Fibrin-derived Peptide B β 15-42 and Earthworm Fibrinolytic Enzyme A Inhibit Atherosclerosis and Inflammation via the VE-cadherin Pathway

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Research Article

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Abstract

Elevated fibrinogen increases risk for cardiovascular and cerebrovascular diseases. The biological effects of the fibrin-derived peptide B β 15–42 are different from fibrin and promote vascular and anti-inflammatory effects. Fragments of fibrinogen cleavage by earthworm fibrinolytic enzyme A (EFEa) are structurally similar to B β 15–42. Therefore, we evaluated if B β 15–42 and EFEa have anti-atherosclerotic effects. To investigate the anti-atherosclerotic effect of B β 15–42 and EFEa, we used New Zealand rabbits fed a high cholesterol diet to promote atherosclerosis. Human umbilical vascular endothelial cells with high expression of VE-cadherin were used to determine the mechanism of action of B β 15–42 and EFEa to inhibit the deleterious effect of fibrin. Both B β 15–42 and EFEa significantly reduced atherosclerosis in rabbits fed a high cholesterol diet. Vascular fibrinogen deposition and inflammatory cell aggregation was significantly reduced in rabbits treated with B β 15–42 or EFEa. In addition, B β 15–42 and EFEa stabilized the structure of endothelial cells and decreased inflammatory cell migration. B β 15–42 and EFEa protected endothelial cell function by reducing the effect of fibrinogen in the VE-cadherin pathway. Therefore, the fibrin-derived B β 15–42 peptide exhibited anti-atherosclerotic effects and reduced vascular fibrinogen deposition and inflammatory cell aggregation in the aorta. Furthermore, EFEa has similar B β 15–42-like anti-atherosclerotic effects.

1. Introduction

Cardiovascular and cerebrovascular diseases are two prominent conditions threatening human health [1], and incidences of these diseases continue to rise in China [2]. Excess fibrinogen is a risk factor for cardiovascular and cerebrovascular diseases, and regulates inflammation in atherosclerosis development [3]. Fibrinogen interacts with multiple cell types, and induces development of pathological conditions including vascular wall disease, stroke, brain trauma, spinal cord injury, multiple sclerosis, bacterial inflammation, and multiple cancers specific to atherosclerosis. Studies have shown that fibrinogen deposition in various stages of atherosclerotic lesions [4] and early deposition of subendothelial fibrinogen hinder the protective function of endothelial cells, promote the accumulation of inflammatory cells, and increase the risk of atherosclerosis [5]. VE-cadherin, a main component of endothelial cell adhesion, forms a homologous linkage with its extracellular region; the intracellular segment forms a complex with various catenin proteins and passes through α -catenin and the cytoskeleton component of F-actin. A dynamic link is formed to jointly regulate the adhesion function of tissue cells [6].

The fibrin B β 15–42 peptide (fibrin-derived peptide B β 15–42) is a 28-amino acid peptide formed from the N-terminus of fibrinogen β . B β 15–42 is produced by fibrinogen degradation in the body and can be artificially synthesized [7]. Previous studies reported that B β 15–42 reduced cardiac ischemia-reperfusion injury in multiple animal models and provided evidence that the beneficial effects of B β 15–42 are mediated through inhibition of fibrinogen-dependent inflammatory responses [8, 9]. In addition, B β 15–42 was shown to be safe in human patients suffering from multiple organ failure after infection with Ebola virus [10]. After combination therapy including B β 15–42 of ventilatory support, antibiotic treatment, and renal replacement therapy for a patient with severe Ebola virus disease, vascular leakage syndrome and

respiratory parameters significantly recover [11]. Based on these data, the role of B β 15–42 in vascular protection deserves further study.

Earthworm fibrinolytic enzymes (EFEs) are closely related to fibrinogen, are structurally similar to B β 15–42, are involved in fibrinolysis and degradation, and have antiviral effects [12]. EFEa is the primary active component of EFEs [13] and hydrolyzes fibrinogen at the N-terminus (Val21-Glu22) of the A α chain. EFEa also contains thrombin splice sites at the N-terminus (Arg16-Gly17) of the A α chain and the N-terminus (Arg14-Gly15) of the B β chain [12]. Some structural similarities between fibrinogen fragments cleaved by lumbrokinase and B β 15–42 suggest that EFEa has some anti-inflammatory effects

Most B β 15–42 studies focused on the relationship between B β 15–42 and the acute inflammatory response and EFEa studies focusing on acute fibrinolysis [7, 8, 9], and studies of the effects of B β 15–42 and EFEa on chronic pathologies or atherosclerosis prevention have not been reported. Previously, our group showed in clinical trials that long-term oral EFEa treatment is beneficial to secondary prevention of ischemic stroke [15]. Therefore, we tested the hypothesis that the fibrin B β 15–42 peptide and the EFE-based drug can prevent atherosclerosis via anti-inflammatory effects of VE-cadherin pathway.

