Original Article

Investigating the Effect of Aspirin on apoAI-Induced ATP Binding Cassette Transporter 1 Protein Expression and Cholesterol Efflux in Human Astrocytes

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Abstract

Background: Neurons need a high amount of cholesterol to maintain the stability of their membrane-rich structures. Astrocytes synthesize and distribute cholesterol to neurons, and ABCA1 is a key mediator of cholesterol efflux to generate HDL for cholesterol transport in the brain. Several studies imply the effect of aspirin on ABCA1 expression in peripheral cells such as macrophages. Here, we compared the effect of aspirin with apoA-I on ABCA1 protein expression and cholesterol efflux in human astrocytes.

Materials and Methods: Human astrocytes were cultured, and the effects of aspirin on the expression and protein levels of ABCA1 were investigated through RT-PCR and Western blot analysis. Additionally, the effect of co-treatment with apoA-I and aspirin on ABCA1 protein level and cholesterol efflux was evaluated.

Results: Dose and time-course experiments showed that the maximum effect of aspirin on ABCA1 expression occurred at a concentration of 0.5 mM after 12 h of incubation. RT-PCR and western blot data showed that aspirin upregulates ABCA1 expression by up to 4.7-fold and its protein level by 67%. Additionally, co-treatment with aspirin and apoA-I increased cholesterol release from astrocytes, indicating an additive effect of aspirin on apoAI-mediated cholesterol efflux.

Conclusions: The results suggest a potential role of aspirin in increasing ABCA1 expression and cholesterol efflux in astrocytes, similar to the effect of apoA-I. This indicates that aspirin could potentially regulate brain cholesterol balance and can be considered in certain neurological diseases, in particular in some neurological disorders related to cholesterol accumulation such as Alzheimer's disease.

Keywords: ABCA1, apolipoprotein A-I, aspirin, astrocytes, cholesterol

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Introduction

The brain requires a steady-state level of cholesterol and intermediates from the mevalonate pathway for normal neurophysiological processes.[1,2] Cholesterol transport between the periphery and brain cells is inhibited due to the blood-brain barrier.[3-5] Astrocytes, a type of brain glial cell, synthesize and transport cholesterol to support neurons in the form of apoE-HDL particles.[6-9] HMGCR and ABCA1 are necessary for cholesterol synthesis and efflux to facilitate cholesterol transport to lipid-poor apoE to generate apoE-HDL particles. Deficiency of ABCA1 can reduce cholesterol secretion to exogenous apoE and promote the accumulation of cholesterol

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in astrocytes.[10,11] Cholesterol accumulation can lead to complications such as Alzheimer's and Niemann-Pick C.[12] Several studies have reported a direct correlation between aspirin and the expression of mediators involved in HDL formation, inducing ABCA1 and SR-B1 as cholesterol efflux mediators in macrophages.[13-16]

The former observations have proved evidence emphasizing the protective effect of aspirin against cholesterol deposition and atherosclerosis in peripheral tissues. There are no reports showing the effects of aspirin on ABCA1 expression in the brain, which is the main player in cholesterol release in astrocytes.[17,18]

The aim of our study is to address whether aspirin affects ABCA1 gene expression and its protein level leading to the enhancement of cholesterol release in human astrocytes. So, we investigate the aspirin effects on both ABCA1 expression and protein level in human astrocytes. Cholesterol release was also determined after treating the cells with aspirin. In western blot and cholesterol efflux experiments, we used apoA-I as a positive control, which has a very well-known effect on ABCA1 and promotes cholesterol efflux. This study may open a window that aspirin treatment can result in brain cholesterol balance in some cholesterol-related neurological disorders through induction of ABCA1 expression in human astrocytes.

