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# The Effects of The Interaction of Vascular Endothelial Cells and Vascular Smooth Muscle Cells on PCSK9 and NF-kB Protein Expression in Vascular Smooth Muscle Cells

## Vasküler Endotel Hücreleri ile Vasküler Düz Kas Hücrelerinin Etkileşiminin, Vasküler Düz Kas Hücrelerinde PCSK9 ve NF-kB Protein Ekspresyonu Üzerindeki Etkileri

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## Abstract

**Aim:** Atherosclerosis is the leading cause of death worldwide. Atherosclerosis is an inflammatory disease that causes significant structural and functional changes in vascular wall cells. It is known that the main cells of the vascular wall, endothelial cells, and smooth muscle cells communicate during atherosclerosis, but the mechanisms of this communication are not fully understood. PCSK9, on the other hand, is a molecule involved in the etiology of hyperlipidemia, and its levels increase in vascular wall cells during atherosclerosis, exerting direct atherosclerotic effects. In our study, we investigated the changes induced by endothelial cells exposed to atherogenic factors in smooth muscle cells and their effects on NF-kB expression—the most important molecule in inflammation—and on PCSK9 protein expression, which has recently been discovered to have direct atherosclerotic effects.

**Materials and Methods:** Cells isolated from human umbilical veins (HUVEC) were incubated for 24 hours with 300 µM hydrogen peroxide ($H_2O_2$) or 40 µg/ml oxidized LDL (Ox-LDL) or 5% cigarette smoke extract (CSE). Following 24 hours, human aortic smooth muscle cells (HASMCs) were incubated with the supernatants of HUVECs which were treated after appropriate processing with these substances. After 48 hours, protein extraction was performed from smooth muscle cells and analyzed by the Western Blot method.

**Results:** We observed no changes in the viability of HUVECs exposed to atherogenic substances. There were significant morphological changes in HASMCs incubated with endothelial cell supernatants exposed to atherogenic substances. Increased expression of NF-kB (p=0.024, p=0.043) and PCSK9 protein (p=0.034, p=0.0137) was found in HASMCs incubated with supernatants exposed to 5% CSE and 300 µM H2O2, respectively. In HASMCs incubated with endothelial cell supernatant exposed

## Öz

**Amaç:** Ateroskleroz dünyada görülen en sık ölüm sebebidir. Ateroskleroz inflamatuar bir hastalık olup, damar duvarı hücrelerinde belirgin şekil ve fonksiyon değişikliklerine neden olur. Damar duvarındaki ana hücreler olan endotel hücreleri ile düz kas hücrelerinin ateroskleroz sırasında iletişim halinde oldukları bilinen bir gerçek olmakla beraber, bu iletişimin mekanizmaları net değildir. PCSK9 ise hiperlipidemi etyolojisinde yer alan bir molekül olmakla birlikte, damar duvar hücrelerinde ateroksleroz sırasında artar ve damar duvar hücrelerine direk aterosklerotik etkileri vardır. Çalışmamızda aterojenik etkenlere maruz kalan endotel hücrelerinin, düz kas hücrelerinde yaptığı değişiklikleri ve inflamasyonun en önemli molekülü olan NF-kB ve direct aterosklerotik etkileri son zamanlarda keşfedilmiş olan PCSK9 protein ekspresyonlarına olan etkilerini araştırdık.

**Gereç ve Yöntem:** İnsan umblikal veninden izole edilen hücreler (HUVEC) ayrı ayrı 24 saat boyunca 300 µM hidrojen peroksit ($H_2O_2$), 40 µg/ml okside LDL (Ox-LDL) %5 sigara duman ekstraktı (SDE) ile inkübe edildi, 24 saat sonra aterojenik etkenlere maruz kalan endotel hücrelerinin süpernatantları uygun işlemlerden sonra insan aortic düz kas hücrelerinin (HAVSMC) inkübe edilmesi için kullanıldı. Düz kas hücrelerinden 48 saat sonra protein ekstraksiyonu yapılarak Western Blot yöntemiyle analiz edildi.

