JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

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Journal of Pharmacy
And Pharmacology

Research Paper

Eugenosedin-A prevents high-fat diet increased adhesion molecules through inhibition of MAPK- and p65-mediated NF-κB pathway in rat model

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Keywords
ACAT-1; adhesion molecules; eugenosedin-A; hyperlipidaemia; MAPKs; NF-κB

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Received February 14, 2012
Accepted August 30, 2012

doi: 10.1111/j.2042-7158.2012.01597.x

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Abstract

Objectives Previous studies have shown eugenosedin-A, a 5-$HT_{1B/2A}$ and $\alpha_1/\alpha_2/\beta_1$-adrenergic blocker, is able to decrease cholesterol levels, hyperglycaemia and inflammation in hyperlipidaemic mice induced by high-fat diet (HFD). The aim of this study is to examine the effects of eugenosedin-A on the inhibition of adhesion molecules of platelets, the aorta and acyl-coenzymeA:cholesterol acyltransferase-1 (ACAT-1) of macrophages in a hyperlipidaemic rat model.
Methods Six-week-old Sprague–Dawley rats were randomly divided into two control and treatment groups. The control rats received either a regular diet or HFD and the treatment groups were fed HFD with either 5 mg/kg eugenosedin-A or atorvastatin for a 10-week period.
Key findings Compared with the two control groups, the HFD group had lower levels of high-density lipoprotein, higher concentrations of triglycerides, total cholesterol, low-density lipoprotein and insulin. The expression of adhesion molecules in platelets, aorta and monocyte-macrophage were enhanced by HFD. HFD also increased upstream proteins and their phosphorylated form in the aorta. In treatment groups, eugenosedin-A and atorvastatin improved HFD-induced hyperlipidaemia and levels of insulin. Eugenosedin-A reduced the upregulation of P-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM, PECAM in platelets and inhibited E-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM protein levels in the aorta. Eugenosedin-A reduced the ACAT-1 protein expression of monocyte-macrophages. The expression of PKCα, MAPKs, IKKα and p65 and their phosphorylated form were reduced in treatment groups.
Conclusions Taken together, hyperlipidaemia enhances the expression of adhesion molecules and ACAT-1 protein, and eugenosedin-A ameliorates those increases. Through inhibition of MAPK- and p-65-mediated NF-κB pathway, eugenosedin-A decreases the quantity of adhesion molecules.

Introduction

Hypercholesterolaemia is a major risk factor for cardiovascular diseases and predisposes the vasculature to thrombogenesis and atherosclerosis. The endothelial cell activation, dysfunction and injury that accompany hypercholesterolaemia appear to lead to the adhesion and arrest of circulating cells (leucocytes and platelets) onto the walls of blood vessels.[1] Circulating platelets become hyperactive under hypercholesterolaemic conditions. The adhesion of activated platelets to the vessel wall can exacerbate the inflammation caused by the endothelial cell phenotype induced by hypercholesterolaemia and may contribute to the development and progression of cardiovascular diseases, including atherosclerosis, thrombosis and acute coronary syndromes.[2,3] Endothelial cells activated by hyperlipidaemia

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release several types of adhesion molecules that support blood cell rolling on the vascular surface and subsequent adhesion to the site of activation.[4]

An important consequence of an elevation in blood cholesterol is the activation of both circulating blood cells and endothelial cells that line the walls of large and microscopic blood vessels.[5] The activated endothelium secretes adhesion molecules involved in the firm attachment of platelets, leucocytes and monocytes, such as selectins, intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) and platelet endothelial cell adhesion molecule (PECAM).[6,7] The elevated P-selectin expression on platelets is known to mediate the adhesion of platelets to endothelium and platelet–leucocyte interactions during hypercholesterolaemia.[8,9] Increased expression of E-selectin, which supports the adhesion of neutrophils, certain T lymphocytes and monocytes to endothelium *in vitro*, has been detected in human atherosclerotic lesions.[10,11] PECAM is expressed on the surfaces of many cells involved in atherosclerotic lesion development, including platelets, endothelial cells and the entire surface of the aorta.[12] ICAM-1 mediates attachment, spreading and migration followed by polymorphonuclear leucocyte rolling, while VCAM-1 may preferentially contribute to monocyte adhesion and plays a critical role in initiating the inflammatory response in atherogenesis.

Monocyte-macrophages play an important role in the early stages of atherosclerosis. They transform into foam cells and release cytokines and growth factors, accelerating the development of atherosclerosis. Acyl-coenzyme A : cholesterol acyltransferase (ACAT), an intracellular enzyme located in the rough endoplasmic reticulum, catalyses cholesterol ester formation from cholesterol and fatty acyl-coenzyme A.[13] ACAT-1 is upregulated during differentiation from monocytes into macrophages, and is expressed at high levels by macrophage-derived foam cells in atherosclerotic lesions.[14] On the other hand, the participation of serotonin (5-HT), which may promote coronary events by adhering to unstable atherosclerotic plaque and initiating thrombotic complication, also affects ACAT-1 expression in monocyte-macrophages.[15]

Hypercholesterolaemia increases inflammatory responses and cell adhesion molecules by stimulating the expression of nuclear factor-κB (NF-κB). NF-κB is activated by protein kinase C (PKC) isoforms and different mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK) and p38.[16,17] Riad *et al*.[18] had shown to inhibit the NF-κB pathway beneficial to the amelioration of hyperlipidaemia-induced adhesion molecules. We have shown that eugenosedin-A, a 5-$HT_{1B/2A}$ and $\alpha_1/\alpha_2/\beta_1$-adrenergic blocker,[19] is capable of reducing inflammation, scavenging free radicals[20] and inhibiting platelet aggregation.[21] Eugenosedin-A also reduces obesity-related hyperglycaemia, hyperinsulinaemia, hyperlipidaemia[22] and MAPKs- and p65-mediated NF-κB-induced inflammation.[23] However, to date, not much research has been performed on the serotonergic and α/β-adrenergic receptor antagonistic activity on hyperlipidaemia-induced adhesion molecules. Regarding the novel findings of this study, we demonstrated the action mechanisms of eugenosedin-A on platelets and aorta adhesion molecules, as well as macrophage ACAT-1, in rats with HFD-induced hyperlipidaemia. We suggest that eugenosedin-A could be developed as an adequate agent used to control HFD-induced metabolic responses.