Materials and Methods

Human astrocytes, experimental groups

Human astrocytes were purchased from Pasteur Institute of Iran, Tehran, Iran, and grown in DMEM Low Glucose (Bioidea, Iran) supplemented with 10% FBS (Gibco, Invitrogen, US), 1% Pen/strep, and 0.1% amphotericin B. Cells were cultured[19] *in vitro* in 75 cm^2 culture flasks and incubated at 37°C in 5% CO2. Upon reaching 80% confluence, the cells were rinsed with DPBS and treated with 0.1% trypsin. Trypsin was then inactivated by adding DMEM/10% FBS in equal volume. Finally, cells were seeded in 7 cm^2 cell culture plates at a density of 3×10^6 cells/plate. The cell culture medium was switched to a serum-free medium for 12 h when the cell confluency reached 80%. Three groups were used in this study. Group A, control, received ethanol as the vehicle, Group B received aspirin, and Group C received aspirin + apoA-I. Initially and for the first three experiments, groups A and B were used, and for the last two experiments, all three groups were used.

Cell treatment

Cells received treatment in a serum-free medium and were treated with aspirin (sigma Aldrich Cat. No: A5376). To check the dose-dependency, astrocytes in the 7 cm^2 cell culture plates were treated in 3 ml media with acetylsalicylic acid at different concentrations (0.25, 0.5, 1.0, and 2.0 mM) for 12 h[20] and for time dependency, astrocytes were treated with 0.5 mM of aspirin for 0–36 h. The same volume of 80% ethanol, as the vehicle, was added to the plate of control cells. Each concentration was tested in triplicate.

RT-PCR

After aspirin treatment, cells were harvested with 0.1% trypsin and washed with PBS three times. Using a Viogene total RNA extraction kit (Cat. No: GR1001), total mRNA was extracted, and its concentration was determined by Thermo Scientific Nanodrop 2000 based on OD260/280 ratio measurements and its quality was assessed by electrophoresis on 1.2% agarose gel.

cDNA was synthesized using dART RT kit (EURX, cat No: E0801-03). RNA Expression levels were analyzed by quantitative RT-PCR using the SYBR Premix Ex Taq™II (cat No: RR820Q) by the Applied Biosystem 7500 fast Real-Time PCR system. The relative amount of mRNA was calculated using the comparative threshold cycle (Ct) method. Primers used for RT-PCR are listed in Table 1. The RT-PCR data was normalized using Rest 2009 V2.0.13 software, with β-actin used as the housekeeping control gene for expression level comparison.

Cholesterol efflux in condition media

Human astrocytes were treated with 0.5 mM of aspirin or 5 μg/ml of apoA-I for 24 h. At the end of incubation time, the culture medium was replaced with fresh DMEM/0.1% FBS for 4 h. Cholesterol efflux in condition media was measured according to the cholesterol kit protocol (MAK043-IKT Sigma).

Western blotting

Cells were treated with 0.5 mM of aspirin or 5 μg/ml of apoA-I for 12 h and then harvested with RIPA buffer (50 mM Tris, 150 mM NaCl, 5 mM EDTA, 1% Triton-x100, 1% SDS, 0.5% Deoxycholate) containing the protease inhibitor cocktail (Sigma) after washing three times with DPBS. Samples were homogenized by a sonicator (Hielscher, UP50F) and passed through a 25-gauge needle. The suspension was clarified by centrifugation at 5,000 rpm for 10 min. The protein concentration of samples was determined by the Lowry method and was separated by SDS-PAGE and then transferred to a PVDF membrane (Roche). The membranes were incubated with anti-ABCA1 (1: 2,000; Invitrogen) and anti β-actin (1: 10,000, Sigma) overnight. Blots were washed with TBST and incubated with a horseradish peroxidase-conjugated secondary anti-mouse antibody. The membranes were developed with the ECL chemiluminescence system. The WB data was analyzed using ImageJ software and normalized with β–actin or GAPDH. All experiments were run in triplicate.