**Bulgular:** Aterojenik maddelere maruz kalan HUVEC'lerde canlılığın korunduğu saptandı. Bu maddelere maruz kalan endotel hücre süpernatantları ile inkübe edilen HASMC'de belirgin bir morfolojik değişiklik saptandı. %5 SDE ve 300 µM H2O2'ye maruz kalan endotel hücre süpernatantları ile inkübe edilen HASMC'lerde NF-kB (sırasıyla p=0.024,p=0.043) ve PCSK9 protein (p=0.034, p=0.0137) ekspresyonları artmış


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to Ox-LDL, an increase in PCSK9 expression was observed (p=0.037), while no significant increase in NF-kB expression was observed (p=0.89).

**Conclusion:** Our study observed that endothelial cells exposed to atherogenic factors induced changes in vascular smooth muscle cells and increased PCSK9 and NF-kB expression in these cells.

**Keywords:** atherosclerosis, vascular smooth muscle cell, endothelial smooth muscle cell, Proprotein subtilisin/kexin type 9 (PCSK9), nuclear factor kappa b (NF-kB)

bulundu. Ox-LDL'ye maruz kalan endotel hücre süpernatantı ile inkübe edilen HASMC'lerde ise PCSK9 ekspresyonu artmış olup (p=0.037), NF-kB ekspresyonunda anlamlı bir artış izlenmemiştir. (p=0.89)

**Sonuç:** Çalışmamızın sonucunda aterojenik etkenlere maruz kalan endotel hücrelerinin, damar düz kas üzerinde değişikliklere neden olduğu, damar düz kas hücrelerindeki PCSK9 ve NF-kB ekspresyonunu arttırdığı gözlenmiştir.

**Anahtar Kelimeler:** ateroskleroz, vasküler düz kas hücresi, vasküler endotel hücresi, proprotein konvertas subtilisin/keksin tip 9 (PCSK9), nükleer faktör kappa b (NF-kB)

## INTRODUCTION

Atherosclerosis is the primary pathophysiological precursor to cardiovascular and cerebrovascular diseases, which are globally recognised as leading causes of mortality (1). Unravelling the mechanisms of atherosclerosis development and progression is paramount to devising more efficacious prevention strategies and treatments for atherosclerotic vascular diseases.

The genesis of atherosclerosis is multifactorial, involving an interplay of genetic predisposition and diverse environmental influences. Identified significant risk factors, such as hypertension, diabetes, tobacco use, and hyperlipidemia, drastically amplify atherosclerotic susceptibility (2). Hyperlipidemia, in particular, has been recognised as a wellestablished risk factor. Numerous therapeutic agents were introduced to manage hyperlipidemia until Abifadel et al. identified a mutation in the gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) protein, leading to high expression of PCSK9 in familial hyperlipidemia patients (3) This discovery has spurred numerous studies on PCSK9, and agents developed to inhibit PCSK9 have been successfully used in the treatment of hyperlipidemia (4). PCSK9, predominantly expressed in the liver, directs low-density lipoprotein receptors (LDLRs) towards lysosomes, leading to the degradation of these receptors and an increase in circulating low-density lipoprotein (LDL) levels (5). PCSK9's contribution to atherosclerosis via hyperlipidemia is well-established; however, recent research has illuminated its direct atherosclerotic effects on vascular wall cells, independent of hyperlipidemia (6). This implies that persistent PCSK9-mediated effects on vascular wall cells could impede atherosclerosis treatment and may precipitate the disease even in nonhyperlipidemic individuals.

Vascular smooth muscle cells (VSMCs) are one of the main cell types that play an important role in carrying out the physiological functions of the blood vessel wall. In atherosclerosis, the proliferation, migration, and phenotypic changes of VSMCs are key steps in the process (7). While PCSK9 leads to the development and progression of all these atherosclerotic changes in vascular smooth muscle cells (VSMCs), it has been found that VSMCs themselves express and secrete PCSK9, and there is increased PCSK9 expression in atherosclerotic VSMCs (8).

