], in recognition of our findings that TO901317 inhibited apoM expression in HepG2 cells, even found that TO901317 could upregulate apoM expression in intestinal cells. In the present study we further revealed the regulative pathway of apoM expression in Caco-2 cells stimulated by TO901317.

Materials and methods

Cells and reagents

Human colorectal adenocarcinoma cell line, Caco-2, was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). TO901317 was purchased from the Cayman Chemical Company (Ann Arbor, MI, USA). Guggulsterone was from the Sigma Chemical Co. Ltd. (Shanghai, China). Six-well cell culture clusters and 75 cm² vented cell culture flasks were purchased from the Nunc (Roskilde, Denmark). Fetal bovine serum (FBS) and Dulbecco’s Modified Eagle Medium (DMEM) were obtained from the Invitrogen (Shanghai, China). Total RNA purification kits were purchased from the Shenery Biocolor BioScience and Technology Company (Shanghai, China). First strand cDNA synthesis kits were obtained from the Fermantas (Vilnius, Lithuania). The LightCycler real-time RT-PCR System was from the Roche Applied Science (Mannheim, Germany).

Cell cultures

Caco-2 cells were cultured in DMEM supplemented with 20% FBS in the presence of 100 U/ml penicillin, 100 μg/ml streptomycin and 1% Glutamax at 37°C under 5% CO₂ atmosphere. Cells were plated in 6-well cell culture clusters at a density of 3 × 10⁵ cells/dish with DMEM containing 20% FBS. Cell monolayer of approximately 50-70% confluence were grown for 24 hrs in the above media prior to experiments, cells were washed twice with phosphate buffered saline (PBS) and once with serum-free DMEM without antibiotics. Then the experimental medium, containing DMEM with 1% bovine serum albumin (BSA) and different concentrations of TO901317 and guggulsterone, or TO901317 together with guggulsterone were added. As TO901317 must be dissolved in the DMSO and then added into the experimental medium (the final concentration of DMSO was at 1% in the present study), 1% DMSO were always applied in the controls. Unless stated otherwise, cells were incubated at 37°C for 24 hrs.

Total RNA extraction and real time RT-PCR

Total RNA of Caco-2 cells were extracted by using the total RNA purification kit according to the manufacturer’s instructions. Primer Express software (Applied Biosystems) was applied to design the primers and probes of human apoM, apoA1, SHP1, LRH-1, ABCA1 and GAPDH (the sequences of primers and probes are shown in Table 1). Quantifications of mRNA levels were performed on a LightCycler in a final volume of 25 μl. Optimal conditions were obtained with 2.5 μl of 10 × PCR buffer, 1.5 μl of 25 mM MgCl₂, 0.5 μl of 10 mM 4 × dNTPs, 0.25 μl of 5 U/μl Taq DNA polymerase, 0.1 μl of 100 μM specific sense primer, 0.1 μl of 100 μM specific antisense primer, 0.1 μl of 100 μM specific probe and 2 μl template cDNA. Finally 17.95 μl H₂O was added to the reaction mixture. The thermal cycling conditions for human apoM, apoA1, SHP1, LRH-1, ABCA1 and GAPDH were as the following steps: 25°C for 10 min, 48°C for 30 min and 95°C for 5 min to do reverse transcription, and then the reaction mixture was preheated for 2 min at 50°C and for 10 min at 95°C to activate Taq polymerase. After that, a 40-cycle two step PCR was performed consisting of 15s at 95°C and 1 min at 60°C. Samples were amplified simultaneously in triplicates in one-assay run. The prospective amplicon of each gene was amplified and purified, then ligated into the pMD19-T vector. The ligated product was transformed into the E. Coli JM109 competent cells. In brief,

