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The neurokinin-1 receptor antagonist aprepitant ameliorates oxidized LDL-induced endothelial dysfunction via KLF2

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ABSTRACT

Atherosclerosis is the main cause of many cardiovascular diseases. Endothelial dysfunction is recognized as an early event in the development of atherosclerosis. Many drugs have been studied to mitigate hyperlipidemia-induced endothelial injury. Studies have demonstrated that neuropeptide substance P (SP) and its preferred receptor neurokinin receptor 1 (NK-1R) are involved in the pathological progression of cardiovascular disease. In this study, we show that aprepitant, a selective NK-1R antagonist, possesses beneficial effects that protect endothelial cells from oxidized low-density lipoprotein (ox-LDL)-induced inflammatory response and injury. Our data demonstrate that NK-1R is expressed in both aortic and vein-originated endothelial cells and that ox-LDL treatment induces NK-1R expression. Treatment with aprepitant suppresses induction of endothelial vascular adhesion molecule (VCAM-1 and E-selectin) and cytokine by ox-LDL. The presence of aprepitant mitigates adhesion of monocytes to endothelial cells and the reduction in eNOS/NO triggered by ox-LDL. Mechanistically, we demonstrate that aprepitant suppresses ERK5-KLF2 axis activation. Silencing of KLF2 abolishes the inhibitory role of aprepitant on ox-LDL-induced inflammatory response, suggesting that its action is dependent on KLF2. Collectively, our data support that aprepitant exerts an anti-inflammatory effect. Further research is required to investigate the therapeutic potential of aprepitant in vascular inflammation resulting from atherosclerosis.

1. Introduction

Atherosclerosis is one of the major causes of cardiovascular disease. Atherosclerosis develops due to buildup of fatty plaque on the artery wall, which leads to narrowing and hardening of the vessel. Heart attack and stroke induced by rupture of atherosclerotic plaques are leading causes of death. Atherosclerotic plaques are the consequence of prolonged hyperlipidemia and lipid oxidation (Rafieian-Kopaei et al., 2014). Low-density lipoprotein (LDL) in the circulation is commonly known as the “bad lipid”. Circulating LDL particles are often retained on the intima of the artery wall, which leads to oxidation of LDL (Steinberg, 2009). It has been known that oxidized LDL (ox-LDL) promotes immune and inflammatory responses, and its deposition on the artery wall is a key event in the development of atherosclerosis (Uchida et al., 2013). Ox-LDL-induced endothelial dysfunction has been implicated in the pathogenesis of atherosclerosis (Di Pietro et al., 2016). Medical application of statins and lifestyle intervention have been applied to relieve damage caused by circulating ox-LDL, but an optimal treatment strategy for reducing circulating ox-LDL levels and the eventual risk of developing cardiovascular diseases remains elusive (Gao and Liu, 2017).

Substance P (SP) is the first identified member of the tachykinin family of peptides and has been called a “pioneering neuropeptide” (Hökfelt et al., 2001). SP prefers to bind to one of its receptor, neurokinin-1 receptor (NK-1R, a seven-transmembrane domain G-protein coupled receptors (GPCR) (Steinhoff et al., 2014). The biological actions of SP are mainly mediated through NK-1R due to it having the highest affinity to NK-1R. Besides its important role in inflammation and pain, SP is involved in the regulation of heart frequency, blood pressure and vessel elasticity. Also, SP plays an important role in ischemia and reperfusion and the cardiovascular response to stress (Mistrova et al., 2016). NK-1R has been shown to be expressed on the human vascular endothelium and binds to SP on the endothelial surface to sensitize inflammatory signals (Greeno et al., 1993; Bowden et al., 1994). This evidence suggests that NK-1R and its ligand, SP, might have an essential biological function in vascular cells. The extensive research

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aimed at achieving blockage of NK-1R has led to the development of hundreds of NK-1R antagonists, including casopitant, netupitant, rolapitant, and aprepitant etc., which have been widely used to prevent cancer chemotherapy-associated nausea and vomiting (Bošnjak et al., 2017). In this study, we report the protective role of the NK-1R antagonist Aprepitant in cultured endothelial cells upon ox-LDL stimulation.