Nuclear Factor kappa B (NF-kB) is a transcription factor that plays a crucial role in inflammation. It controls the transcription of many genes involved in the inflammatory response, including cytokines, chemokines, adhesion molecules, acute-phase proteins, apoptosis regulators, and genes involved in cell proliferation (9). In several studies, it has been demonstrated that NF-kB expression particularly promotes proliferation in smooth muscle cells and is associated with arterial hyperplasia (10). Furthermore, NF-kB inhibition has been demonstrated to reduce intimal hyperplasia in arteries (11). In atherosclerotic lesions, PCSK9 increases the expression of NF-kB, thereby increasing the production of inflammatory cytokines such as tumour necrosis α (TNF-α), Interleukin 1 β (IL-1β), and Monocyte chemoattractant protein 1 (MCP-1). On the other hand, PCSK9 inhibition has been shown to reduce NF-kB expression induced by lipopolysaccharide (LPS), oxidised LDL (Ox-LDL), and TNF-α

in macrophages (12). Based on these studies, it is obvious that the atherosclerotic effects of PCSK9 in vascular wall cells are closely associated with NF-kB. Endothelial cells, the primary cellular population exposed to atherosclerotic molecules within the vascular lumen, orchestrate the atherosclerotic process by modulating the activities of other cell types implicated in disease progression. The cross-talk between endothelial cells and VSMCs is crucial to the evolution and progression of atherosclerosis (13). Nevertheless, the role of endothelial cells in augmenting PCSK9 expression in VSMCs during atherosclerosis remains nebulous. Our study aims to elucidate the alterations in VSMCs, the associated inflammatory response, NF-kB and PCSK9 expression in relation to endothelial cell activity in atherosclerosis.

## MATERIAL and METHODS

*Cell culture*:

Two different cell types were used for the experiments: human aortic smooth muscle primary cell line (HASMC) and primary endothelial cells derived from the umbilical cord vein (HUVEC). HASMC was cultured in smooth muscle cell medium (Lonza), while HUVEC cells were cultured in endothelial cell medium (Lonza). The basal mediums were supplemented with the recommended amounts of fetal bovine serum and growth factors. The cells were incubated at 37 °C and 5% $CO_2$ in a $CO_2$ incubator for optimal cell growth. Smooth muscle cells were expanded in T25 and T75 flasks, while endothelial cells were expanded in gelatincoated T25 flasks. For the experiments, HUVECs were seeded onto gelatin-coated 6 well plates, and HASMCs were seeded onto tissue culture treate 6-well plates. When the cells reached 80% confluence, they were passaged and subcultured.

*Atherogenic substances for cell treatment*:

HUVECs were treated with atherogenic substances for 24 hours, and then the supernatants were collected and used to incubate HASMCs. Hydogen peroxide ($H_2O_2$), cigarette smoke extract (CSE), and oxidised LDL (Ox-LDL) were chosen as atherogenic substances. A 30% wt $H_2O_2$ solution (Sigma) was diluted to obtain 300μM $H_2O_2$. Purchased Ox-LDL (Thermo Fisher) was added to the cells at a concentration of 40μg/ml. Cigarette smoke extract (CSE) was prepared as described in previous publications (14). A plastic tube and pipette tip were attached to a 50ml syringe. The syringe was filled with 10 ml of basal endothelial medium. A cigarette containing 0.8 mg nicotine and 10 mg tar was attached to the pipette tip. Smoke was drawn into the syringe every 30 seconds for a total of 35ml, and the syringe was shaken. This process was repeated 10 times for one cigarette. To obtain 100% cigarette smoke extract, the smoke of 2 cigarettes was added to 10ml of the medium. The pH of the prepared solution was adjusted to 7.4, and it was then sterilised using a 0.22μm filter. Based on previous publications, HUVECs were treated with 10%, 5%, and 2.5% CSE, but significant cell death was observed with 10% CSE. Therefore, the experiments continued with the highest dose at which the cells remained viable, which was 5% CSE.

