

# Simvastatin and atorvastatin attenuate VCAM-1 and uPAR expression on human endothelial cells and platelet surface expression of CD40 ligand

Ksenija Stach<sup>1,4</sup>, Xuan Duc Nguyen<sup>2</sup>, Siegfried Lang<sup>1</sup>, Elif Elmas<sup>1</sup>, Christel Weiß<sup>3</sup>,  
Martin Borggrefe<sup>1</sup>, Joachim Fischer<sup>4</sup>, Thorsten Kälsch<sup>1</sup>

<sup>1</sup>1<sup>st</sup> Department of Medicine, University Medical Center Mannheim,  
Medical Faculty Mannheim, University of Heidelberg, Germany

<sup>2</sup>Institute of Transfusion Medicine and Immunology, University Medical Center Mannheim,  
Medical Faculty Mannheim, University of Heidelberg, Germany

<sup>3</sup>Department of Medical Statistics, University Medical Center Mannheim,  
Medical Faculty Mannheim, University of Heidelberg, Germany

<sup>4</sup>Institute of Public Health, University Medical Center Mannheim,  
Medical Faculty Mannheim, University of Heidelberg, Germany

## Abstract

**Background:** *In addition to their cholesterol lowering ability, statins have proven pleiotropic effects in the cardiovascular system. Chronic inflammation with interactions between platelets and endothelial cells leads to an upregulation of activity markers of atherosclerosis. The purpose of this study was to investigate the effects of simvastatin and atorvastatin on platelets and endothelial cells in an in vitro endothelial cell model.*

**Methods and Results:** *After a 24 h incubation period with either simvastatin or atorvastatin (1 µmol/L), human umbilical vein endothelial cells were stimulated for 1 h with lipopolysaccharide (LPS), and were then incubated in direct contact with activated platelets. Platelet surface expression of CD40L and CD62P and expression of ICAM-1, VCAM-1, uPAR and MT1-MMP on endothelial cells were measured by flow cytometry. Supernatants were analyzed by ELISA for soluble MMP-1. The increased expression of VCAM-1 and uPAR on endothelial cells by stimulation with LPS and by direct contact with activated platelets was significantly reduced to a similar extent through pre-incubation with both atorvastatin and simvastatin ( $p < 0.05$ ). Platelets without endothelial cell contact, but in direct contact with either statin, showed similar significant reductions in surface expression of CD40L ( $p < 0.005$ ).*

**Conclusions:** *These effects may explain the ability of statins to reduce the progression of atherosclerosis in addition to their cholesterol-lowering properties. (Cardiol J 2011; 18, X: xx–xx)*

**Key words:** platelets, endothelial cells, simvastatin, atorvastatin, atherosclerosis

Address for correspondence: Thorsten Kälsch, MD, PhD, Assistant Professor of Medicine, 1<sup>st</sup> Department of Medicine, University Medical Center Mannheim, Medical Faculty Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany, tel/fax: 0049-6213832204/0049-6213833821, e-mail: thorsten.kaelsch@umm.de; T.Kaelsch@yahoo.de

Received: 23.06.2011

Accepted: 12.09.2011

## Introduction

The pathogenesis of atherosclerosis, the leading cause of morbidity and mortality in industrial countries, is multifactorial. Cardiovascular (CV) risk factors such as hypertension, diabetes, cigarette smoking, family history and elevated serum lipid levels contribute to the initiation and progression of atherosclerosis [1–5]. Interactions between platelets and endothelium play an important role in the pathogenesis of atherosclerosis and CV diseases, leading to severe clinical events such as myocardial infarction (MI) and stroke. It is characterized by the formation of plaque consisting of foam cells, immune cells, vascular endothelial cells, smooth muscle cells, platelets, extracellular matrix and a lipid-rich core [2, 6, 7]. Moreover, interactions between platelets and endothelial cells mediate essential processes in the development of atherosclerosis by an increased expression of vascular cell adhesion molecules and their ligands [8–12]. CD62P and CD40L are expressed on activated platelets and are directly involved in the interaction of platelets with leukocytes and endothelial cells [13]. Binding of CD40L to endothelial CD40, which is the counter-receptor for platelet-derived CD40L, leads to the release of interleukin-8, tissue factor and MCP-1, the major chemoattractants for neutrophils and monocytes [14–16]. In addition, activation of CD40 on endothelial cells leads to increased expression of various endothelial adhesion receptors such as E-selectin, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 [17–19]. Upregulation of endothelial VCAM-1 and ICAM-1 expression increases vulnerability of the atherosclerotic vascular plaque to fissuring rupture and superimposed thrombosis, leading to the clinical scenario of unstable angina, acute MI or sudden cardiac death [20–22]. Engagement of the endothelial CD40 receptor and platelet CD40L results in an increased production of inflammatory cytokines, adhesion molecules and matrix-degrading proteases (MMPs). MMPs degrade various proteins of the extracellular matrix and promote inflammation and the destruction of the inflamed tissue. Additionally, imbalance in the plasminogen and matrix metalloproteinase activation systems may lead to destabilization of the vulnerable fibrous cap of the atherosclerotic plaque [23–25].