2. Materials and methods

2.1. Cell culture and treatment experiment

Primary human umbilical vascular endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) were purchased from Lonza (CC-2517 and CC-2635). These two endothelial cell lines were grown in 2% serum endothelial growth media (EGM2) in low passage numbers (less than 10). Human monocyte cell line U937 cells were from ATCC stock (CRL-1593.2™) and grown in 10% fetal serum containing DMEM media. All cell cultures were maintained in a 5% (v/v) CO_2/95% (v/v) nitrogen incubator at 37 °C. Ox-LDL was purchased from Yiyuan Biotechnologies (#YB-002), Guangzhou, China. This lot of ox-LDL is derived from human LDL. Oxidized LDL was prepared by incubation of LDL with 2 mM Cu_2SO_4 at 37 °C overnight. Oxidation was terminated by adding excess EDTA-Na_2 and agarose gel electrophoresis was performed to determine migration versus LDL. This lot of ox-LDL migrates 1.2-fold further than the native LDL. To determine the expression of NK-1R in response to ox-LDL treatment, HAECs were incubated with 50, 100, or 200 mg/L of ox-LDL for 24 h. To determine the protective effects of aprepitant against ox-LDL treatment, HAECs were stimulated with 100 mg/L ox-LDL in the presence or absence of aprepitant (5, 10 μM) for 24 h.

For ox-LDL-mediated immune cell and endothelial adhesion experiments, we followed the published method (Frostegård et al., 1990). Briefly, U937 cells were pre-labeled with the cell-permeant dye calcein-AM with green fluorescence. The cell adhesion experiment was performed by mixing 5×10^5 U937 cells with 1×10^5 confluent HAECs for 2 h. The HAEC-bound U937 cells were visualized by fluorescent signal.

2.2. Semi-quantitative and quantitative PCR

The total RNA from the cells were extracted using a micro RNeasy Micro Kit from Qiagen (Cat.74004) in accordance with the manufacturer's manual. The RNA concentrations were quantified by Nanodrop. A total of 1 μg of RNA was used to synthesize cDNA using iScript™ Reverse Transcription Supermix (BioRad, #1708840) for RT-qPCR from Invitrogen. For quantitative PCR, SYBR-based real-time PCR experiments were performed to detect the total mRNA transcripts of human NK-1R, VCAM-1, E-selectin, eNOS and KLF2 by an ABI 7500 platform.

2.3. Western blot analysis

Cells from the different conditions were lysed by RIPA buffer with protease and phosphatase inhibitors. The nuclear extracts were obtained by lysing the cells with hypotonic buffer to remove the cytoplasm. A total of 20 μg cell lysates or nuclear extracts were immobilized by PAGE gel. The separated protein mixture was then transferred to PVDF membranes and blotted against their specific antibodies and corresponding secondary antibodies. The immunoblots were visualized by Pierce™ ECL Plus western blotting substrate (Catalog # 32132).

2.4. ELISA

To measure the secreted levels of VCAM-1 and E-selectin levels in the different conditions, HAEC culture media were collected for analyses. Two ELISA kits were purchased from R&D Systems. The experiments were performed by following the manufacturer's instructions. The data were collected using 96-plate reader spectrometry. The absolute values were obtained from a standardized 4-PL curve. The relative levels of VCAM-1 and E-selectin were normalized to total protein amounts and are represented by fold change.

2.5. Intracellular NO measurement

We measured intracellular NO levels based on cell permeable fluorescent probe staining. The fluorescent stain 4-Amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM DA) was purchased from Sigma-Aldrich (254109-22-3, St. Louis, MO). Briefly, DAF-FM DA was added to HAEC cell media and permeated into living cells for 30 min. Upon entry of the cells, DAF-FM DA was diacetylated by esterases to DAF-FM. The latter was reacted with NO to form green-fluorescence. The fluorescence intensity of DAF-FM was quantified as the percentage of change from the initial value.

2.6. Lentivirus knockdown of KLF2

We constructed a human KLF2 gene-specific silence and control lentivirus vector and produced the corresponding lentivirus particles in the package cell line HEK293 T. The KLF2 and control knockdown lentiviruses were then concentrated by density gradient centrifuge and titrated for virus efficiency. To silence HAECs, the lentivirus was added to 50% confluent cells for 48–72 h at 10 MOI. Knockdown efficiency was assessed by immunoblotting with KLF2 antibody.

2.7. Statistical analysis

We designed our experiment with an adequate sample size from each group to ensure the statistical test power. Comparisons between two groups were analyzed using the Student two-tail two-side *t*-test Comparisons between three groups were performed by analysis of variance (ANOVA) testing, and p values less than 0.05 were considered statistically significant.