The cells were incubated with 300 μM $H_2O_2$ or 40 μg/ml Ox-LDL or 5% CSE for 24 hours. At the end of the 24-hour period, the supernatant of endothelial cells was collected in Eppendorf tubes using a pipette. To remove dead cells, the supernatant was centrifuged at 1000g for 10 minutes. The pellet was discarded, and the supernatant was preserved. The medium on top of HASMCs was aspirated, and the cells were washed with PBS. The supernatant obtained from endothelial cells was added to the HASMCs. After 48 hours of incubation, the cellular protein was extracted. The viability of endothelial cells in the presence of atherogenic substances was demonstrated using an MTT assay (Roche).

*Protein Isolation and Western Blot Analysis:*

For protein isolation, a commercially purchased RIPA lysis buffer was used, and on the day of use, 100X protease inhibitor and phosphatase inhibitor were added to the RIPA buffer at a 1:100 ratio. Following extraction, the protein concentration was measured using the Pierce BCA Protein Assay. The samples were prepared for SDS-PAGE, and the proteins were separated by electrophoresis on a 5-20% acrylamide gel. The proteins on the gel were transferred to a nitrocellulose membrane using wet transfer apparatus. The nitrocellulose membrane was first blocked then incubated overnight at +4 °C with the primary antibody targeting the protein of interest. The following day, the membrane was incubated with an appropriate secondary antibody for the primary antibody. To visualize the bands, the membrane was incubated with ECL(Thermo Fisher) and imaged with a ChemiDoc imager.

The quantitative analysis was performed by using the

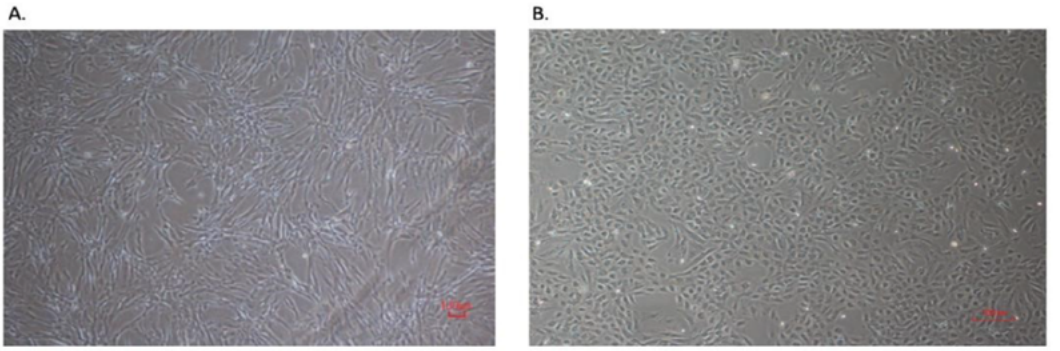


**Figure 1.** The images of HASMC and HUVEC.
**A.** Image of HASMC were captured at passage 8 when they reached 80% confluence.
**B.** Image of HUVEC were captured at passage 6 when they reached 80% confluence.The scale bar represents a measurement of 100 µm. The images were taken at a magnification of 10X.

Image J program.

The primary antibodies used in the Western blot experiments were as follows:

NF-kB p65 Polyclonal Antibody (Thermo Fisher, 51-0500, PCSK9 Polyclonal Antibody

(Cayman, 10007185), GAPDH antibody (Abcam, ab9485)

*Data analysis*

The data analysis was performed using GraphPad Prism version 9.4.1. The data were presented as means and standard error of the mean (S.E.M). Differences between means were compared using Student's t-test when comparing two groups; for comparisons involving more than two groups, analysis of variance (ANOVA) with Tukey's post hoc test was used. Statistical significance was considered at $p < 0.05$.

## RESULTS

The purchased HASMC and HUVECs were cultured in the appropriate culture medium.