Table 1: Forward (F) and reverse (R) primer sequences of β-actin (human), GAPDH (Mouse), and ABCA1 used in real-time PCR

Gene	Sequence	PCR product size (pb)
ABCA1 F	5′-CCCAGAGCAAAAAGCGACTC-3′	
ABCA1 R	5′-GGTCATCATCACTTTGGTCCTTG-3′	102
β-actin F	5′- TGGACTTCGAGCAAGAGATG-3′	
β-actin R	5′- GAAGGAAGGCTGGAAGAGTG-3′	137

Statistical data analysis

To evaluate the statistical significance, the differences between groups were determined by using one-way ANOVA in spss 16.0 statistical software. Differences were considered significant at a value of $P < 0.05$ for all tests.

Result

Aspirin increases ABCA1 gene expression in human astrocytes

To investigate the impact of aspirin on the expression of ABCA1, the human astrocytes were treated with different doses of aspirin and mRNA levels of ABCA1 were analyzed by RT-PCR. Quantification of RT-PCR data revealed that ABCA1 gene expression has been significantly increased compared with the control (***$P < 0.0001$), with a 4.7-fold increase in mRNA of ABCA1 at the 0.5 mM of aspirin treatment [Figure 1]. Therefore, in the next experiments, cells were exclusively treated with 0.5 mM of aspirin which showed the most stimulatory effects on ABCA1 expression.

To determine how long it may take for aspirin to exhibit its effects, time-course experiments were carried out. The maximum effect of aspirin was observed after 12 h of incubation, (*** $P < 0.0001$), and interestingly suppression of ABCA1 was observed after 36 h of incubation with 0.5 mM of aspirin in human astrocyte [Figure 2].

Aspirin increases ABCA1 protein level in human astrocytes

To check whether ABCA1 mRNA Levels are reflected in its protein levels, a western blot was done. Western blot data analysis revealed a 67% increase in AB CA1 protein levels in human astrocytes when they were treated with 0.5 mM of aspirin for 12 h compared to that of the untreated control cells, *$P < 0.05$ [Figure 3].

Aspirin enhanced apoAI-mediated cholesterol efflux by upregulation of ABCA1 protein levels

Exposure of human astrocytes to aspirin results in the upregulation of ABCA1 at both mRNA and protein levels. To investigate if this effect on ABCA1 is functional and reflected in its activity, astrocytes were treated with aspirin and apoA-I, then the cell protein levels of ABCA1 [Figure 4] and cholesterol secretion in condition media [Figure 5] were determined. ApoA-I is known as a powerful inducer of ABCA1 stability through interaction with ABCA1 to promote cholesterol efflux. To evaluate the effects of aspirin on ABCA1 activity in lipid release, we treated the cells with apoA-I which is a positive stimulator for increased cholesterol release through ABCA1. Treatment of cells with aspirin alone or aspirin + apoA-I caused a significant increase in ABCA1 protein levels [Figure 4, *$P < 0.05$ and **$P < 0.001$) and in cholesterol secretion [Figure 5, *$P < 0.05$] as compared to the cells treated with aspirin alone, confirming the additive effect of aspirin on apoAI-induced ABCA1 and its ability to enhance cholesterol efflux [Figures 4 and 5].

Discussion

It has been reported that aspirin might have a primary prevention for cardiovascular disease and also a secondary prevention for individuals who have already been diagnosed with some disease such as cardiovascular disease or stroke.[21,22] ABCA1 is a key protein in the cell membrane that plays a very essential role in cellular cholesterol secretion to prevent cell cholesterol accumulation and cardiovascular disease. Several studies have reported the effect of aspirin on induction of ABCA1 expression in peripheral cells[23,24] Despite these findings, there are not any reports showing the effect of aspirin in the brain which is the most cholesterol rich organ in the body.