3. Results

3.1. Neurokinin-1 receptor is expressed in the endothelial cells

To test the action of NK-1R and its antagonist in endothelial cells, we tested the expression patterns of NK-1R in two classic primary endothelial cells: HUVECs and HAECs. HUVECs represents widely used umbilical vein endothelial cells, while HAECs are originated from the aortic endothelium. By reference with U251 glioblastoma cells which is

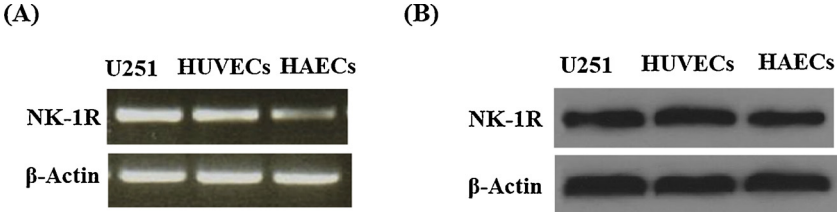

Fig. 1. Neurokinin-1 receptor (NK-1R) is expressed in primary human aortic endothelial cells (HAECs) and human umbilical vein endothelial cells (HUVECs). The human glioblastoma cell line U251 was used as a positive control. (A). RT-PCR analysis of NK-1R; (B). Western blot analysis of NK-1R.

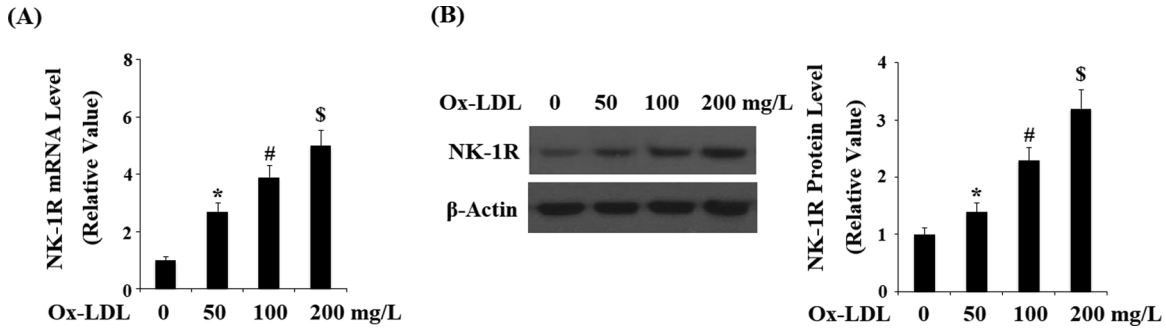

Fig. 2. Ox-LDL increases NK-1R expression in HAECs in a dose-dependent manner. (A). Real-time PCR analysis of NK-1R at the mRNA level; (B). Western blot analysis of NK-1R at the protein level. HAECs were stimulated with 50, 100, and 200 mg/L of ox-LDL for 24 h (*, #, $, P < 0.01 vs. previous column group).

known to have NK-1R expression, our data indicate that NK-1R is fairly expressed in both endothelial cell types based on the profile from both mRNA transcripts and protein production (Figs. 1A and B).

3.2. Ox-LDL increases NK-1R expression in endothelial cells

Next, we applied ox-LDL to challenge the cultured endothelial cells. As the most pathologically relevant stimuli in atherosclerosis development, ox-LDL is well-characterized as being harmful to endothelial cells. We treated HAECs with a series of diluted ox-LDL to mimic the hyperlipidemia challenge to aortic burden. Our results show that ox-LDL induced NK-1R in a dose-dependent manner. When HAECs were treated with 50, 100 and 200 mg/mL of ox-LDL and compared with non-treated cells, NK-1R mRNA was induced by approximately 3-, 4-, and 5-fold on average, respectively (Fig. 2A). Similarly, the protein expression of NK-1R was induced by approximately 1.6-, 2.3- and 3.2-fold, respectively, when compared with non-treatment conditions (Fig. 2B).

3.3. The NK-1R antagonist aprepitant ameliorates ox-LDL-induced adhesion of monocytes to endothelial cells

Based on the inducible response of NK-1R to ox-LDL, we investigated the biological effect of blockage of NK-1R by its selective antagonist, aprepitant. Ox-LDL is known to activate the inflammatory pathways and attract immune cells to vascular endothelial cells. In the monocyte U937 and HAECs co-culture adhesion experiment, we added aprepitant and assessed its influence on immune cell adhesion. Compared with non-treated cells, ox-LDL induced binding of roughly 3.5-fold more U937 cells to HAECs, while the addition of 5 and 10 μM of aprepitant resulted in binding of only about 2- and 1.5-fold more U937 cells, respectively (Fig. 3). This experiment indicates that aprepitant exerts significant suppression on ox-LDL-induced adhesion of immune cells to endothelial cells.