When the cell density reached 80%, images were captured under a microscope. (Figure 1)

The endothelial cells were treated with 300µM $H_2O_2$, 40µg/ml OxLDL, and 5% cigarette smoke extract (CSE) to simulate atherogenic conditions. A group of cells without any atherogenic substances was considered as the control group. After confirming the viability of the cells following a 24-hour incubation with these agents using the MTT assay, the experiment was initiated. As shown in Figure 2, there were no significant differences between the viabilities of control vs treated cells.

Following proving the viability of HUVECs with these atherogenic molecules, endothelial cells were incubated with 300µM $H_2O_2$, 40µg/ml OxLDL, and 5% cigarette smoke extract (CSE) for 24 hours. The mediums of HASMC grown on separate plates were discarded, and the mediums from endothelial cells, which were incubated with these substances for 24 hours, following the steps specified in the methods, were added to the smooth muscle cells. After 48 hours of incubation, protein extraction was performed from the vascular smooth muscle cells, and the expression of PCSK9 and NF-kB proteins was evaluated.

Images of the HASMCs were captured under a microscope before protein extraction. It was observed that the morphology of the HASMCs incubated with endothelial cell medium containing atherogenic substances differed from the cells incubated with endothelial cell medium without atherogenic substances, and the smooth muscle cells incubated with endothelial cell medium containing atherogenic substances exhibited similarities to a synthetic phenotype (Figure 3).

Following protein extraction, Western Blot analysis was performed to understand the effect of HUVECs on HASMCs' PCSK9 and NF-kB protein expressions.

In HASMCs which were incubated with endothelial cell medium treated with 5%CSE, the expression of PCSK9 ($p=0.034$) and NF-kB p65 ($p=0.024$) in HASMCs were

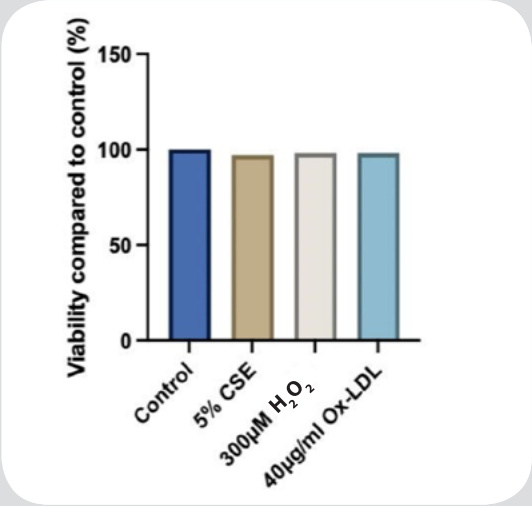


**Figure 2.** The MTT analysis of HUVECs The viability of endothelial cells was analysed using the MTT assay. Compared to the control group, the viability in the 5% SDE group was found to be 96%; in the $H_2O_2$ group, it was 98%; and in the ox-LDL group, it was 97%.

significantly higher than in HASMCs incubated with endothelial cell medium without any additions (Figure 4).

The expression of PCSK9 (p=0.0137) and NF-kB p65(p=0.043) proteins in HASMCs incubated with endothelial cell medium treated with 300μM $H_2O_2$, was also higher than in HASMCs incubated with endothelial cell medium without the addition of atherogenic substances (Figure 5).

In HASMCs incubated with endothelial cell medium treated with 40μg/ml Ox-LDL, the expression of PCSK9 was higher than in HASMCs incubated with control endothelial cell medium (p=0.037), but the difference in NF-kB expression between the two groups was not statistically significant (p=0.89) (Figure 6).

## Discossion

PCSK9 expression in vascular wall cells and its effects on these cells have recently gained attention, leading to associated studies. There are conflicting pieces of information regarding PCSK9 expression in endothelial cells, but researchers agree that vascular smooth muscle cells are the main cells in the vascular wall that exhibit PCSK9 expression (6).