2. Materials And Methods

2.1. Reagents and antibodies

Fibrin was formed using fibrinogen and thrombin purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were purchased from Sangon Biotech (Shanghai, China). Primary antibodies against VE-cadherin(SAB4301448) were obtained from Sigma, and the other antibodies were purchased from Abcam (Cambridge, UK). Cell culture reagents were obtained from Gibco (Grand Island, NY, USA). The lentivirus (CDH5-GV358,) was obtained from the Genechem (Shanghai, China).

2.2. Animal model

All animal experimental procedures were performed in accordance with the institutional ethics committee regulations and guidelines on animal welfare and were approved by the Institutional Animal Care and Use Committee of Soochow University. Animals were housed in a controlled environment (temperature of 22 $\pm$ 2°C and a 12 h day/night cycle) with free access to food and water. New Zealand rabbits (4-months-old, 2.4 $\pm$ 1kg)used in the experiments were fed a normal diet during the first-week and a 1%-cholesterol diet (Shuangshi Animal Feed Technology, Suzhou, China) during the next 8 weeks. At 9-weeks, the rabbits were divided into four groups as follows: (1) Control, without extra treatment (CTRL group), (2) B β 15–42, with B β 15–42 treatment for 4 weeks (B β 15–42 at 2.5 mg/kg, intravenous injection every three days), (3) EFEa, with EFEa treatment for 4 weeks (EFE at 20 mg/kg, feeding daily), and (4) Ros (Rosuvastatin), with Ros treatment for 4 weeks (Ros at 5 mg/kg, feeding daily). Blood was drawn through the middle ear artery into blood collection tubes and biochemical analysis was immediately performed on the last day of the experiment.

2.3. Analysis of atherosclerotic lesions

The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and serum fibrinogen (Fg) were measured in the blood samples. The heart and aortic tissues were removed from the aortic root to the iliac bifurcation and placed in 4% paraformaldehyde for 6 h. To evaluate atherosclerotic lesion areas, aortas were cut longitudinally to expose the intimal surface; the plaque-containing area was visualized by fat staining (Oil Red O, Shanghai Sangon Biological Engineering Technology & Services Co. Ltd.). The proximal aorta was quantified by obtaining a section of the aorta beginning and ending at 5 mm and 3 cm from the heart, respectively, and embedded in paraffin. Five-micrometer sections were stained to analyze atherosclerotic lesions in the aorta. Aortic lesion areas were quantified using Image J software (National Institutes of Health) by a blinded observer.

2.4. Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) and murine macrophages (RAW264.7) were purchased from Shanghai Gene Chem (Shanghai, China). HUVECs and macrophages were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich) with 3.7 g/L NaHCO₃, 10% (v/v) fetal bovine serum (FBS), and 1% (w/v) penicillin and streptomycin at 37°C. B β 15–42 was purchased from Pepmic (Suzhou, China); a final concentration of 0.6 mg/mL was used for experiments. EFE was obtained from the Institute of Biophysics, Chinese Academy of Sciences, China; a final concentration of 2 μ g/mL was used for experiments. HUVECs are infected by lentivirus (48h) and then screened by puromycin.

2.5. Western blot analysis

Cells were washed with phosphate-buffered saline (PBS) and homogenized in ice-cold lysis buffer (150 mmol/L NaCl, 25 mmol/L Tris, 5 mmol/L ethylenediaminetetraacetic acid (EDTA), 1% Nonidet P-40, pH 7.5) with protease inhibitor cocktail tablets (Roche Diagnostics, Penzberg, Germany). Protein concentrations were determined using bicinchoninic acid (BCA) protein assay kits (Pierce, Rockford, IL, USA). Protein samples were separated on 8% sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose membranes. Membranes were blocked in 5% (w/v) non-fat dry milk powder in 0.1% Tris-buffered saline/Tween 20 (TBST) for 1 h and then incubated with primary antibodies at optimized dilutions (1:500) described above at 4°C overnight. Membranes were washed with TBST and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h. Membranes were visualized using ECL chemiluminescence (Thermo Fisher, Waltham, USA). The band densities were analyzed using *Image J software* by a blinded observer.