Materials and Methods

Animals

Male Sprague–Dawley rats were provided by the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan) and were housed under constant temperature and illumination (light on 07:30–19:30 h). Water and regular diet were freely available. After an acclimatization period, the 6-week-old rats were randomly divided into four groups (two control groups and two treatment groups) and fed different diets for 10 weeks. One group of control rats was fed a regular diet ($n = 8$) and the other was fed an HFD ($n = 8$) (cat. no. 58G9; TestDiet, Richmond, VA, USA). The HFD contained 60% fat, 21.4% carbohydrates and 18.6% protein. The treatment groups were fed HFD supplemented with either eugenosedin-A or atorvastatin (5 mg/kg). Body weight was measured weekly. At the end of the study, the rats were anaesthetized by urethane (25 mg/kg, i.p.) and blood samples and aortic tissues were collected for the subsequent experiments. Blood samples were used for biochemical assay and flow cytometry. Aortic tissues were stored in buffer solution at −80°C until analysis. This study was approved on 1 August 2010 by the Animal Care and Use Committee of Kaohsiung Medical University IACUC Approval no. 98126.

Drugs and chemicals

Atorvastatin was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Eugenosedin-A (synthesized in our laboratory) and other agents were solubilized in 5% absolute alcohol. Further dilutions of these agents were made in distilled water.

Measurement of biochemical parameters

Blood samples were collected for measurement of lipid profiles (triglycerides, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL)), glucose, glycated haemoglobin (HbA_{1c}) and insulin concentrations.

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Platelet isolation

The heparinized blood was centrifuged at 800 *g* for 10 min. After centrifugation, the upper layer containing predominantly platelets was removed using a clean pasteur pipette. Platelets were counted, resuspended in phosphate-buffered saline (PBS) and stored at room temperature for use within 3 h.

Measurement of adhesion molecules in the platelets

Monoclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Platelets were stained by P-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM antibody-conjugated fluorescein isothiocyanate (FITC) for 30 min, respectively. The positive cell counts and the mean fluorescent intensities were analysed by flow cytometry (Beckman Coulter, Miami, FL, USA) using 488 nm excitation with an argon-ion laser for FITC. Data were processed using E4win-ESP analysis software program (Version 2.5, Beckman Coulter, Miami, FL, USA).

Western blot analysis of adhesion molecule expression in the thoracic aorta tissue

Following previously described procedures,[23] the homogenized tissues were centrifuged at 10 000 *g* for 30 min and the supernatants were stored at −70°C until further analysis. Samples of tissue homogenates were used for protein assay (Bio-Rad protein assay reagent) and Western blot analysis. The aorta tissues were identified by E-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM antibody (1 : 500 dilution; Santa Cruz Biotechnology) and IgG-conjugated antibody (1 : 10 000 dilution; Santa Cruz Biotechnology). The relative expression of those proteins in each tissue was quantified by densitometric scanning of the Western blots using Image-pro plus software (Media Cybernetics, MD, USA).

Western blot analysis of ACAT-1 expression in monocyte-macrophages

Rat monocytes were isolated by Ficoll-Paque PLUS gradient centrifugation as described previously.[24] Purified monocytes were suspended in RPMI medium. After 1 h incubation (37°C, 5% CO_2) for adherence onto dishes, the medium was replaced with RPMI medium containing 10% fetal bovine serum, streptomycin (0.1 mg/ml) and penicillin G (100 U/ml). Adhering monocytes were incubated for 7 days to induce differentiation into macrophages.[15]

Monocyte-macrophages were extracted using 100 μl of 10% sodium dodecyl sulfate (SDS). Thirty-microgram samples of protein were used for protein assay and Western blot analysis. The monocyte-macrophage was identified by ACAT-1 antibody (1 : 500 dilution; Santa Cruz Biotechnology) and IgG-conjugated antibody (1 : 10 000 dilution; Santa Cruz Biotechnology). The relative expression of ACAT-1 was quantified by densitometric scanning of the Western blots using Image-pro plus software (Media Cybernetics).

Western blot analysis of PKCα, IKKα, p65 and MAPK expression

Thoracic aorta were obtained and frozen at −80°C before assay. Frozen tissues were homogenized on ice with a polytron PT 3000 homogenizer (Kinematic AG, Littau, Switzerland) in a buffer composed of (in mM): Tris-HCl 50, EDTA 0.1, EGTA 0.1, 2-mercaptoethanol 12 and phenylmethylsulphonyl fluoride 1 (pH 7.4). The homogenized tissues were centrifuged at 10 000*g* for 30 min and the supernatants were stored at −80°C until further analysis. Samples of tissue homogenates were used for protein assay (Bio-Rad protein assay reagent) and Western blot analysis. Tissue homogenates containing 20 μg of protein were reduced and separated by SDS-polyacrylamide gel electophoresis (SDS-PAGE) on a 7.5% gel using PhastSystem with PhastGel (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). The membranes were blocked with 1% bovine serum albumin in Tris buffer solution (TBS) containing 0.1% Tween-20 for 2 h; it was then incubated with PKCα, IKKα, p65, p38α, JNK1, ERK-1/2 and their phosphorylated antibodies (1 : 500 dilution; Santa Cruz Biotechnology) in TBS containing 0.1% Tween-20 for 2 h. The membrane was washed and finally incubated with a 1 : 1000 dilution of IgG conjugated to horseradish antibody for 2 h. After successive washings, the immunocomplexes were developed using an enhanced horseradish peroxide/luminol chemiluminescence reaction (ECL Western blotting detection reagents; GE Healthcare Bio-Sciences Corp.) and exposed to X-ray film for 10 min. The relative expression of those proteins in each tissue was quantified by densitometric scanning of the Western blots using Image-pro plus software (Media Cybernetics) as previously described.[23]

Statistical evaluation of data

Results are expressed as mean ± SE. Statistical differences were determined by independent and paired Student's *t*-test in unpaired and paired samples, respectively. Whenever a control group was compared with more than one treatment group, one-way analysis of variance or two-way repeated measures analysis of variance was used. If a significant difference was found, we used Dunnett's or Student–Newman–Keuls test for further analysis. $P < 0.05$ was considered significant in all experiments. Analysis of data and plotting of figures were performed using SigmaStat: Version 2.03 and SigmaPlot: Version 8.0 (Systat Software, Point Richmond, CA, USA).