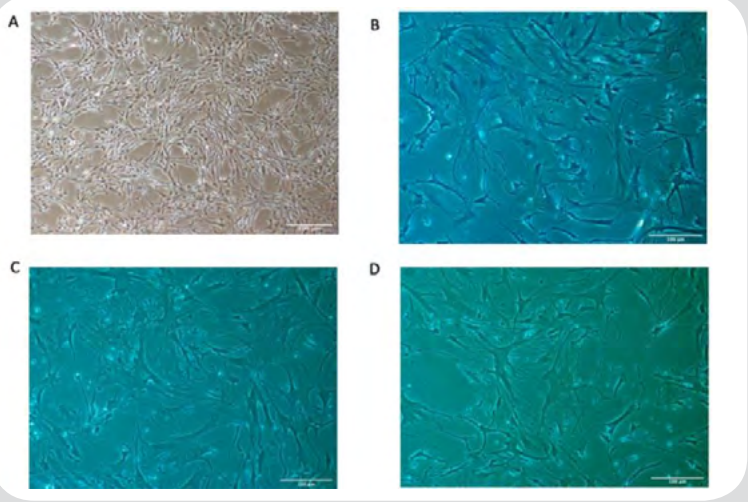


**Figure 3.** The appearance of HASMC cells after 48 hours of incubation with HUVEC supernatants exposed to atherogenic stimuli. The appearances of HASMCs incubated with HUVEC supernatants for 48 hours are as follows
**A.** Smooth muscle cells incubated with HUVEC medium without any added substance.
**B.** Smooth muscle cells incubated with HUVEC medium supplemented with 5% SDE (cigarette smoke extract).
**C.** Smooth muscle cells incubated with HUVEC medium supplemented with 300 μM $H_2O_2$.
**D.** Smooth muscle cells incubated with HUVEC medium supplemented with 40 μg/ml Ox-LDL.The images were captured at 20X magnification, and the scale bar represents 100 μm.

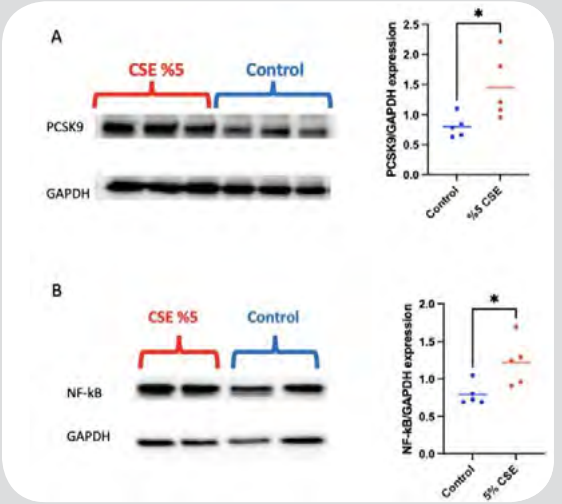


**Figure 4.** The protein expression of PCSK9 and NF-kB p65 in HASMCs incubated with endothelial cell medium supplemented with 5% CSE. Following protein extraction, HASMCs incubated with endothelial cell medium supplemented with 5% CSE and HASMCs incubated with endothelial cell medium without any additions (control) were evaluated using Western Blot analysis. .* p<0.05

**A.** The expression of PCSK9 was significantly higher in the %5 CSE group compared to the control group (p=0.034).

**B.** The expression of NF-kB p65 was significantly higher in the %5CSE group compared to the control group (p=0.024)

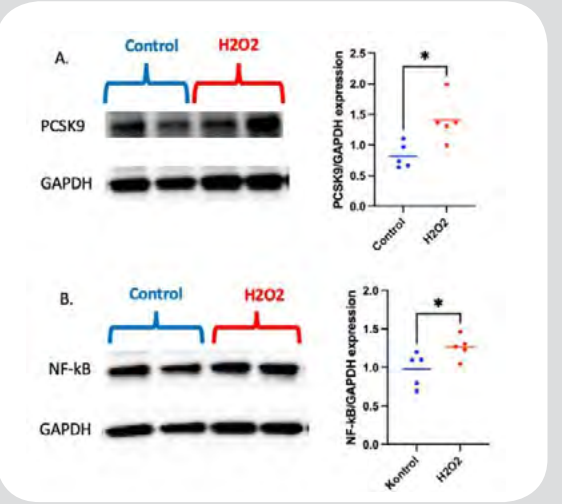


**Figure 5.** The protein expression of PCSK9 and NF-kB p65 in HASMCs incubated with endothelial cell medium supplemented with 300μM $H_2O_2$, Following protein extraction, HASMCs incubated with endothelial cell medium supplemented with 300μM $H_2O_2$, and HASMCs incubated with endothelial cell medium without any additions (control) were evaluated using Western Blot analysis. * p<0.05