2.6. Cell migration assay

To assess the migration of macrophages through the endothelium, HUVECs were pre-plated on transwell upper chambers and cultured to 80% confluence. Fibrinogen or fibrin was placed in the lower chamber to induce migration in conjunction with treatment by B β 15–42 or EFEa intervention for 24 hours. Similar experiments were performed on macrophages. Cells that migrated to the lower side of the transwell membrane were stained and counted under a Zeiss microscope (Axio Scope A1, Goettingen, Germany). The results are expressed as the mean number of cells per optical field.

2.7. Statistical analysis

Data are expressed as mean $\pm$ SEM. Statistical significance was analyzed using Student's *t* tests or one-way analysis of variance followed by a post-hoc analysis (Tukey's test) where applicable. The results were considered statistically significant at $P < 0.05$.

3. Results

3.1. B β 15–42 And EFEd treatment reduces plaque formation in a rabbit model of atherosclerosis

Fibrinogen staining is consistent with aortic atherosclerotic lesions[3, 4, 5]. EFEd and Ros treatment significantly reduced the fibrinogen areas in histological sections compared with the control group ($P < 0.05$ and $P < 0.01$, respectively). However, B β 15–42 treatment resulted in a non-significant reduction in fibrinogen area compared with the control group (Fig. 1.A-B). Fibrinogen aortic protein expression in each of the drug intervention groups was significantly lower compared with the control group (Fig. 1.I).

Aortic atherosclerotic lesions were associated with marked intimal infiltration by foam and inflammatory cells in the control group. EFEd and Ros significantly reduced inflammatory cell infiltrations. However, B β 15–42 treatment resulted in a non-significant decrease in inflammatory cell infiltration. (Fig. 1.C-D).

Based on these data, we deduce that fibrinogen deposition and inflammatory cell infiltration in the aorta are mainly present in the regions of atherosclerotic tissue. These regions can be reduced by treatment with EFEd and Ros.

3.2. B β 15–42 and EFEd treatment reduces the deposition of plaques in vivo.

B β 15–42 and EFEd treatment did not affect low-density lipoprotein (LDL) or fibrinogen levels in the animals (Fig. 1.G-H). Tissue sections stained with hematoxylin and eosin show reduced aortic lesions and thinner arteries in EFEd-treated animals compared with control animals; a non-significant decrease was seen in animals treated with B β 15–42 (Fig. 1.E,F,J).

Visualization of the aortic intimal outcrop by Oil Red O staining showed extensive atherosclerotic tissue in the control group that was significantly reduced by B β 15–42, EFEd, and Ros treatments to 32.30%, 72.94%, and 48.92% of control, respectively (Fig. 2.A-B).

3.3. Stable VE-cadherin overexpression in endothelial cells

Because cadherin expression levels differed between cell types (Fig. 2.C), examining the mechanisms of action *ex vivo* was difficult. Therefore, we established a CDH5-positive VE-cadherin expressing endothelial cell line (HUVEC-CDH5+) by lentivirus infection that is capable of stably expressing high VE-cadherin protein and mRNA (Fig. 2.E-F). Compared with common HUVECs, HUVEC-CDH5+ cells are tightly connected and are more likely to form a stable endothelial monolayer mimicking the vascular intima (Fig. 2.G).

3.4. Fibrinogen reduces VE-cadherin in endothelial cells and increases migration of inflammatory cells in vitro

Serum-free medium containing 6 mg/mL fibrinogen or fibrin was added to HUVEC-CDH5 + cells for 3, 6, 12, or 24 hours, and the amount of VE-cadherin protein was monitored by western blot. Fibrinogen treatment decreased total VE-cadherin protein in a time-dependent manner, and reached statistical significance at 24-hours compared with the control group (Fig. 3.A, C). Fibrin significantly decreased total VE-cadherin protein after 12 and 24 hours of treatment compared with control cells (Fig. 3.B,D). Next, we exposed HUVEC-CDH5 + cells to fibrinogen and fibrin (1 mg/mL, 3 mg/mL, and 6 mg/mL) for 24 hours. Fibrinogen and fibrin significantly reduced VE-cadherin protein expression at 6 mg/mL and 1 mg/mL, respectively. (Fig. 3.F-H).