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Results

Body weight and weight gain

The untreated HFD control group gained significantly more weight in total than the regular diet control group. The HFD groups treated with the test agents gained weight at a more gradual rate, suggesting that eugenosedin-A and atorvastatin (5 mg/kg) inhibited weight gain to some degree (Table 1).

Table 1 Effect of eugenosedin-A on body weight and weight gain in rats fed high-fat diet

Group	Body weight (g)		Weight gain (%)
	Initial	Final	
Regular diet	273.6 ± 13.1	442.2 ± 27.6	61.6
HFD	271.4 ± 14.3	551.3 ± 38.2#	103.1#
HFD + eugenosedin-A	275.6 ± 15.5	462.3 ± 25.5*	67.7*
HFD + atorvastatin	272.9 ± 15.3	457.3 ± 24.3*	67.6*

HFD, high-fat diet. Eugenosedin-A or atorvastatin was supplemented with 5 mg/kg. Values represent the mean ± SE, $n = 8$.#$P < 0.05$ vs regular diet;*$P < 0.05$ vs HFD.

Plasma biochemical parameters

The untreated HFD group had a 1.7-fold higher plasma triglyceride level, 1.9-fold higher total cholesterol level, 2-fold higher LDL level and a 1.4-fold lower HDL level than the untreated regular diet group (Table 2). The eugenosedin-A-treated group were found to have clear ameliorated levels of triglycerides (21%), cholesterol (30%), LDL (38%) and HDL (44%). The insulin concentration of the untreated HFD group was 1.5-fold higher than that of the untreated regular-diet group. Eugenosedin-A reversed the HFD-induced increases of insulin levels. However, we did not find any appreciable differences in the levels of blood glucose and HbA_{1c} between any of the groups (data not shown).

Analysis of adhesion molecules in the platelets

As shown in Figure 1, the expression of ICAM-1, ICAM-2 and ICAM-3 on isolated platelets of the untreated HFD group was increased, while eugenosedin-A and

Table 2 Effect of eugenosedin-A on plasma biochemical variables in rats fed high-fat diet

Group	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Insulin (μg/l)
Regular diet	95.2 ± 7.1	49.5 ± 8.6	16.1 ± 4.1	32.5 ± 3.3	0.27 ± 0.04
HFD	163.7 ± 8.6#	93.1 ± 8.5#	32.4 ± 4.5#	23.7 ± 3.2#	0.42 ± 0.08#
HFD + eugenosedin-A	128.6 ± 8.3*	65.4 ± 9.5*	20.2 ± 5.6*	34.2 ± 3.8*	0.24 ± 0.08*
HFD + atorvastatin	110.4 ± 9.4*	68.7 ± 9.4*	19.8 ± 5.3*	37.6 ± 3.1*	0.26 ± 0.07*

HFD, high-fat diet. Eugenosedin-A or atorvastatin was supplemented with 5 mg/kg. Values represent the mean ± SE, $n = 8$.#$P < 0.05$ vs regular diet;*$P < 0.05$ vs HFD.

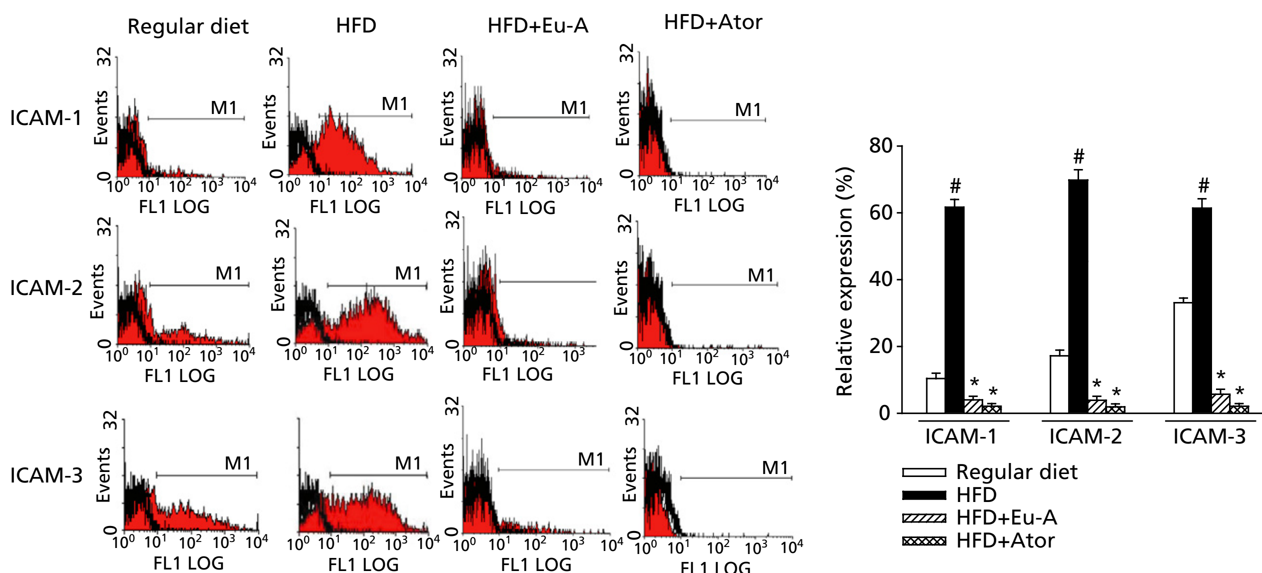

Figure 1 Effect of eugenosedin-A (Eu-A) and atorvastatin (Ator) on ICAM-1, ICAM-2 and ICAM-3 of platelets in high-fat diet (HFD)-fed rats. Each value represents the mean ± SE, $n = 8$. #$P < 0.05$ vs regular diet; *$P < 0.05$ vs HFD.