3.4. Aprepitant inhibits ox-LDL-induced expression of vascular adhesion molecules

Next, we assessed the action of aprepitant on the expression of vascular adhesion molecules. As shown in Fig. 4A, as compared to non-treated cells, ox-LDL treatment resulted in approximately 12-fold higher VCAM-1 mRNA transcripts. However, in the presence of 5 and 10 μM of aprepitant, ox-LDL triggered only approximately 6- and 4-fold higher VCAM-1 mRNA. Compared to the control, ox-LDL gave rise to approximately 10-fold higher expression of another vascular adhesion molecule, E-selectin. While ox-LDL caused only approximately 5.4- and 4-fold higher E-selectin transcripts under the condition of 5 and 10 μM of aprepitant. We were able to confirm this inhibitory effect of aprepitant on the same two molecules at the protein level. As shown in Fig. 4B, under the basal non-treated condition, there was only very low

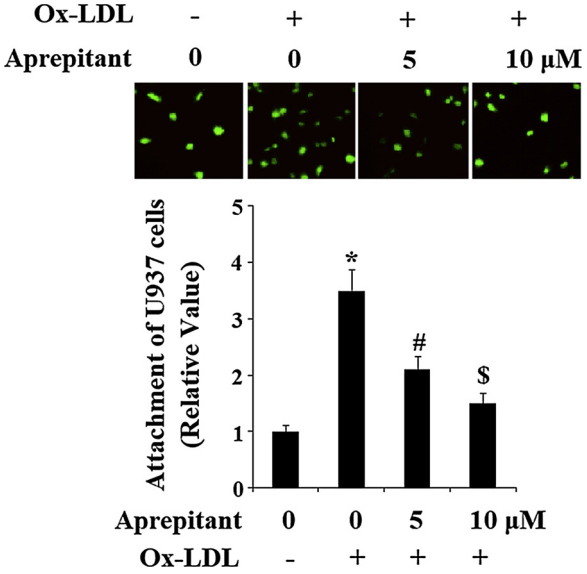

Fig. 3. The neurokinin-1 receptor (NK-1R) antagonist aprepitant ameliorates ox-LDL-induced attachment of human monocytes cell line U937 cells to HAECs. (A). Representative images of attached U937 cells; (B). Quantification of attached U937 cells. HAECs were stimulated with 100 mg/L ox-LDL in the presence or absence of aprepitant (5, 10 μM) for 24 h (*, #, $, P < 0.01 vs. previous column group).

levels of detectable VCAM-1 and E-selection proteins in the cultured media. Meanwhile ox-LDL treatment gave rise to ample production of VCAM-1 and E-selectin proteins, and the presence of the two doses of aprepitant significantly suppressed the production of these proteins induced by ox-LDL in a dose-dependent manner.

3.5. Aprepitant mitigates ox-LDL-induced reduced endothelial KLF2

We explored the molecular mechanism of aprepitant involved in its effect on endothelial cells. Kruppel like factor 2 (KLF2) has been reported to be one of the signature regulators of endothelial function. Our exploratory experiments identified KLF2 to be one of the factors affected by aprepitant. As compared with non-treated cells, ox-LDL treatment resulted in an approximate 50% reduction in KLF2 mRNA. However, the presence of 5 and 10 μM aprepitant recovered KLF2 mRNA by approximately 70% and 95% (Fig. 5A). Furthermore, we were able to confirm this action of aprepitant at the protein level. Compared with non-treated cells, ox-LDL caused an approximate 50% reduction in KLF2 protein, while the addition of 5 and 10 μM of aprepitant recovered KLF2 protein by approximately 75% and 95%, respectively (Fig. 5B).

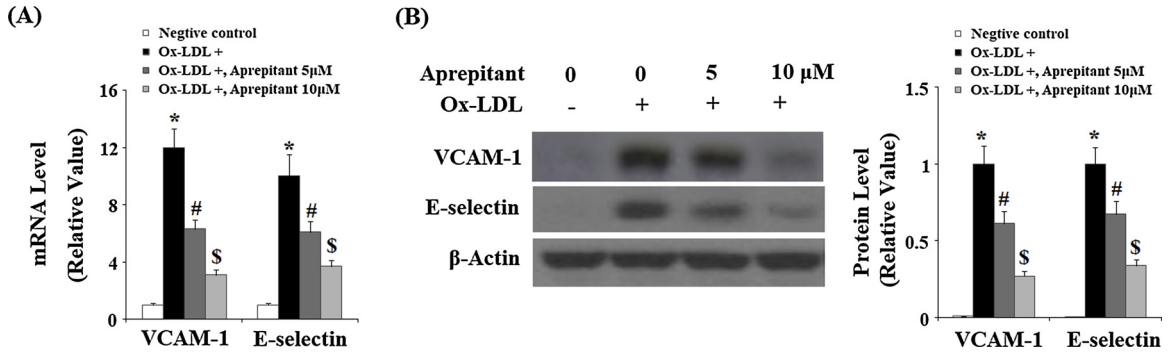

Fig. 4. The neurokinin-1 receptor (NK-1R) antagonist aprepitant ameliorates ox-LDL-induced expression of VCAM-1 and E-selectin. (A). Real-time PCR analysis of VCAM-1 and E-selectin at the mRNA level; (B). Western blot analysis of VCAM-1 and E-selectin at the protein level. HAECs were stimulated with 100 mg/L ox-LDL in the presence or absence of aprepitant (5, 10 μM) for 24 h (*, #, $, P < 0.01 vs. previous column group).