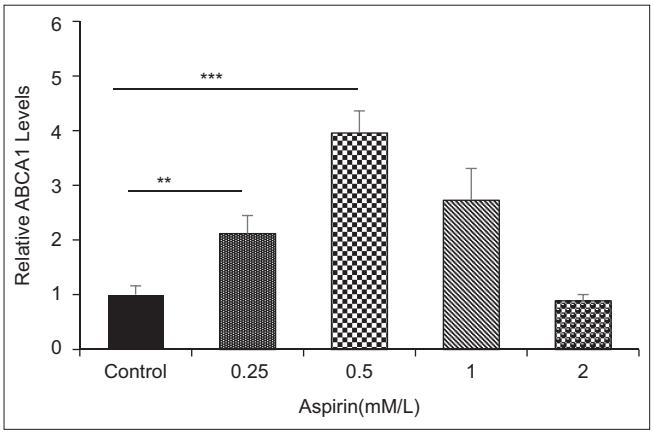

Figure 1: Quantitative real-time PCR analysis of ABCA1 mRNA expression in human astrocytes. Cells were treated with different doses of aspirin for 12 h, and RT-PCR was carried out for mRNA expression of ABCA1. β-actin mRNA was used as a reference. Values are expressed as fold changes relative to the controls. The data represent mean ± SEM from three independent experiments (** $P < 0.001$, *** P,0.0001) versus the control group

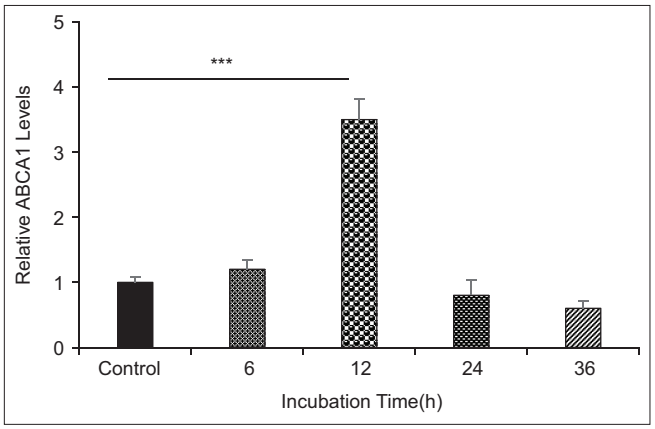

Figure 2: Time course quantitative real-time PCR analysis of ABCA1 m RNA expression in human astrocytes. Cells were treated for the time period of 0, 6, 12, 24, and 36 h with the 0.5 mM aspirin, and RT-PCR was carried out for mRNA expression of ABCA1. β-actin mRNA was used as an internal reference. Values are expressed as fold changes relative to the controls. The data represent mean ± SEM from three independent experiments **$P < 0.0001$ versus the control group

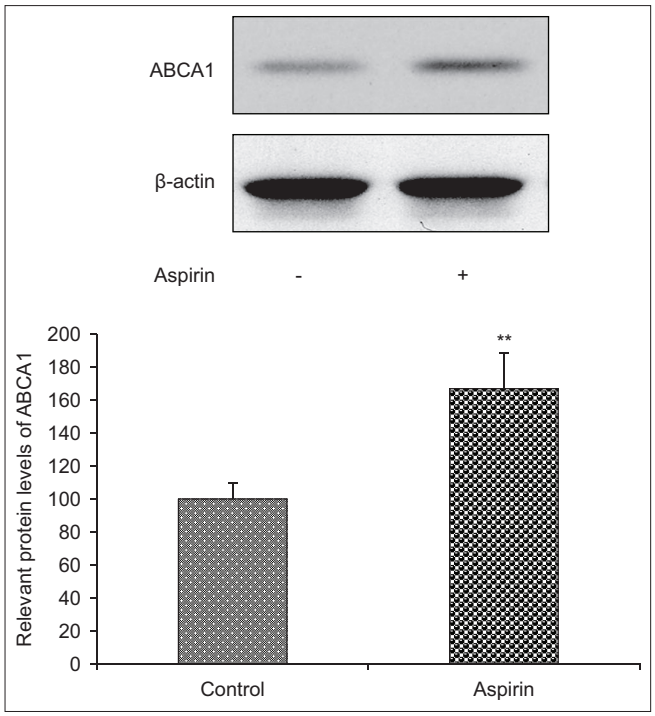

Figure 3: Effects of aspirin on ABCA1 protein levels in human astrocytes. Values are expressed as percent changes compared to the controls. The data represent means ± SEM ($n = 3$). * $P < 0.05$ versus control group. Human astrocytes were treated for 12 h with medium 0.5 mmol/l aspirin and after treatment, cells were harvested by RIPA buffer, and ABCA1 was detected by a specific mouse anti-ABCA1 antibody following the SDS PAGE. Western blotting with an anti-β-actin antibody was used to confirm equal protein loading