| Gene    | Forward primer | Reverse primer | Probe                     |
|---------|----------------|----------------|---------------------------|
| GAPDH   | gagagtgtaagtgctagtc | cgggctcagcagggt | 5′-FAM-ttgggctgtttgagggcctgctg-TAMRA |
| ApoM    | tgcctccttcgagcctgagc | cgggctcagcagggt | 5′-FAM-ttgggctgtttgagggcctgctg-TAMRA |
| ApoA1   | tgggatactctgttaagggac | cgggctcagcagggt | 5′-FAM-ttgggctgtttgagggcctgctg-TAMRA |
| SHP1    | aaagggcaccacctcccttc | cgggctcagcagggt | 5′-FAM-ttgggctgtttgagggcctgctg-TAMRA |
| LRH-1   | ttagctcagcagttgacctcc | cgggctcagcagggt | 5′-FAM-ttgggctgtttgagggcctgctg-TAMRA |
| ABCA1   | caaggggttaggaaagagagac | cgggctcagcagggt | 5′-FAM-ttgggctgtttgagggcctgctg-TAMRA |
a serial dilution of extracted plasmid DNA was used to generate a standard curve by plotting the cycle threshold versus the log initial copy number of input plasmid DNA. Standard curves of apoM, apoA1, SHP1, LRH-1, ABCA1 and GAPDH achieved a very high correlation coefficient (r = 1.00). The ratio between the target gene and GAPDH was calculated as the relative gene expression.

Statistical analysis
Data are expressed as means ± SE. All experiments were repeated twice and the data were represented one of the experiments. Statistical analyses were performed with the software Prism (version 5.0). Multiple comparisons were performed with one-way ANOVA/dunnett-t, and comparisons between two groups were statistically evaluated by the unpaired t-test. Significance was established at a P value less than 0.05.

Results
As shown in Figure 1, TO901317 could significantly upregulate mRNA levels of ABCA1, apoA1, apoM, LRH-1 and SHP1 with dose-dependent manners. At 1.0 μM TO901317, apoM mRNA level was increased by 24% (P <

![Graphs showing effects of TO901317 on mRNA levels of ABCA1, apoA1, apoM, LRH-1, and SHP1 in Caco-2 cells.](image-url)
0.05), and at 5.0 μM TO901317, increased by more than 100% \((P < 0.01)\) compared to the controls. When Caco-2 cells were cultured with guggulsterone alone, mRNA levels of SHP1, LRH-1 and apoM were significantly inhibited \((P < 0.05)\); apoA1 decrease by 6% and 16% respectively; However, ABCA1 were significant upregulated (Figure 2), which were also dose dependents. As shown in Figure 3, it demonstrated that guggulsterone could abolish TO901317 induced upregulation of apoA1, SHP1, LRH-1 and apoM. When Caco-2 cells cultured with 5 μM TO901317 together with 3.0 μg/ml guggulsterone, the mRNA levels of apoA1, SHP1, LRH-1 and apoM were only 85%, 70%, 77% and 74% compared to the cells cultured with TO901317 alone, respectively, whereas ABCA1 were not affected compared to the cells cultured with TO901317 alone (Figure 3).

**Discussion**

ApoM has firstly been identified from human postprandial lipoproteins by Xu and Dahlbäck in 1999 [6]. Recent observations strongly suggest that apoM is predominantly confined to the HDL particles in human plasma and it may have antiatherogenic properties involving in the conversion of large HDL to pre-β HDL, the later mainly functions as an acceptor of peripherally deposited cholesterol that is described as the revise cholesterol transportation [9,17]. Moreover other researches indicated that apoM might be also involved in the immunity, inflammation, and neoplasia [18-20]. However, the physio-pathological functions of apoM are still not fully revealed. Observation of the regulation of apoM expression may reveal clinical importance of apoM in humans.

![Figure 2](image-url) Effects of guggulsterone on mRNA levels of SHP1, LRH-1 and apoM in Caco-2 cells. Caco-2 cells were cultured with different concentrations of guggulsterone for 24 hrs. The mRNA levels of ABCA1, apoA1, SHP1, LRH-1 and apoM were determined by real-time RT-PCR in triplicates. The controls were represented as 100%. Data represents one of two similar experiments (means ± SE, n = 4-6). *\(p < 0.05\); **\(p < 0.01\); ***\(p < 0.001\) vs. controls.
Previous studies have demonstrated that hepatic apoM expression could be regulated by certain cytokines and nuclear factors. In our previous study [15], we demonstrated that LXR agonist, TO901317, could significantly downregulate apoM expression in the hepatic cell line, HepG2 cells. Moreover, Calayir., et al. [16] confirmed our findings that TO901317 did inhibit apoM expression in HepG2 cells, however, they found that TO901317 could significantly upregulate apoM expression in intestinal cell, which suggest that TO901317 regulating apoM expression is cellular dependent. Moreover, Mosialou., et al. [12] reported that the natural LXR ligand oxysterols could significantly upregulate apoM mRNA level and protein level in HepG2 cells, and LXR could bind to the HRE in the proximal apoM promoter examined by the Chromatin Immunoprecipitation Assays (CHIP). They found that TO901317 caused an increased mRNA level of Short Heterodimer Partner (SHP) in HepG2 cells. In contrast, the SHP mRNA level was not affected by the oxysterols. Enhanced expression of SHP could inhibit LRH-1-mediated trans-activation of apoM promoter in HepG2 cells [11]. SHP is an inhibitory nuclear receptor activated by the FXR that interacts physically with many nuclear receptors including LRH-1. Furthermore, it has been reported that TO901317 is a dual LXR/FXR agonist that activates FXR more efficiently than its natural ligand, the bile acids [21]. Based on the findings described above, the TO901317 downregulating apoM expression in hepatic cells may be due to the activation of the FXR/SHP pathway that inhibits LRH-1.