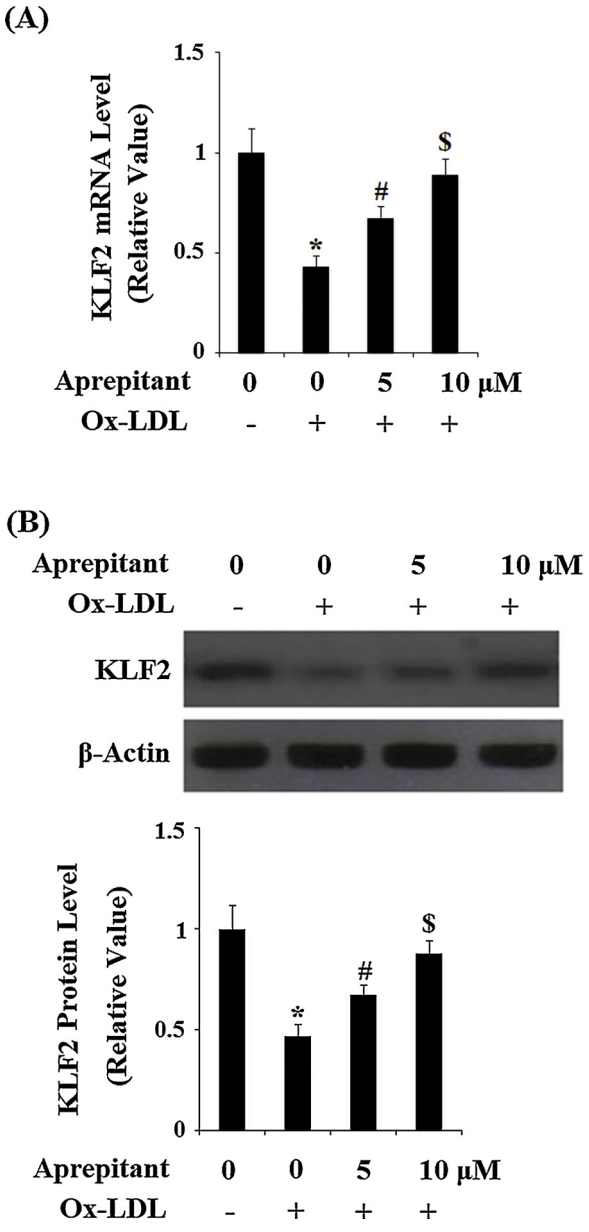

Fig. 5. The neurokinin-1 receptor (NK-1R) antagonist aprepitant rescued ox-LDL-induced reduced KLF2 expression. (A). Real-time PCR analysis of KLF2 at the mRNA level; (B). Western blot analysis of KLF2 at the protein level. HAECs were stimulated with 100 mg/L ox-LDL in the presence or absence of aprepitant (5, 10 μM) for 24 h (*, #, $, P < 0.01 vs. previous column group).

3.6. Aprepitant mitigates ox-LDL-induced reduced eNOS and NO

KLF2 has been reported to regulate the expression of endothelial nitric oxide synthase (eNOS). Next, we assessed nitric oxide (NO) production and eNOS expression as the production of NO and its modulation on vasodilation, the most important function for the endothelium. The production of NO is dependent on the availability of eNOS. In our experiment, 100 mg/mL ox-LDL caused a 40% reduction in eNOS mRNA as compared to non-treated cells, while the addition of 5 and 10 μM aprepitant recovered eNOS mRNA by approximately 70% and 95%, respectively (Fig. 6A). At the protein level, the same dose of ox-LDL caused a reduction in eNOS to 45% of the normal level. However, the presence of 5 and 10 μM of aprepitant recovered eNOS protein by approximately 75% and 95%, respectively (Fig. 6B). We then measured NO production under these different circumstances. As compared to the non-treatment control, ox-LDL treatment caused an approximate 50% reduction in NO, however, the presence of 5 and 10 μM aprepitant recovered NO production in HAECs by approximately 75% and 95%, respectively, even in the presence of ox-LDL (Fig. 6C). These data suggest that aprepitant has a protective effect on endothelial NO production and its synthase eNOS.