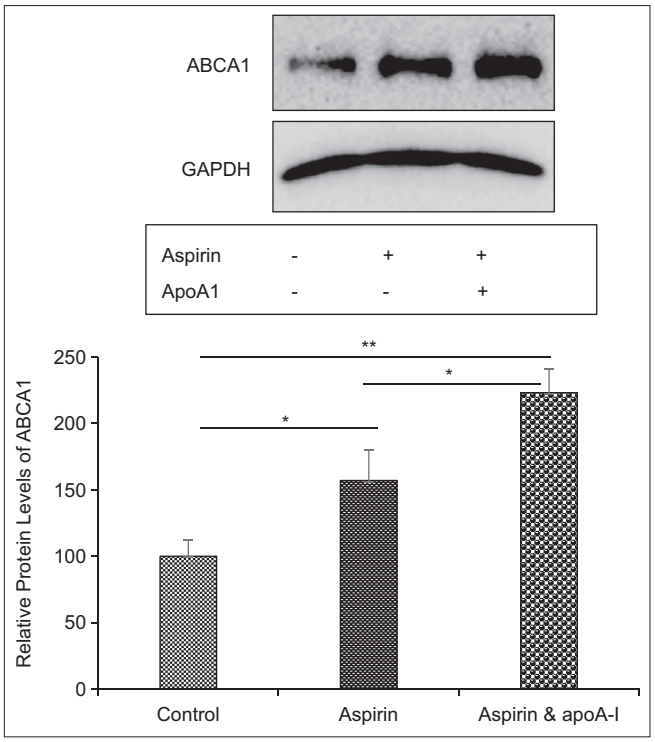

Figure 4: Effects of aspirin and Apo-A1 on ABCA1 protein levels in human astrocytes. Values are expressed as percent changes compared to the controls. The data represent means ± SEM (n = 3), (*$P < 0.05$, **$P < 0.001$) versus the control group. Human astrocytes were treated for 12 h with 0.5 mmol/l aspirin and 5 μl of apoA-I. After treatment, cells were harvested by RIPA buffer, then ABCA1 was detected by a specific mouse anti-ABCA1 antibody following SDS-PAGE. Western blotting with an anti-GAPDH antibody was used to confirm equal protein loading.

Our findings showed that: 1) aspirin increases ABCA1 at the mRNA and protein expression in human astrocytes and 2) aspirin has an additive effect on apoAI-induced ABCA1 expression and cholesterol efflux in human astrocytes. Since ABCA1 is involved in cholesterol efflux, our findings point to the positive effect of aspirin on the cell cholesterol release and reduction of cholesterol accumulation in astrocytes.

Here, we reported that aspirin induces the mRNA and protein expression of ABCA1 in a dose-dependent manner, and these findings are consistent with the previous reports showing the ABCA1-induction after treating macrophages with aspirin.[25,26]

Our RT-PCR data showed that aspirin is able to sharply increase the ABCA1 gene expression by 4.7-fold; however, the ABCA1 protein level showed a 67% increase. A considerable and significant difference between the ABCA1 gene expression and its protein levels indicates that firstly, the protein levels of ABCA1 are highly controlled by a post-transcriptional regulatory mechanism. Secondly, it has been shown that miRNAs have crucial and regulatory roles in ABCA1 translation processes. So, we believe that some specific miRNAs may prevent the translation of ABCA1-mRNA into its functional protein.[27]

ApoA-I can directly interact with ABCA1 and increases its protein stability.[28,29] The western blot and cholesterol release data showed that aspirin, like apoA-I, can significantly induced ABCA1 protein expression. Co-treating the cells with aspirin/apoA-I causes an additional increase in protein level of ABCA1 and cholesterol efflux, suggesting an additive and beneficiary effect of aspirin on apoA-I function. This capability of aspirin leads us to suggest a new role for aspirin in relation to the brain cholesterol homeostasis by enhancing ABCA1 expression which is a very important mediator in the cell cholesterol efflux.