Immunofluorescent analysis of VE-cadherin and cytoskeletal proteins showed structural changes in fibrin-treated cells, including shortened and thickened F-actin and cell separation due to reduced VE-cadherin (Fig. 3.I-K). Based on these data, transwell invasion assays were performed. Fibrin, but not fibrinogen, induced inflammatory cell migration through an endothelial internal surface to the lower chamber (Fig. 3.L).

3.5 B β 15–42 and EFEa protect endothelial cells and inhibit migration of macrophages

Immunofluorescence experiments revealed that B β 15–42 (0.6 mg/mL) and EFEa (2 μ g/ml) reduced changes in cytoskeleton formation and VE-cadherin expression, stabilized the state of inner endothelial cells, and maintained the structure of cell monolayers (Fig. 4.A). VE-cadherin protein expression was increased after B β 15–42 and EFEa treatment (Fig. 4.B). Cell migration experiments showed that B β 15–42 and EFEa significantly reduced fibrin-induced migration of macrophages through endothelial cells by 83.3% and 56.1%, respectively (Fig. 4.C-D).

4. Discussion

Fibrinogen increases the development of atherosclerosis. The fibrin-derived peptide B β 15–42 prevents leukocyte accumulation, protects endothelial function, and reduces inflammatory osmosis [9, 16]. EFEa cleaves fibrinogen, and it has been suggested that, similar to B β 15–42, EFEa has clinical value for reducing fibrinogen expression and has anti-tumor, anti-virus, and anti-inflammatory effects [12]. Our study focused on understanding the anti-atherosclerotic mechanisms of these two drugs.

We found no difference in LDL levels after treatment of rabbits with B β 15–42 and EFEa. However, fibrinogen protein levels were reduced in aortic lesions after drug treatment. The EFEa effects are likely due to molecular antagonism like B β 15–42, rather than direct effects on fibrinogen expression. Previous studies demonstrated that fibrinogen primarily accumulates in early atherosclerotic tissues and this deposition results in destruction of endothelial cells and progression of atherosclerotic lesions [4, 5].

Therefore, treatment with B β 15–42 or EFEd reduces fibrinogen deposition and overall atherosclerotic burden.

Local inflammation and cell activation are important underlying mechanisms of atherosclerosis. Macrophage expression is significantly increased in atherosclerotic plaques, and is closely related to the severity of plaque formation, development, and rupture [17]. We used immunohistochemistry to label macrophages on the aorta and found that Ros treatment significantly reduced inflammatory cells, demonstrating that statins account for at least part of the anti-inflammatory effects in our model; this is consistent with the effect of Ros given by Tziakas et al. in a rabbit model of atherosclerosis [18]. In addition, these data suggest that EFEd also has anti-inflammatory effects as seen by a reduction in inflammatory cells. B β 15–42 and EFEd treatment reduced aortic atherosclerotic lesion areas, demonstrating that both drugs have potent anti-atherosclerotic effects.

However, the specific mechanisms of action of these drugs remain largely unknown. The Zbharowski group first confirmed in vitro that the fibrin fragment N-terminal disulfide knot (NDSK)-II can promote migration of human peripheral blood mononuclear cells through HUVEC monolayers [19]. The effect of B β 15–42 treatment on the amino-terminus of the NDSK-II β chain can be blocked by antibodies against recombinant VE-cadherin, CD11b, and the amino terminal sequence A α GPRP of the NDSK-II α chain [19]. Therefore, we propose that the B β 15–42/VE-cadherin pathway is involved in the leukocyte transendothelial cell monolayer as a "molecular bridge". We used real-time fluorescence quantitative PCR, protein immunoblot, and immunofluorescence to show VE-cadherin expression in HUVECs by successfully expressing VE-cadherin protein in HUVECs through a lentivirus vector (HUVEC-CDH5+). HUVEC-CDH5+ cells linked tightly to each other and provided a good model to simulate the state of vascular endothelium in the body.