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atorvastatin significantly inhibited the expression of ICAM-1, ICAM-2 and ICAM-3 on platelets of the hyperlipidaemic rats.

Flow cytometric analysis on isolated platelets showed that the untreated hyperlipidaemic rat group had greater increases in VCAM, PECAM and P-selectin than the untreated regular-diet control group (Figure 2). Eugenosedin-A and atorvastatin clearly decreased the expression of VCAM, PECAM and P-selectin in the hyperlipidaemic rats.

E-selectin protein expression in thoracic aorta

Western blot analysis revealed that the untreated HFD group had increased expression of E-selectin proteins in vascular tissues, where it was found to be 1.8-fold higher than in the regular diet control group. Eugenosedin-A and atorvastatin markedly decreased the elevated E-selectin protein levels by 44% and 32%, respectively, in hyperlipidaemic rats (Figure 3).

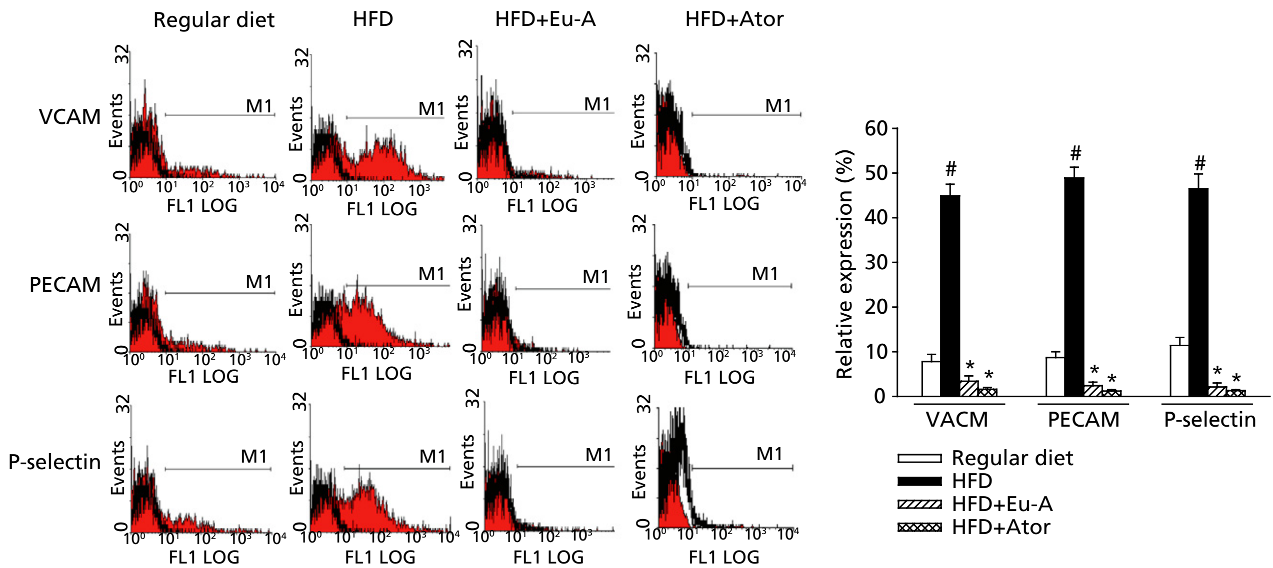

Figure 2 Effect of eugenosedin-A (Eu-A) and atorvastatin (Ator) on VCAM, PECAM and P-selectin of platelets in high-fat diet (HFD)-fed rats. Each value represents the mean ± SE, $n = 8$. #$P < 0.05$ vs regular diet; *$P < 0.05$ vs HFD.

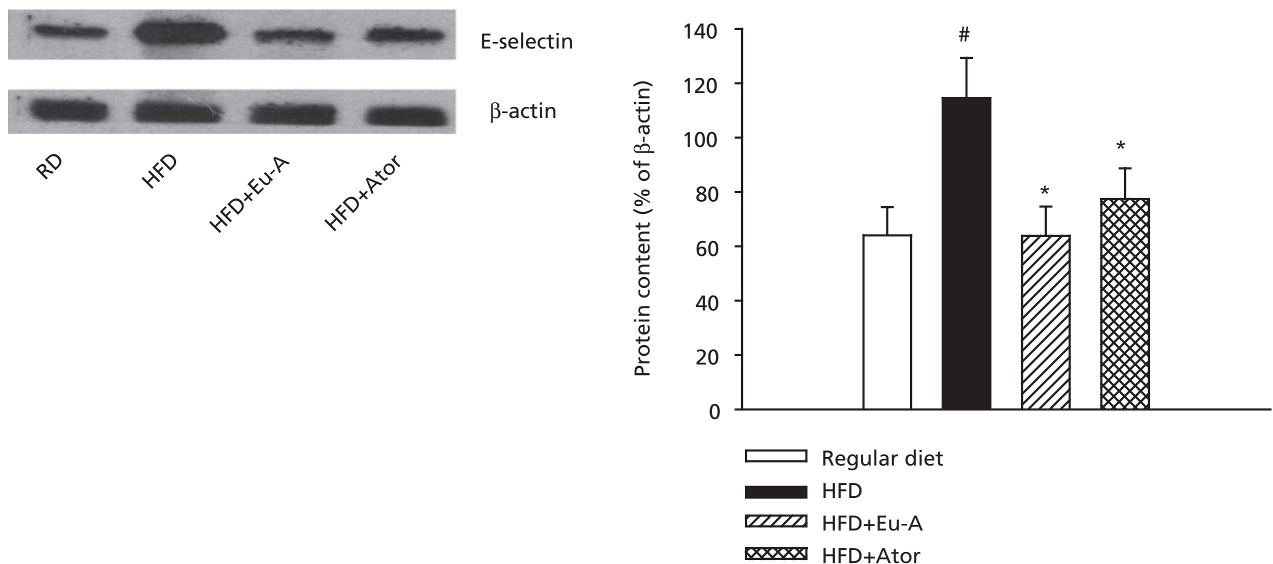

Figure 3 Effect of eugenosedin-A (Eu-A) and atorvastatin (Ator) on E-selectin in the aorta of high-fat diet (HFD) fed rats. Each value represents the mean ± SE, $n = 8$. #$P < 0.05$ vs regular diet; *$P < 0.05$ vs HFD.