Although, we did not provide evidences for signal transduction related to the effect of aspirin on the ABCA1 expression, according to the literatures it is rational to suggest that the effect of aspirin on ABCA1 expression might be through PPARα activation. Yoshikawa *et al*. reported that ABCA1 gene transcription is highly regulated by some metabolites, such as cAMP, sterols, and peroxisome proliferator-activated receptor (PPAR) agonists.[30] It is reported that treatment of NIH 3T3 fibroblasts or RAW264 macrophages with oxysterols lead to a sharp induction of ABCA1 mRNA. Many studies support the involvement of PPAR agonists in the induction of ABCA1[31-35] in different cell types; however, further studies are necessary to approve this hypothesis.

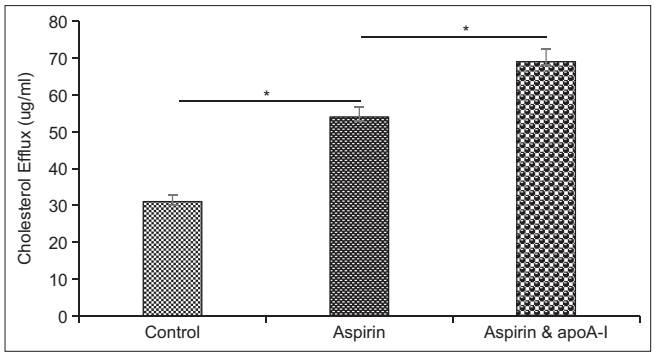

Figure 5: The effect of aspirin and apoA-I on cholesterol efflux. Human astrocytes were treated with 0.5 mM of aspirin or 5 μg/ml of apoA-I for 12 h. At the end of incubation time, the cholesterol efflux in media was measured. Values are expressed as percent changes compared to the controls. The data represent means ± SEM. (*$P < 0.05$) indicate statistical significance

Cholesterol in the astrocytes, as the main supporting cell in the brain, is segregated from the circulation and regulated via endogenous mechanisms.[36,37] including three important pathways: cholesterol synthesis, cholesterol efflux, and cholesterol degradation. Our study highlights a protective role for aspirin in regulating cholesterol efflux in astrocytes which is mediated by increasing ABCA1 protein levels [Figure 4] and cholesterol efflux [Figure 5]. Because apoE (apolipoprotein E) is the predominant acceptor for cholesterol release in the brain,[38] it is interesting to speculate that aspirin might prevent cholesterol accumulation through apoE-HDL generation in astrocytes.

In conclusion, our results revealed a further property and another aspect of aspirin's broad functions. The present study demonstrates that aspirin has a positive and additive effect on the function of apoA-I in ABCA1 induction which plays a very important role in the cholesterol metabolism in astrocytes. To approve this effect of aspirin and investigate the possible therapeutic role of aspirin in relation to brain cholesterol homeostasis, further studies are necessary.

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Conflict of interest

The authors have no conflicts of interest.