**A.** The expression of PCSK9 was significantly higher in the 300μM H2O2 group compared to the control group (p=0.0137)

**B.**The expression of NF-kB p65 was significantly higher in the 300μM $H_2O_2$ group compared to the control group (p=0.043).

Atherosclerosis is triggered when vascular endothelial cells are exposed to atherogenic stimuli, followed by the involvement of inflammatory cells and vascular smooth muscle cells in the process (2). Understanding the effects of endothelial cells on other cells when exposed to atherosclerosis-triggering stimuli is important for understanding the mechanism of atherosclerosis. Changes induced by endothelial cells in smooth muscle cells, which are one of the most important cells in the vascular wall, are being investigated through various coculture studies (15, 16). Communication between endothelial cells and smooth muscle cells mainly occurs through paracrine signalling and via exosomes (17). Therefore, it is considered appropriate to use the stimulated medium method to observe the effects of paracrine molecules secreted by endothelial cells on smooth muscle cells when exposed to an atherosclerotic stimulus (18).

It is well-established that smoking causes atherosclerosis (19). Using cigarette smoke extract in cell culture experiments studying the atherosclerotic effects of smoking is a common method (14). One of the atherogenic substances we applied to endothelial cells was 5% cigarette smoke extract (CSE). CSE contains various reactive oxygen species that induce oxidative stress and disrupts NO biosynthesis by causing eNOS dysfunction (20). CSE leads to increased expression of adhesion molecules and the release of inflammatory cytokines such as TNF-α and IL-6 in endothelial cells (21). It has also been shown to induce NF-kB activation in bronchial epithelial cells (22). In our study, we observed an increased expression of NF-kB in HASMCs incubated with the medium from endothelial cells treated with 5% CSE. This finding supports previous studies indicating that CSE increases in-

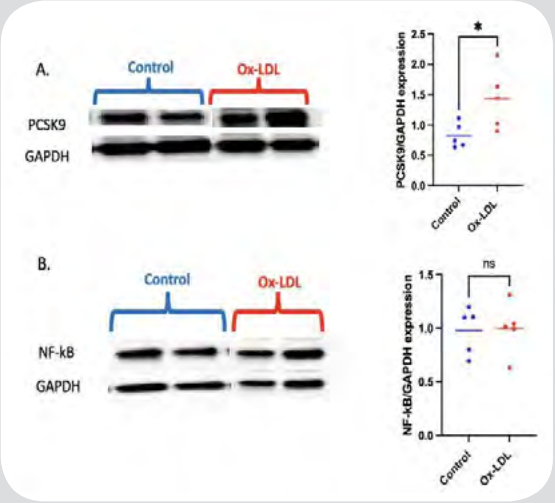


**Figure 6.** The protein expression of PCSK9 and NF-kB p65 in HASMCs incubated with endothelial cell medium supplemented with 40µg/ml Ox-LDL Following protein extraction, HASMCs incubated with endothelial cell medium supplemented with 40µg/ml Ox-LDLand HASMCs incubated with endothelial cell medium without any additions (control) were evaluated using Western Blot analysis. .* p<0.05, ns: nonsignificant **A.** The expression of PCSK9 was significantly higher in the 40µg/ml group compared to the control group (p=0.037)). **B.**The expression of NF-kB p65 was not different between Ox-LDL and the control group (p=0.89)