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ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM protein expression in thoracic aorta

Western blot analysis revealed that the thoracic aorta isolated from untreated HFD control group had higher levels of ICAM-1 (1.3 fold), ICAM-2 (1.3 fold), ICAM-3 (2 fold), VCAM (1.3 fold) and PECAM (1.4 fold) proteins than the regular diet group. Eugenosedin-A and atorvastatin reduced the elevated expression of those proteins. Eugenosedin-A significantly reduced ICAM-1 (by 25%), ICAM-2 (22%), ICAM-3 (44%), VCAM (23%) and PECAM (39%), and atorvastatin reduced ICAM-1 (22%), ICAM-2 (24%), ICAM-3 (32%), VCAM (18%) and PECAM (32%) in thoracic aorta of HFD-fed rats (Figure 4).

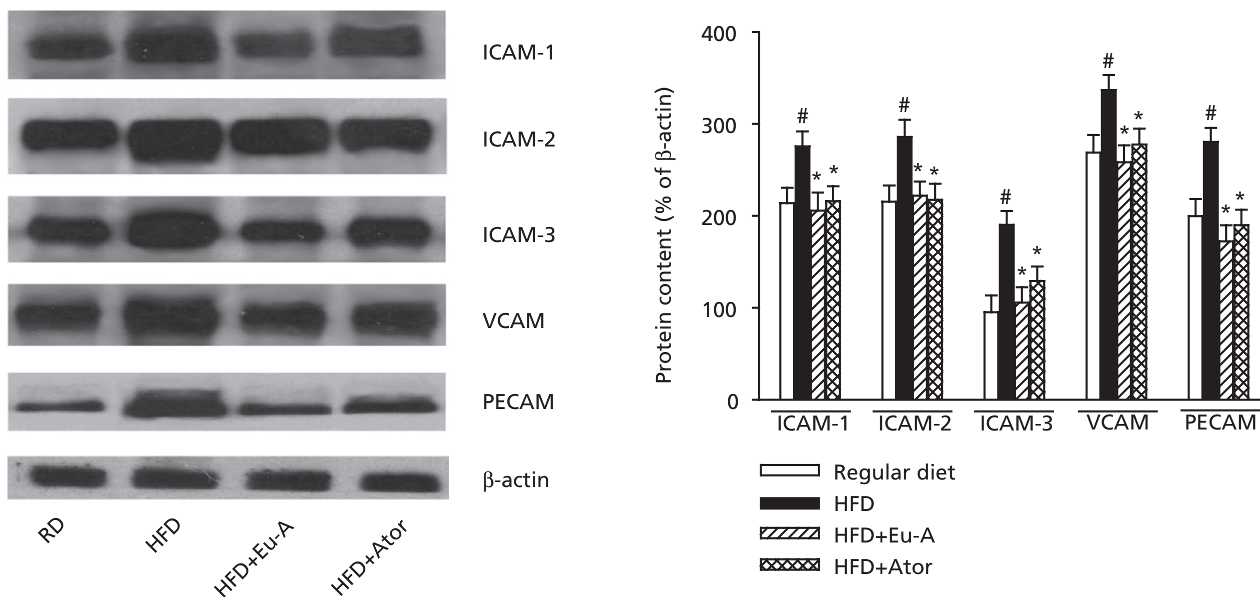

Figure 4 Effect of eugenosedin-A (Eu-A) and atorvastatin (Ator) on ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM of aorta in high-fat diet (HFD)-fed rats. Each value represents the mean ± SE, $n = 8$. #$P < 0.05$ vs regular diet; *$P < 0.05$ vs HFD.

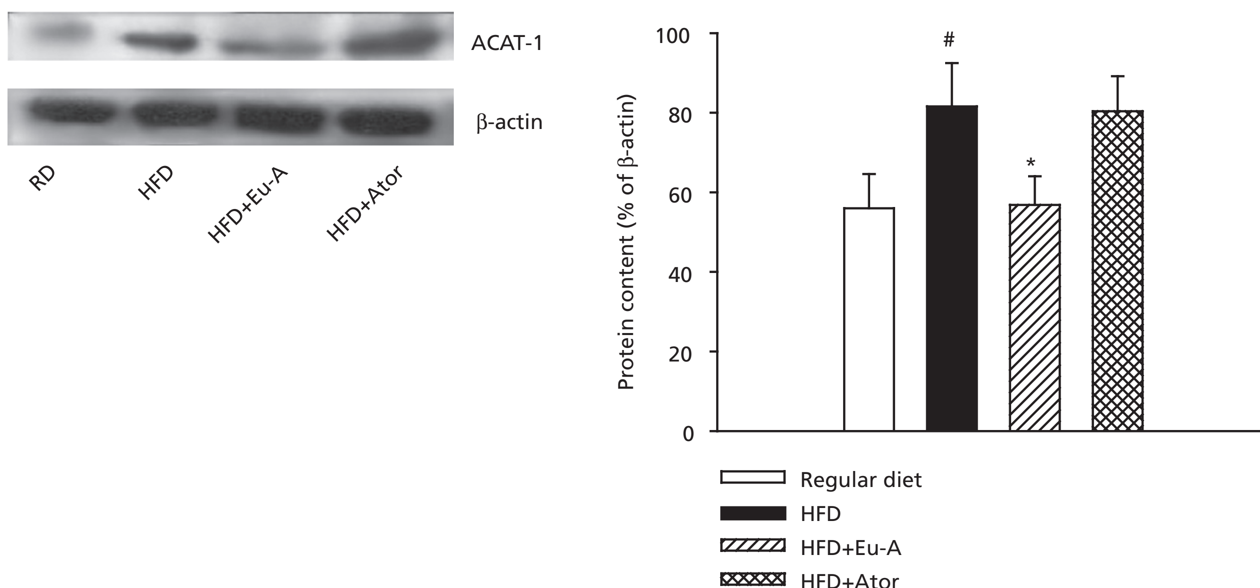

Figure 5 Effect of eugenosedin-A (Eu-A) and atorvastatin (Ator) on ACAT-1 of macrophages in high-fat diet (HFD)-fed rats. Each value represents the mean ± SE, $n = 8$. #$P < 0.05$ vs regular diet; *$P < 0.05$ vs HFD.