References

1. Phillips RE, Yang Y, Smith RC, Thompson BM, Yamasaki T, Soto-Feliciano YM, *et al*. Target identification reveals lanosterol synthase as a vulnerability in glioma. Proc Natl Acad Sci U S A 2019;116:7957-62.
2. Ho WY, Hartmann H, Ling SC. Central nervous system cholesterol metabolism in health and disease. IUBMB Life 2022;74:826-41.
3. Rhea EM, Banks WA. Interactions of lipids, lipoproteins, and apolipoproteins with the blood-brain barrier. Pharm Res 2021;38:1469-75.
4. Chernick D, Ortiz-Valle S, Jeong A, Qu W, Li L. Peripheral versus central nervous system APOE in Alzheimer's disease: Interplay across the blood-brain barrier. Neurosci Lett 2019;708:134306.
5. Zhou B, Zuo YX, Jiang RT. Astrocyte morphology: Diversity, plasticity, and role in neurological diseases. CNS Neurosci Ther 2019;25:665-73.
6. Kheirollah A, Ito J, Nagayasu Y, Lu R, Yokoyama S. Cyclosporin A inhibits apolipoprotein A-I-induced early events in cellular cholesterol homeostasis in rat astrocytes. Neuropharmacology 2006;51:693-700.
7. Hartmann H, Ho WY, Chang JC, Ling SC. Cholesterol dyshomeostasis in amyotrophic lateral sclerosis: Cause, consequence, or epiphenomenon?. FEBS J 2022;289:7688-709.
8. Papotti B, Adorni MP, Marchi C, Zimetti F, Ronda N, Panighel G, *et al*. PCSK9 Affects astrocyte cholesterol metabolism and reduces neuron cholesterol supplying *In Vitro*: Potential implications in Alzheimer's disease. Int J Mol Sci 2022;23:12192.
9. Gliozzi M, Musolino V, Bosco F, Scicchitano M, Scarano F, Nucera S, *et al*. Cholesterol homeostasis: Researching a dialogue between the brain and peripheral tissues. Pharmacol Res 2021;163:105215.
10. Ogura M. HDL, cholesterol efflux, and ABCA1: Free from good and evil dualism. J Pharmacol Sci 2022;150:81-89.
11. Azizidoost S, Babaahmadi-Rezaei H, Nazeri Z, Cheraghzadeh M, Kheirollah A. Amyloid beta increases ABCA1 and HMGCR protein expression, and cholesterol synthesis and accumulation in mice neurons and astrocytes. Biochim Biophys Acta Mol Cell Biol Lipids 2022;1867:159069.
12. Hammond N, Munkacsi AB, Sturley SL. The complexity of a monogenic neurodegenerative disease: More than two decades of therapeutic driven research into Niemann-Pick type C disease. Biochim Biophys Acta Mol Cell Biol Lipids 2019;1864:1109-23.
13. Liu H, Yang Y, Liu Y, Cui L, Fu L, Li B. Various bioactive peptides in collagen hydrolysate from salmo salar skin and the combined inhibitory effects on atherosclerosis *in vitro* and *in vivo*. Food Res Int 2022;157:111281.
14. Hu W, Luo Y, Yang X. Inappropriate use of proton pump inhibitors increases cardiovascular events in patients with coronary heart disease. Int J Gen Med 2022;15:8685-91.
15. Wu Y, Yan B, Xu W, Guo L, Wang Z, Li G, *et al*. Compound C enhances the anticancer effect of aspirin in HER-2-positive breast cancer by regulating lipid metabolism in an AMPK-independent pathway. Int J Biol Sci 2020;16:583-97.
16. Sirtori CR, Ruscica M, Calabresi L, Chiesa G, Giovannoni R, Badimon JJ. HDL therapy today: From atherosclerosis, to stent compatibility to heart failure. Ann Med 2019;51:345-59.
17. Yanai H, Adachi H, Hakoshima M, Katsuyama H. Molecular biological and clinical understanding of the statin residual cardiovascular disease risk and peroxisome proliferator-activated receptor alpha agonists and ezetimibe for its treatment. Int J Mol Sci 2022;23:3418.
18. Milasan A. Assessment and modulation of the lymphatic function throughout the onset and progression of atherosclerosis. 2020.
19. Galland F, Seady M, Taday J, Smaili SS, Gonçalves CA, Leite MC. Astrocyte culture models: Molecular and function characterization of primary culture, immortalized astrocytes and C6 glioma cells. Neurochem Int 2019;131:104538.
20. Madan RK, Levitt J. A review of toxicity from topical salicylic acid preparations. J Am Acad Dermatol 2014;70:788-92.
21. Sperkowska B, Murawska J, Przybylska A, Gackowski M, Kruszewski S, Durmowicz M, *et al*. Cardiovascular effects of chocolate and wine-narrative review. Nutrients 2021;13:4269.