3.7. Aprepitant activates endothelial ERK5

Based on its action on endothelial KLF2 and eNOS, we tested extracellular-signal-regulated kinase 5 (ERK5) activity. Compared to non-treated cells, ox-LDL caused an approximate 55% reduction in the phosphorylation of ERK5 kinase, while 5 and 10 μM of aprepitant recovered p-ERK expression by approximately 50% and 90%, respectively (Fig. 7). These data indicate that aprepitant activates the axis of ERK5-KLF2 and ameliorates damage from ox-LDL.

3.8. The inhibitory role of aprepitant on ox-LDL-induced inflammatory response is dependent on endothelial KLF2 expression

Finally, we assessed whether the inhibitory effect of aprepitant on vascular inflammatory molecules is dependent on endothelial KLF2. We constructed a KLF2-specific ShRNA virus approach and silenced KLF2 in HAECs, which we then verified by its protein expression (Fig. 8A). We compared the response of KLF2-silent HAECs and KLF2 non-silent cells upon exposure to ox-LDL and aprepitant. As shown in Fig. 8B and normalized to non-treated cells, ox-LDL treatment resulted in approximately 12-fold higher VCAM-1 expression in non-KLF2-silent cells, while the addition of aprepitant reduced the level of VCAM-1 to approximately 4-fold higher than baseline. However, aprepitant failed to have any influence on ox-LDL action in KLF2-silent cells, and the VCAM-1 level remained at 12-fold higher than baseline. Meanwhile, ox-LDL induced approximately 10-fold higher E-selectin mRNA, which was reduced to approximately 3-fold higher than baseline upon treatment

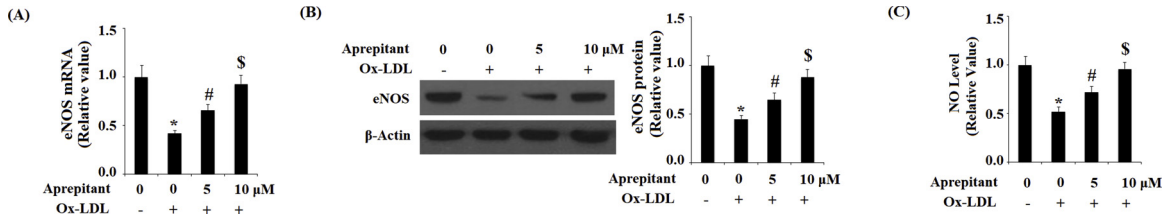

Fig. 6. The neurokinin-1 receptor (NK-1R) antagonist aprepitant increases eNOS expression and NO production. (A). Real-time PCR analysis of eNOS at the mRNA level; (B). Western blot analysis of eNOS at the protein level; (C). NO levels. HAECs were stimulated with 100 mg/L ox-LDL in the presence or absence of aprepitant (5, 10 μM) for 24 h (*, #, $, $P < 0.01$ vs. previous column group).

with aprepitant in KLF2 non-silent cells. Notably, the level of E-selectin remained high in the presence of both aprepitant and ox-LDL in KLF2-silent cells. In the immune cell adhesion experiment shown in Fig. 8C, ox-LDL gave rise to an approximate 3.5-fold increase in the number of U937 cells attached to ECs. The number of attached U937 cells was reduced about half upon treatment with aprepitant in non-silent HAECs, but the number of attached U937 cells remained equally high in KLF2-silent cells upon exposure to both ox-LDL and aprepitant. These data imply that the inhibitory role of aprepitant in ox-LDL-induced inflammatory response is dependent on endothelial KLF2 expression.

3.9. Discussion

The SP/NK-1 receptor is involved in many pathological processes, and NK-1R antagonists have shown many promising therapeutic indications (Hoffman et al., 2006). In endothelial cells, SP has been shown to induce the internalization of endothelial NK-1R and desensitization to SP, limiting the amount of plasma leakage at sites of inflammation (Bowden et al., 1994). SP binds dermal microvascular endothelial NK-1R and stimulates the secretion of a variety of inflammatory factors, which leads to vasodilation and neurogenic inflammation (Scholzen et al., 1998). NK-1R on dermal microvascular endothelial cells responds to SP signaling to activate nuclear factor κB (NF-kB) and promote the expression of vascular endothelial adhesion molecules (Lindsey et al., 2000). SP and NK-1R have been shown to promote neovascularization and angiogenesis both *in vivo* and *in vitro* (Ziche et al., 1990; Song et al., 2010). Aprepitant is the first NK-1R antagonist developed by Merck to prevent chemotherapy-induced nausea and vomiting (Patel and Lindley, 2003). In general, clinically approved NK-1R antagonists are efficacious, safe, and are well-tolerated among different types of populations (Quartara and Altamura, 2006). Several NK-1R antagonists are commercially available to treat chemotherapy-induced nausea and vomiting in cancer patients, which include aprepitant, netupitant and rolapitant. Aprepitant has a much shorter elimination half-life than netupitant and rolapitant, but also has a more prolific drug interaction profile (Rapoport and Smit, 2017).