flammation in the vascular wall. Studies conducted on smooth muscle cells have shown that CSE acts as a mitogen (22). However, none of these studies has demonstrated increased NF-kB expression in smooth muscle cells due to exposure to CSE, as we have shown in our study. Ma et al. demonstrated that CSE increases PCSK9 expression in HepG2 cells and associated this finding with the mechanism of smoking-related dyslipidemia (23). However, there is no study available on the effect of smoking on PCSK9 expression in vascular wall cells. Our study provides new evidence that endothelial cells exposed to cigarette smoke increase PCSK9 expression in vascular smooth muscle cells. This finding is consistent with the knowledge that cigarette smoke induces a phenotypic change in smooth muscle cells, leading to a synthetic phenotype, as increased PCSK9 expression in smooth muscle cells is also associated with a synthetic phenotype (24).

When we applied $H_2O_2$ to endothelial cells, we observed an increase in NF-kB and PCSK9 expression in smooth muscle cells as well. $H_2O_2$ is a reactive oxygen species and plays a role in the development of atherosclerosis by inducing oxidative stress (25). Similar to other reactive oxygen species, it can disrupt cellular functions by causing DNA damage, lipid peroxidation, protein modifications, and impairment of the signalling system, leading to apoptosis in cells (26). Numerous studies have shown the association between increased NF-kB expression and $H_2O_2$ (27, 28). In our study, we hypothesize that the increased NF-kB expression in smooth muscle cells is associated with the release of inflammatory cytokines such as TNF-α and ROS from endothelial cells, which are exposed to H2O2. It has been demonstrated that ROS and inflammatory cytokines contribute to increased PCSK9 expression in smooth muscle cells (29). Therefore, observing increased PCSK9 expression in vascular smooth muscle cells is an expected finding. Additionally, it has been shown that PCSK9 inhibition can prevent the atherosclerotic effects caused by $H_2O_2$ (30).

The uptake/cholesterol efflux balance and accumulation of Ox-LDL stimulate the endothelial cells to produce pro-inflammatory molecules (31). The inflammatory molecules produced by endothelial cells also cause inflammatory changes in smooth muscle cells. Therefore, an expected finding is an increase in PCSK9 expression in smooth muscle cells incubated with the medium from endothelial cells exposed to Ox-LDL. Ding et al. have shown an increased expression of PCSK9 in vascular smooth muscle cells exposed to Ox-LDL (32). An increase in NF-kB expression in smooth muscle cells due to the release of inflammatory cytokines from endothelial cells was also expected, but we did not observe a statistically significant increase in NF-kB. This may be due to the lower concentration of Ox-LDL (40µg/ml) used in our study compared to other studies. Chen et al. have shown increased NF-kB expression with 50µg/ml Ox-LDL in their experiments (33). Ding et al. observed that when NF-kB gene expression was inhibited by siRNA in vascular smooth muscle cells, the induced PCSK9 expression by Ox-LDL decreased (32). This provides evidence for the association of PCSK9 expression in smooth muscle cells with the NF-kB pathway, and an expected

finding is an increase in NF-kB along with PCSK9 expression. However, Ding et al. demonstrated increased PCSK9 expression with 60µg/ml Ox-LDL, but did not observe a statistically significant rise with lower doses (32). On the other hand, Robbesyn et al. have shown that high doses of Ox-LDL can suppress NF-kB expression (34).

An important limitation of our study is that we incubated vascular smooth muscle cells with the medium from endothelial cells exposed to atherogenic stimuli. Demonstrating the molecules released by endothelial cells, especially inflammatory cytokines and PCSK9, in the medium through ELISA could have shed light on endothelial cell-smooth muscle cell communication. We aim to investigate this aspect in future studies. In the future, the communication between endothelial cells and smooth muscle cells, particularly regarding PCSK9 and inflammatory pathways, is aimed to be further evaluated in more detail.

## CONCLUSION

Endothelial cell smooth muscle cell interaction is one of the key mechanisms in atherosclerosis. In our study, we have shown that endothelial cells which were exposed to atherogenic stimuli induce PCSK9 and NF-kB expression in vascular smooth muscle cells.

In the future, more studies will shed light on the role of PCSK9 in this interaction.

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