In the present studies, we further investigated effects of TO901317 on apoM expression in Caco-2 cells mediated by ABCA1, ApoA1, ApoM, LRH-1, and SHP1. Caco-2 cells were cultured with 5 µM TO901317 together with 3.0 µg/ml guggulsterone for 24 hrs. The mRNA levels of ABCA1, apoA1, SHP1, LRH-1 and apoM were determined by real-time RT-PCR in triplicates. The cells cultured with TO901317 alone were represented as 100%. Data represents one of two similar experiments (means ± SE, n = 4-6). *p < 0.05; **p <0.01; ***p <0.001 vs. controls.
via the FXR/SHP/LRH-1 pathway. TO901317 could significantly upregulate the mRNA levels of ABCA1 that is a classic LXR downstream gene [14]. LXR can bind to the HRE upstream in the proximal apoM promoter to upregulate apoM gene expression. As SHP levels were enhanced simultaneously and SHP is a classic FXR downstream gene, which suggests that FXR has been activated simultaneously [22], whereas the LRH-1 was not inhibited by the overexpressed SHP. LRH-1 was induced by the SHP and the apoM mRNA levels were upregulated. The similar phenomenon was seen in apoA1 expression [23], TO901317 could downregulate apoA1 mRNA levels in HepG2 cells but it could be upregulated in the Caco-2 cells. Furthermore we demonstrated that FXR antagonist, guggulsterone, could abolish TO901317 increased expressions of apoA1, apoM, SHP1 and LRH-1 in Caco-2 cells, although guggulsterone alone might also decrease mRNA levels of apoA1, apoM, SHP1 and LRH-1. All these findings strongly suggest that activating FXR may mediate upregulative apoM expression in the Caco-2 cells.

Acknowledgements

This research project was supported by the national Natural Science Foundation of China (NSFC) (39072955), the research grant of Jiangsu province (BK2008140) and the research grant of the Changzhou Health Bureau (B200901).

Authors’ contributions

ZCW participated in the assay of RT-PCR. JW performed the statistical analysis. YPS participated in cell culture. DD, XYZ, GHL, MBS, PNE and NX participated in the project design. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 22 September 2011 Accepted: 4 November 2011 Published: 4 November 2011