During our investigation of the potential role of the NK-1 receptor in cardiovascular diseases, we were intrigued by findings from IMPACT National Benchmark Database. The study shows that cancer patients who use aprepitant generally do not experience higher frequencies of cardiovascular events as compared to patients who do not use aprepitant for chemotherapy-induced nausea and vomiting (Barni et al., 2016). These findings suggest that aprepitant has an unidentified role in cardiovascular complications. We further explored its biological effect in cultured endothelial cells. We found that aprepitant has a unique biological function and appears to be beneficial in endothelial cells. Our data demonstrate that endothelial cells express a decent amount of NK-1R and that it is responsive to ox-LDL-induced cellular stress. Blockage of NK-1R by aprepitant efficiently reduced expression of the vascular adhesion molecules VCAM-1 and E-selectin. Moreover, the presence of aprepitant in cultured endothelial media could block the adhesion of monocytes to endothelial cells, suggesting its inhibitory effect on ox-LDL-induced vascular inflammation. The high induction of VCAM-1 has been shown to be closely associated with the development of atherosclerosis (Cybulsky et al., 2001), and the inhibitory role of aprepitant on VCAM-1 elevation indicates its protective role in endothelial cells under inflammatory conditions. Additionally, aprepitant appears to be vascular protective as it recovers ox-LDL-induced NO reduction by increasing eNOS expression, suggesting its protective role in ox-LDL-induced endothelial dysfunction. eNOS plays a pivotal role in the maintenance of endothelial function and acts as a central modulator of vascular tone and homeostasis through regulating the generation of the gasotransmitter NO (Shu and Keller, 2015). A functional eNOS enzyme exerts a protective action against pathological vascular remodeling, hypertension, atherosclerosis, and complications associated with diabetes (Erkens et al., 2017). eNOS uncoupling and subsequent reduced NO bioavailability are associated with endothelial dysfunction, which is a hallmark of many cardiovascular diseases (Siragusa and Fleming, 2016). Long-term exposure to ox-LDL induces impairment of endothelial cell relaxation, migration, and vasculogenesis through reducing eNOS expression (Wang et al., 2018). In the endothelium, eNOS activity is tightly regulated by different mechanisms, including transcriptional and post-transcriptional regulation. KLF2 has been shown to be the major regulator of eNOS and NO production (SenBanerjee et al., 2004). Our findings suggest that aprepitant is able to restore endothelial cell function through activating eNOS/NO signaling via KLF2.

Mechanistically, our findings show that the inhibitory role of aprepitant is mediated by the transcriptional factor KLF2, and silencing

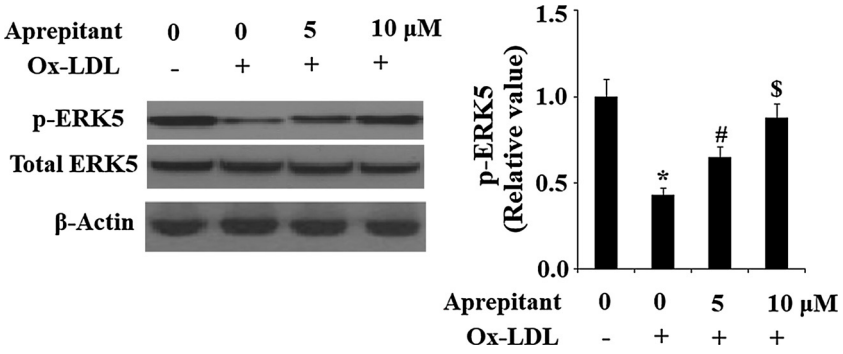

Fig. 7. The neurokinin-1 receptor (NK-1R) antagonist aprepitant increases phosphorylation of ERK5. Phosphorylated and total levels of ERK5 were determined by western blot analysis. HAECs were stimulated with 100 mg/L ox-LDL in the presence or absence of aprepitant (10 μM) for 2 h (*, #, $, $P < 0.01$ vs. previous column group).