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ACAT-1 expression in the monocyte-macrophage

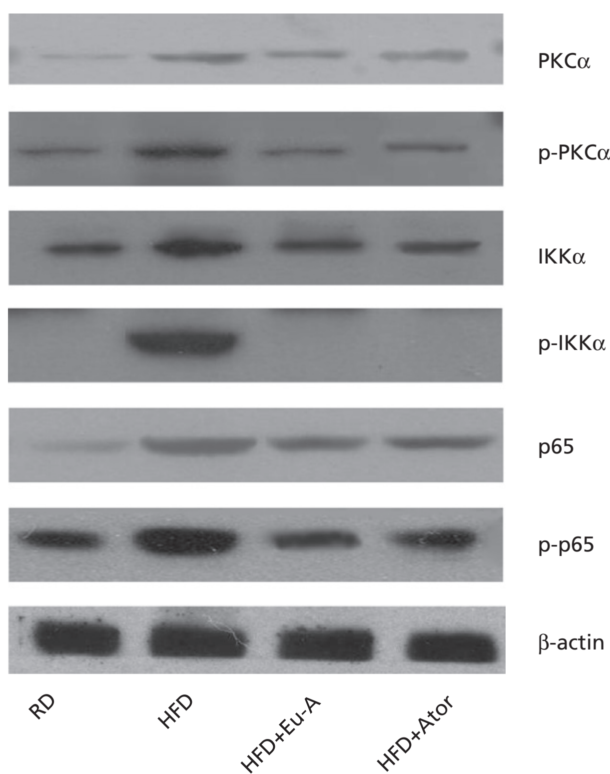
Figure 5 depicts the expression of ACAT-1 protein during differentiation from monocytes into mature macrophages in the rats. Compared with the regular diet group, ACAT-1 protein expression was increased by 1.5 fold in the monocyte-macrophages of the HFD group. Eugenosedin-A significantly reduced (by 30%) the ACAT-1 protein enhancement in HFD-fed rats, whereas atorvastatin did not.

PKCα, MAPKs, IKKα and p65 protein expression in thoracic aorta

Comparing the expression of proteins in the two control groups, the HFD-fed group had significant increases in PKCα, IKKα, p65 and their phosphorylated form in the aorta (Figure 6). Eugenosedin-A, like atorvastatin, clearly decreased those protein levels in HFD-fed rats in aorta. The expression of p38α, JNK-1 and ERK-1/2, as well as their phosphorylated forms, were also enhanced in the HFD-fed rats (Figure 7). Eugenosedin-A and atorvastatin significantly reduced the elevated levels of these proteins. Eugenosedin-A and atorvastatin also decreased the activity of the proteins.

Discussion

We have demonstrated that eugenosedin-A, a 5-$HT_{1B/2A}$ and $\alpha_1/\alpha_2/\beta_1$-adrenergic blocker, was able to decrease the cholesterol levels and hyperglycaemia of hyperlipidaemic mice induced by HFD.[22] Eugenosedin-A was also found to prevent HFD-induced inflammation and ameliorate the downregulation of endothelial nitric oxide synthase (eNOS) in mice.[23] In this study, SD rats fed with HFD for 10 weeks were found to be significantly heavier than those fed a regular diet (Table 1). The HFD group was found have higher levels of plasma triglyceride, total cholesterol and LDL and lower levels of HDL. Treatment with



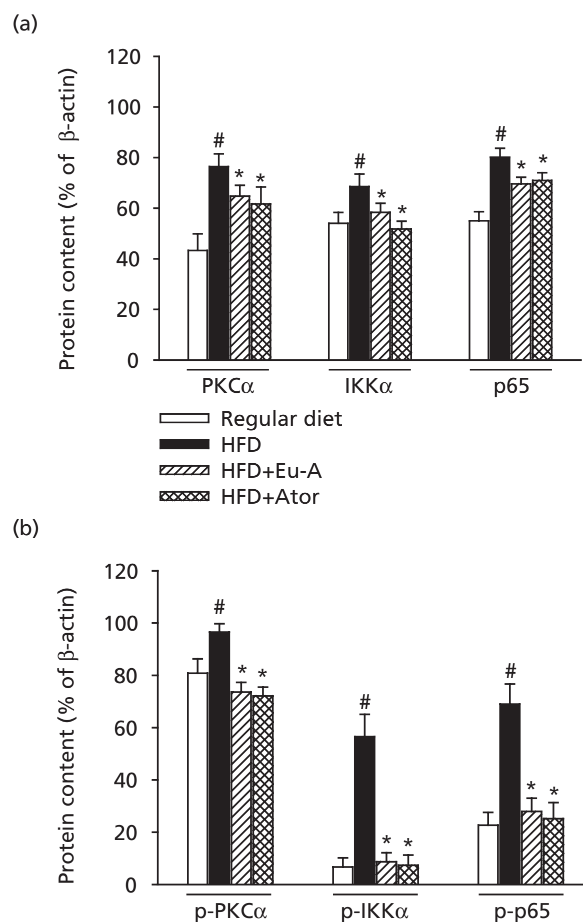


Figure 6 Effect of eugenosedin-A (Eu-A) and atorvastatin (Ator) on PKCα, IKKα, p65, p-PKCα, p-IKKα and p-p65 of aorta in high-fat diet (HFD)-fed rats. The bar charts represent the data derived from the Western blots of the proteins in (a) unphosphorylated form and (b) phosphorylated form. Each value represents the mean ± SE, $n = 8$. #$P < 0.05$ vs regular diet; *$P < 0.05$ vs HFD.