To test fibrinogen- and fibrin-mediated effects on VE-cadherin expression, we exposed HUVEC-CDH5+ cells to varying lengths of time and concentrations of fibrinogen and fibrin. The high-dose fibrinogen treatment (6 mg/mL for 24 hours) reduced VE-cadherin protein expression. Consistent with our results, Lominadze et al. [20] demonstrated that high concentrations of fibrinogen (4 mg/mL) decreased VE-cadherin expression in cerebral vascular endothelial cells and led to cerebral vascular dysfunction. In MMP9 knockout mice, the use of ICAM-1 function-blocking antibodies can antagonize fibrinogen-induced reduction in VE-cadherin expression, suggesting that ICAM-1 is involved in the interaction between fibrinogen and endothelial cells. The reduction in the amount of VE-cadherin protein caused by fibrinogen injection in vivo is associated with MMP9 [21]. Our results suggest that the effect of fibrin on reducing VE-cadherin protein is even more pronounced. Low-dose fibrin (1 mg/mL) treatment significantly reduced VE-cadherin protein expression and high-dose fibrin (6 mg/mL) treatment reduced VE-cadherin after 12 hours of treatment compared with 24-hours by fibrinogen, and by a greater extent. Therefore, we speculated that in vivo, fibrin deposited in the blood vessel or fibrinogen that was exposed to the β 15–42 sequence [22] could reduce VE-cadherin expression, destroy the integrity of the original endothelium, and accelerate atherosclerosis.

We used immunofluorescence to observe the distribution of VE-cadherin and F-actin in HUVEC-CDH5 + treated with high concentrations of fibrinogen or fibrin. In response to the high dose of fibrinogen and, to a greater extent, fibrin, the total amount of VE-cadherin was decreased and aggregated, the structure of F-actin was disordered, and co-localization of F-actin with VE-cadherin at the cell junction was reduced. Higher concentrations of fibrinogen (4mg/mL) can activate the ERK signaling pathway and increase the formation of endothelial cytoskeletal protein F-actin, thereby increasing intercellular permeability. In addition, the activation of the ERK pathway in endothelial cells with fibrinogen alters the relationship between ICAM-1 and integrin $\alpha 5\beta 1$ [23]. Our results suggest that fibrin has a more pronounced effect on VE-cadherin redistribution and F-actin morphological changes than does fibrinogen.

The central E region of fibrin consists of $\alpha 17-78$, $\beta 15-122$, and $\gamma 1-62$. Its homologue NDSK-II ($\alpha 17-51$, $\beta 15-118$, $\gamma 1-78$) promotes transmigration of leukocytes through the endothelium in vitro experiments. To understand the role of migration through cell monolayers [19], we stimulated HUVEC-CDH5 + cells with fibrin and observed an increase in macrophage migration through endothelial cells; however, fibrinogen did not have this effect. These results indicate that the $\alpha 17$ - $\beta 15$ -terminal exposure of fibrin is necessary to promote inflammation and intracellular migration. Next, we used B $\beta 15-42$ and EFEd to interfere with fibrin-induced macrophage migration. Both drugs showed an inhibitory effect on fibrin-induced cell migration through endothelial cell monolayers and prevented loss of VE-cadherin. Several experiments have shown that B $\beta 15-42$ reduces ischemia reperfusion injury and protects the integrity of the vascular endothelium. The main mechanism may be that B $\beta 15-42$ mimics the amino-terminal sequence of the fibrin β chain and binds competitively to VE-cadherin, thereby preventing leukocyte migration through the endothelial monolayer. B $\beta 15-42$ induces release of Fyn, inhibits RhoA activity, inhibits rearrangement of skeletal proteins, maintains the continuity of VE-cadherin between cells, and reduces inflammatory cell infiltration [19, 24]. EFEd has trypsin-like activity and multiple hydrolytic sites for plasminogen and fibrin that can hydrolyze fibrinogen [25]. EFEd cleaves the fibrinogen alpha chain at Val(21)-Glu(22). Our results suggest that the B $\beta 15-42$ /VE-cadherin "molecular bridge" links the amino terminus of the alpha chain of leukocyte CD11c and CD18 [26] and that B $\beta 15-42$ has the effect of antagonizing fibrin mediated pro-inflammatory cell migration.