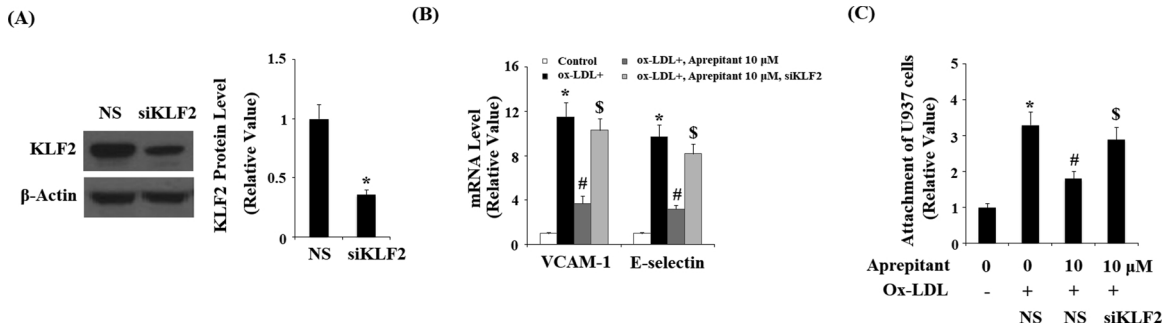

Fig. 8. Knockdown of KLF2 abolishes the inhibitory effect of aprepitant on the expression of VCAM-1 and E-selectin as well as adhesion of U937 cells to HAECs. (A). Successful knockdown of KLF2; (B). Expressions of VCAM-1 and E-selectin at the mRNA level were determined by real-time PCR; (C). Representative quantification of the attachment of U937 cells to HAECs. HAECs were infected with lenti-KLF2 shRNA or empty control. After 24 h, cells were treated with ox-LDL (100 mg/l) in the presence or absence of aprepitant (10 μM) for another 24 h (*, #, $, $P < 0.01$ vs. previous column group).

of endothelial KLF2 abolished aprepitant-mediated suppression of ox-LDL-induced inflammation. The action of aprepitant appears to also be dependent on ERK5 activation. The mechanisms of KLF2 and ERK5 activation in atherosclerotic animals have been documented in previous publications, and atherosclerotic flow-induced ERK5 activation has been shown to be protective in endothelial cells (Kim et al., 2012). The axis of the ERK5-KLF2 regulation mechanism appears to suppress adhesion of immune cells to atherosclerotic tissue and was shown to play a protective role in an animal model study (Deng et al., 2018). Thus, our data disclose a new mechanism of aprepitant in vascular endothelial cells. KLF2 has been identified as a "molecular switch" that regulates endothelial health and disease by differentially controlling the expression of factors that confer anti-inflammatory, antithrombotic, vasodilatory, and anti-proliferative effects in endothelial cells (Chang et al., 2017). One important mechanism of the action of KLF2 in endothelial cells is the inhibition of cytokine-mediated induction of cell adhesion molecules such as VCAM-1 and E-selectin. It should be noticed that KLF2 is able to interplay with of NF-κB, the central regulator of inflammation signaling. The transcriptional activity of NF-κB and KLF2 is antagonistically regulated in human umbilical vein endothelial cells. On one hand, KLF2 inhibits NF-κB activation by competing for the common coactivators p300 and PCAF (SenBanerjee et al., 2004), thereby serving as an inhibitor of vascular inflammation. On the other hand, NF-κB inhibits the expression of KLF2 in the presence of TNF-α stimulation dependent on nuclear translocation of p65 (Kumar et al., 2005). It has been reported that NK-1R is capable of mediating the activation of NF-κB and AP-1 through MAPK and Akt signaling in glioblastoma cells (Mou et al., 2013). Additionally, SP activates the transcription factor NF-κB by promoting nuclear translocation of p65 (Bardelli et al., 2005). Therefore, it is possible that the crosstalk between NF-κB and KLF2 plays a role in NK-1R-mediated expression of VCAM-1 and E-selectin. However, the transcriptional regulation of adhesion molecules in endothelial cells is complicated. Another adhesion molecule p-selectin contributes to acute and chronic inflammation by promoting leukocyte tethering/rolling on endothelial cells. It has been reported that SP upregulates p-selectin through the STAT6 pathway in human endothelial cells (Miyazaki et al., 2006). Ox-LDL-induced expression of human p-selectin through degradation of IκBα and activation of NF-κB plays a role in the regulation of foam cell formation (Wang et al., 2011). However, murine p-selectin is down-regulated by NF-κB. It remains unknown whether NK-1R and aprepitant have an impact on the expression of p-selectin. Future investigations are necessary to clarify the underlying molecular mechanisms.

In conclusion, NK-1R antagonists may have the potential to modulate vascular inflammation in the development of atherosclerosis. Particularly, aprepitant appears to be pleiotropic and has shown promise in clinical trials in terms of cardiovascular outcomes.

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