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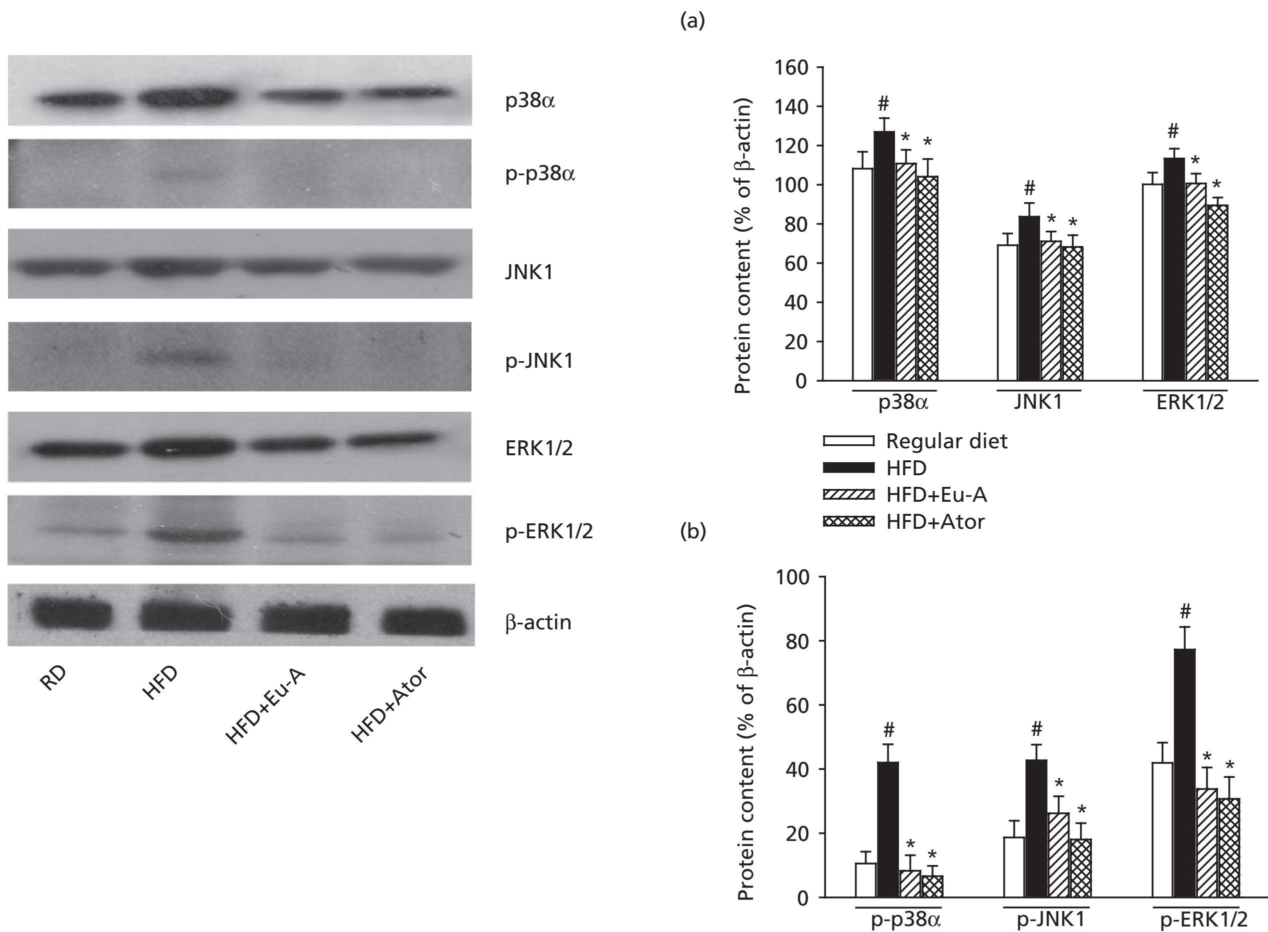

Figure 7 Effect of eugenosedin-A (Eu-A) and atorvastatin (Ator) on p38α, JNK1, ERK1/2, p-p38α, p-JNK1 and p-ERK1/2 of aorta in high-fat diet (HFD)-fed rats. The bar charts represent the data derived from the Western blots of the proteins in (a) unphosphorylated form and (b) phosphorylated form. Each value represents the mean ± SE, $n = 8$. #$P < 0.05$ vs regular diet; *$P < 0.05$ vs HFD.

eugenosedin-A and atorvastatin clearly ameliorated the HFD-induced body weight and hyperlipidaemia. Increased plasma levels of insulin were noted in the HFD group, but blood glucose and HbA_{1c} concentrations were not increased. Eugenosedin-A and atorvastatin significantly improved HFD-induced hyperinsulinaemia (Table 2).

Of importance, this study found that a 10-week HFD markedly induced platelet expression of the adhesion molecules P-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM, and also E-selectin, ICAM-1, ICAM-2, ICAM-3, VCAM and PECAM expression in the aorta of rats (Figures 2–4). However, Diacovo *et al.*[25] demonstrated that ICAM-2, but not ICAM-1 or ICAM-3, is present on the surface of resting and activated platelets and that ICAM-2 has significant clinical consequences in facilitating the inflammation and haemostatic processes at sites of vascular injury. Interestingly, not only ICAM-2 but also ICAM-1 and ICAM-3 were augmented in activated platelets, in contrast to those reports. Eugenosedin-A, like atorvastatin, could inhibit those adhesion molecules, suggesting that both agents might be used to ameliorate hyperlipidaemia-induced cell adhesion.