Our results together with previous studies indicate that fibrinogen is closely related to the occurrence and development of atherosclerosis. The fibrin B $\beta 15-42$ /VE-cadherin pathway participates in the migration process of macrophages through endothelial cells. The mechanism may be related to the down regulation of VE-cadherin protein expression and the rearrangement of F-actin. B $\beta 15-42$ treatment protects endothelial function, reduces the deposition of local fibrinogen, reduces inflammatory cell levels in the aorta, and is anti-atherosclerotic. Our data suggest that the inhibitory effect of EFE on atherosclerosis in rabbits is caused by the peptide effect of sheared products like B $\beta 15-42$.

Declarations

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Contributions

JX and GX conceived and designed the experiments. JX, GX, XY, YL, XS and FW performed the experiments. JX and GX analyzed the data. GX and XY wrote the draft; YC and CL checked and revised the draft. All authors approved to submit this version to this publication.

Consent to participate

This article does not contain any studies with human participants by any of the authors.

Ethical approval

The study was approved by the Ethics committee of Soochow University and The Second Affiliated Hospital of Soochow University.

Consent for publication

All authors read and approved the final manuscript.

Conflict of interest

The authors have no financial or proprietary interests in any material discussed in this article.

Data availability

All data generated and analyzed during our study are included in the published article.

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Figures

Figure 1

Effect of Bβ15-42 and EFEa on the aorta in a rabbit model of atherosclerosis. (A) Representative immunohistochemical image of an aortic root section stained for fibrinogen. (B) Quantitative analysis of fibrinogen-positive lesion sizes in aortic root sections (C, D) Representative immunohistochemical (CD68) image of inflammatory cells within aortic root sections & cell count. Scale bar = 100 μm. (E,F,J) Representative images of hematoxylin and eosin staining and quantitative analysis of atherosclerotic lesion size in aortic root sections, scale bar = 200 μm. (G) Blood low-density-lipoprotein cholesterol level in rabbits. (H) Blood fibrinogen level in rabbits. (I) Western blot for fibrinogen isolated from the aortic root. All data are shown as mean±SEM. * indicates P<0.05, ** indicates P<0.01 compared to the control group.

Figure 2

B β 15-42 and EFEa affect the deposition of plaques in vivo & VE-cadherin of human umbilical vascular endothelial cells (A) Representative photographs of Oil Red O staining in aorta, scale bar = 1 cm. (B) Quantitative analysis of atherosclerosis lesion sizes in aortic root sections. (C) VE-cadherin expression in different cell types. (D) Quantitative PCR analysis of VE-cadherin mRNA expression in HUVEC-CDH5+ cells. (E) Representative western blots from CDH5+ lentivirus-infected HUVECs. (F) Quantitative western blot analysis of VE-cadherin in HUVEC-CDH5+ cells. (G) Representative endothelial cell optical fields. Red, endothelial cells immune-positive for VE-cadherin. Blue, nuclear counterstain, scale bar = 50 μ m. All data are shown as mean $\pm$ SEM. * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, NS indicates not significant compared to the control group.

Figure 3

Effect of fibrinogen and fibrin on human umbilical vascular endothelial cells (A)-(D) Effect of increasing fibrinogen and fibrin concentration on VE-cadherin expression in HUVEC-CDH5+ cells. (E)-(H) Effect of increasing fibrinogen and fibrin exposure time on VE-cadherin expression in HUVEC-CDH5+ cells. (I)-(K) Representative optical fields of fibrinogen and fibrin intervention on VE-cadherin/F-actin in endothelial cells, scale bar = 20 μm. (L) Representative optical fields and quantitative analysis of macrophages that migrated through endothelial cells. Red, macrophages immuno-positive for F4/80. Blue, nuclear counterstain, scale bar = 20 μm. All data are shown as mean±SEM. *indicates P<0.05, **indicates P<0.01, ***indicates P<0.001, NS indicates not significant compared to the control group.

Figure 4

Effects of B β 15-42 and EFEd in vitro. (A) Representative optical fields of fibrinogen and fibrin intervention on VE-cadherin/F-actin in endothelial cells. (B) Representative western blot of VE-cadherin in endothelial cells, scale bar = 50 μ m. (C-D) Representative optical fields and quantitative analysis of macrophages that migrated through endothelial cells. Red, macrophage cells immuno-positive for F4/80. Blue, nuclear counterstain, scale bar = 20 μ m. All data are shown as mean $\pm$ SEM. *indicates $P < 0.05$, **indicates $P < 0.01$, ***indicates $P < 0.001$, NS indicates not significant compared to the control group.