22. Kotlyarov S, Kotlyarova A. Molecular pharmacology of inflammation resolution in atherosclerosis. Int J Mol Sci 2022;23:4808.
23. Deng Q, Li XX, Fang Y, Chen X, Xue J. Therapeutic potential of quercetin as an antiatherosclerotic agent in atherosclerotic cardiovascular disease: A review. Evid Based Complement Alternat Med 2020;2020:5926381.
24. Wang J, Xiao Q, Wang L, Wang Y, Wang D, Ding H. Role of ABCA1 in cardiovascular disease. J Pers Med 2022;12:1010.
25. Wang F, Stappenbeck F, Tang LY, Zhang YE, Hui ST, Lusis AJ, *et al*. Oxy210, a semi-synthetic oxysterol, exerts anti-inflammatory effects in macrophages via inhibition of toll-like receptor (TLR) 4 and TLR2 signaling and modulation of macrophage polarization. Int J Mol Sci 2022;23:5478.
26. Lorkowski SW, Brubaker G, Li L, Li XS, Hazen SL, Smith JD. A novel cell-free fluorescent assay for HDL function: Low apolipoprotein a1 exchange rate associated with increased incident cardiovascular events. J Appl Lab Med 2020;5:544-57.
27. Bhattarai A, Likos EM, Weyman CM, Shukla GC. Regulation of cholesterol biosynthesis and lipid metabolism: A microRNA management perspective. Steroids 2021;173:108878.
28. Ito J, Nagayasu Y, Kheirollah A, Abe-Dohmae S, Yokoyama S. ApoA-I enhances generation of HDL-like lipoproteins through interaction between ABCA1 and phospholipase Cγ in rat astrocytes. Biochim Biophys Acta 2011;1811:1062-9.
29. Shen X, Zhang S, Guo Z, Xing D, Chen W. The crosstalk of ABCA1 and ANXA1: A potential mechanism for protection against atherosclerosis. Mol Med 2020;26:84.
30. Yoshikawa T, Ide T, Shimano H, Yahagi N, Amemiya-Kudo M, Matsuzaka T, *et al*. Cross-talk between peroxisome proliferator-activated receptor (PPAR) alpha and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signaling. Mol Endocrinol 2003;17:1240-54.
31. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, *et al*. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med 2001;7:53-8.
32. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, *et al*. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 2001;7:161-71.
33. Patel D, Roy A, Kundu M, Jana M, Luan CH, Gonzalez FJ, *et al*. Aspirin binds to PPARα to stimulate hippocampal plasticity and protect memory. Proc Natl Acad Sci U S A 2018;115:E7408-17.
34. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, *et al*. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med 2001;7:53-8.
35. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, *et al*. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 2001;7:161-71.
36. Geffken SJ, Moon S, Smith CO, Tang S, Lee HH, Lewis K, *et al*. Insulin and IGF-1 elicit robust transcriptional regulation to modulate autophagy in astrocytes. Mol Metab 2022;66:101647.
37. Wang H, Kulas JA, Wang C, Holtzman DM, Ferris HA, Hansen SB. Regulation of beta-amyloid production in neurons by astrocyte-derived cholesterol. Proc Natl Acad Sci U S A 2021;118:e2102191118.
38. Hirsch-Reinshagen V, Zhou S, Burgess BL, Bernier L, McIsaac SA, Chan JY, *et al*. Deficiency of ABCA1 impairs apolipoprotein E metabolism in brain. J Biol Chem 2004;279:41197-207.