References

1. Briffa TG, Sanfilippo FM, Hobbs MS, Ridout SC, Katzenellenbogen JM, Thompson PL, Thompson SC. Under-ascertainment of Aboriginality in cardiovascular disease in hospital morbidity and mortality data in Western Australia: a record linkage study. BMC Med Res Methodol 2010, 10:11-116.
2. Vardavas CI, Linardakis MK, Hatzi CM, Saris WH, Kafatos AG. Cardiovascular disease risk factors and dietary habits of farmers from Crete 45 years after the first description of the Mediterranean diet. Eur J Cardiovasc Prev Rehabil 2010, 17(4):440-446.
3. Andrikoula M, McDowell IF. The contribution of ApoB and ApoA1 measurements to cardiovascular risk assessment. Diabetes Obes Metab 2008, 10(4):271-278.
4. Panayiotou A, Griffin M, Georgiou N, Bond D, Tyllis T, Tzakouri-Shakkall C, Fessas C, Nicolas A. ApoB/ApoA1 ratio and subclinical atherosclerosis. Int Angiol 2008, 27(1):74-80.
5. Arsenault BJ, Boekholt SM, Kastelen JJ. Lipid parameters for measuring risk of cardiovascular disease. Nat Rev Cardio 2011, 8(4):197-206.
6. Xu N, Dahlback B. A novel human apolipoprotein (apoM). J Biol Chem 1999, 274(44):31286-31290.
7. Christoffersen C, Nielsen LB, Axler O, Andersson A, Johnsen AH, Dahlback B. Isolation and characterization of human apolipoprotein M-containing lipoproteins. J Lipid Res 2006, 47(8):1833-1843.
8. Christoffersen C, Obinata H, Kumaraswamy SB, Galvani S, Ahnstrom J, Sxevana M, Eggerer-Sieber C, Muller YA, Hia T, Nielsen LB, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. Proc Natl Acad Sci USA 2011.
9. Wolfrum C, Poy MN, Staffel M. Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis. Nat Med 2005, 11(4):418-422.
10. Christoffersen C, Jauhiainen M, Moser M, Porse B, Enhholm C, Boesi M, Dahlback B, Nielsen LB. Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low density lipoprotein receptor knock-out mice. J Biol Chem 2008, 283(4):1839-1947.
11. Venteclaf N, Hanoriti A, Toutain JJ, Tallandits I, Delverze P. Regulation of anti-atherogenic apolipoprotein M gene expression by the orphan nuclear receptor LRH-1. J Biol Chem 2008, 283(7):3964-3970.
12. Mosialou I, Zarmis VI, Kardassi D. Regulation of human apolipoprotein m gene expression by orphan and ligand-dependent nuclear receptors. J Biol Chem 2010, 285(40):30719-30730.
13. Zhang Y, Chen CJ, Yang QL, Chen LQ, Wang H, Huang LZ. Effect of interfering hepatocyte nuclear factor-1 alpha in HepG2 on the expressions of apolip, apoA-I and the correlative key enzyme of cholesterol metabolism. Zhonghua Gan Zang Bing Za Zhi 2011, 19(2):121-126.
14. Lu X, Yeh V, Molteni V, Liver X receptor modulators: a review of recently patented compounds (2007 - 2009). Expert Opin Ther Pat 2010, 20(4):535-562.
15. Zhang X, Zhu Z, Luo G, Zheng L, Nilsson-Ehle P, Xu N. Liver X receptor agonist downregulates hepatic apoA1 expression in vivo and in vitro. Biochem Biophys Res Commun 2008, 371(1):114-117.
16. Calayir E, Becker TM, Kratzer A, Ebner B, Panzenbock U, Stefujl J, Kostmer GM. LXR-agonists regulate ApoM expression differentially in liver and intestine. Curr Pharm Biotechnol 2008, 9(6):516-521.
17. Huang XS, Zhao SP, Hu M, Luo YP. Apolipoprotein M likely extends its anti-atherogenic via anti-inflammation. Med Hypotheses 2007, 69(1):136-140.
18. Feingold KR, Shigenaga MK, Chen L, Goldberg W, Grunfeld C. Infection and inflammation decrease apolipoprotein M expression. Atherosclerosis 2008, 199(1):19-26.
19. Luo G, Zhang X, Mu Q, Chen L, Zheng L, Wei J, Berggren-Soderlund M, Nilsson-Ehle P, Xu N. Expression and localization of apolipoprotein M in human colorectal tissues. Lipids Health Dis 2010, 9(10).
20. Jiang J, Wu C, Luo G, Zheng L, Chen L, Zhang X, Xu N. Expression of apolipoprotein M in human hepatocellular carcinoma tissues. Acta Histochem 2011, 113(1):53-57.
21. Houck KA, Borchert KM, Hepler CD, Thomas JS, Bramlett KS, Michael LF, Burns TP. TO901317 is a dual LXR/FXR agonist. Mol Genet Metab 2004, 83(1-2):184-187.
22. Liu G, Thomas AM, Hart SN, Zhong X, Wu P, Guo QL. Farnesoid X receptor activation mediates head-to-tail chromatin looping in the Nr0b2 gene encoding small heterodimer partner. Mol Endocrinol 2010, 24(7):1404-1412.
23. Huuskonen J, Vihma M, Chau P, Fielding PE, Fielding CJ. Liver X receptor inhibits the synthesis and secretion of apolipoprotein A1 by human liver-derived cells. Biochemistry 2006, 45(50):15068-15074.