Activation of NF-κB is implicated in the regulation of genes involved in inflammation, degenerative changes and growth control. NF-κB may perform this role by regulating the expression of cytokines, and adhesion molecules such as VCAM, ICAM and PECAM. Several mechanisms, such as ERK, JNK, p38 and IKKα/NF-κB pathways, are activated in hypercholesterolaemia; this enhances the production of adhesion molecules.[16,17] Protein kinase C (PKC) plays a major role in several mechanisms that promote atherosclerosis. Hypercholesterolaemia could activate PKC in cells and induce their adhesion to endothelial cell.[26] Inhibition of PKC could be useful for anti-inflammatory and anti-angiogenic effects.[27] In this study, we found that eugenosedin-A significantly reduced the expression of PKCα, IKKα, p65, p38, ERK and JNK1 in the aorta. Together these findings suggest that, like atorvastatin,

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eugenosedin-A can markedly ameliorate hyperlipidaemia-induced cell adhesion through inhibiting the PKCα, IKKα, p65 and MAPKs/NF-κB pathway.

Serotonin (5-HT) has been found to be a mitogen for vascular smooth muscle cells and has been implicated in platelet activation and vasoconstriction; these processes contribute to arterial thrombosis in atherosclerotic diseases. Even low concentrations of very low density lipoprotein, intermediate density lipoprotein or LDL in hypercholesterolaemic plasma may significantly potentiate the mitogenic effect of 5-HT released by aggregating platelets at sites of vascular damage.[28] Meanwhile, 5-HT upregulates ACAT expression at both the protein and mRNA levels. This elevates the ACAT enzyme activity during differentiation of monocytes into macrophages, in turn contributing to the formation of macrophage-derived foam cells. ACAT, an intracellular enzyme located in the rough endoplasmic reticulum, catalyses cholesterol ester formation from cholesterol and fatty acyl-coenzyme A. Of the two human ACAT isozymes (ACAT-1 and ACAT-2), ACAT-1 is the dominant isozyme in monocyte-macrophages. ACAT-1 is expressed at high levels by macrophage-derived foam cells in atherosclerotic lesions. Suguro *et al.*[15] has shown that 5-HT may play a crucial role in macrophage-derived foam cell formation by upregulating ACAT-1 expression via the 5-HT_{2A} receptor/G protein/c-Src/PKC/MAPK pathway, contributing to the progression of atherosclerotic plaque. Our results indicate that ACAT-1 expression of macrophages was induced by hyperlipidaemia. Eugenosedin-A, but not atorvastatin, significantly inhibited ACAT-1 protein expression in HFD-fed rats. Since the number of macrophages is limited, the pathway of PKC/MAPKs or p65 remains unresolved. Based on the findings of previous reports,[15,20] we suggest that the decrease in ACAT-1 expression might be related to its 5-HT_{2A} antagonism and PKC/MAPK pathway inhibition.

Conclusions

In conclusion, eugenosedin-A was found to diminish HFD-induced adhesion molecules in this study. Through the antagonistic effect of 5-HT_{2A}, eugenosedin-A inhibits HFD-induced macrophage ACAT-1 protein expression. As we know, the expression of adhesion molecules was increased by the activation of NF-κB. The PKC/MAPKs (p38, ERK and JNK) and p65 (Akt/IKKα) were proven to stimulate NFκB to translate inflammatory and adhesion molecules of mRNA to proteins. According to our results, eugenosedin-A prevented HFD-induced adhesion molecules and ACAT-1 formation, which is closely related to the reduction of PKC/MAPKs (p38, ERK and JNK) and p65 (Akt/IKKα) pathways (Figure 8). Taken together, we suggest that eugenosedin-A could be developed as an adequate pharmacotherapeutic agent used to control HFD-induced metabolic syndrome.

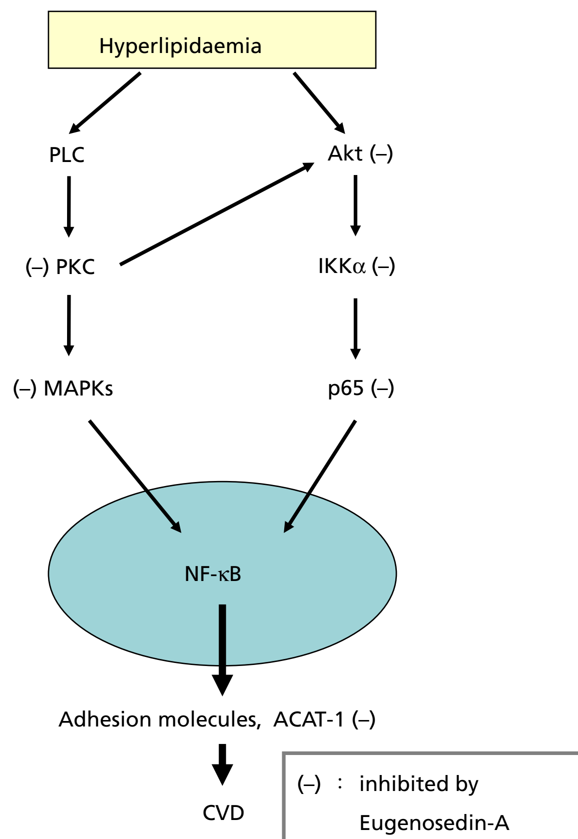

Figure 8 Hypothetical mechanisms of eugenosedin-A in hyperlipidaemia-induced adhesion molecules and acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT-1). Eugenosedin-A decreases hyperlipidaemia-induced adhesion molecules and ACAT-1 formation, which is related to the inhibition of PKC/MAPKs (p38, ERK and JNK) and p65 (Akt/IKKα) pathways. CVD, cardiovascular disease.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by grants NSC-98–2320-B037-017-MY3 to Dr Bin-Nan Wu from the National Science Council, Taiwan, AMH-100-DFN-002, and to Dr Kuo-Ping Shen from Meiho University, Pingtung, Taiwan.

Acknowledgement

The authors thank the IPSF exchange student Yuchu Yan at Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta for his editorial assistance with the manuscript.

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