Statins are highly effective lipid-lowering agents, widely used in medical practice [26]. Simvastatin and atorvastatin are representative of this class and are commonly prescribed and applied statins. Hypercholesterolemia impairs endothelial function

and, by blocking 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), statins inhibit an early rate-limiting step in cholesterol biosynthesis [27]. Furthermore, statins decrease smooth muscle cell migration and proliferation [28], decrease pre-pro endothelin-1 (ET-1) mRNA expression and increase basal nitric oxide dilator functions of the endothelium [29]. Because of the strong association between elevated serum cholesterol levels and coronary atherosclerotic disease, it has been suggested that the reduction of serum cholesterol levels by statins is the main mechanism underlying their beneficial effects [30].

However, recent evidence suggests that statins have pleiotropic effects, independent of lipid balance regulation, and that this may partially explain their role in decreasing CV mortality and morbidity. The influence of statin treatment on various soluble cellular adhesion molecules (CAMs), including ICAM-1, has already been described in hypercholesterolemic patients [31]. Pleiotropic statin effects may be due to an attenuation of interactions between platelets and vascular endothelial cells and the release and expression of activity markers of atherosclerosis. Therefore, the purpose of the present study was to investigate the effects of simvastatin and atorvastatin on platelets and endothelial cells in an *in vitro* endothelial cell model.

## Methods

### Coincubation of simvastatin or atorvastatin pre-incubated endothelial cells with platelets

Human umbilical vein endothelial cells (HUVEC) were prepared as previously described [32, 33] and cultured in endothelial cell basal medium (PromoCell) containing 2% fetal calf serum (FCS); 1 µg/mL hydrocortisone (HC-500); 0.4% endothelial cell growth supplement (ECGS/H-2); 0.1 ng/mL epidermal growth factor (hEGF-0.05); 1 ng/mL basic fibroblast growth factor (hbFGF-0.5); 5 ng/mL amphotericin and 50 µg/mL gentamicin. The confluent endothelial cells were added to 12-well plates and incubated for 24 h with 1 mmol/L nicotinic acid (Merck, Hohenbrunn, Germany). Platelets were prepared from the blood of healthy probands as described [34, 35]. Washed platelets were stimulated for 30 min with thrombin (0.5 U/mL) and lipopolysaccharide (LPS) (1,000 ng/mL). Before coincubation experiments, thrombin activity was antagonized by hirudin (5 U/mL). Pretreated platelets (final concentration  $2 \times 10^8$ /mL) were added to confluent endothelial monolayers with and without nicotinic acid. After 60-min coincubation under

cell culture conditions, all platelets were removed by gentle washing, which was confirmed by light microscopy. After six more hours of incubation of the endothelial cells, the supernatant was aspirated, centrifuged at 2,000 g and stored at  $-80^{\circ}\text{C}$  [36]. Following this incubation, the expression of activity markers on platelets, as well as that on endothelial cells, was measured by flow cytometry.

### Flow cytometric analysis

Flow cytometric analysis of platelets and endothelial cells was performed by gating in forward and side scatter. Platelets were gated back for determination of the expression of CD40L and CD62P. For the analysis of platelets, 100  $\mu\text{L}$  of each sample were stained for 15 min at room temperature with 10  $\mu\text{L}$  aliquots of mouse anti-human CD40L-FITC antibodies (BD Pharmingen, Heidelberg, Germany) and mouse anti-human CD62P-PE antibodies (Beckman-Coulter, Krefeld, Germany). Endothelial cells were gated back for determination of the surface expression of ICAM-1, VCAM-1, urokinase receptor uPAR and membrane type 1 matrix metalloprotease (MT1-MMP). For the analysis of endothelial cells, 100  $\mu\text{L}$  of each sample were stained for 15 min at room temperature with 10  $\mu\text{L}$  aliquots of anti-human CD54 PE-Cy5 (ICAM-1 from BD Pharmingen, Heidelberg, Germany), anti-human CD106-FITC (VCAM-1 from R&D Systems, Inc., Wiesbaden, Germany), anti-human CD87-FITC (uPAR from American Diagnostica Inc., Stamford, CT, USA), anti-human MT1-MMP (Ab-1) Mouse mAb (114-IF2) (Merck Chemicals Ltd., Nottingham, UK). Corresponding isotypes (Beckman Coulter, Marseille, France) were used as a control. All flow cytometric analyses were performed on an EPICS XL-MCL analyzer (Beckman Coulter, Krefeld, Germany) equipped with an argon laser tuned at 488 nm. Mean fluorescence intensity was measured and all FACS data is expressed as MFI in this manuscript. System II Version 3.0 software was used for data acquisition and evaluation. Compensation of the four channel fluorescence was adjusted for precision using Cyto-Comp<sup>TM</sup> reagents and Cyto-Trol<sup>TM</sup> control cells (Coulter Immunotech, Krefeld, Germany).

### Enzyme linked immunosorbent assay (ELISA)

The concentration of MMP-1 (Human, Biotrak ELISA System, GE Healthcare Ltd, UK) in the supernatants was determined by sandwich-type immunoassay following the manufacturer's instructions. All concentration analyses were performed

on an ELISA-Reader — Lab Systems Multiskan RC (Labsystems, Finland). Genesis Lite Software, ELISA Multiskan RC was used for data acquisition and evaluation.

### Statistical analysis

All calculations were performed using SAS release 9.2 (SAS Institute Inc. Cary, NC, USA). Numerical data was expressed as means  $\pm$  standard deviation. A Dunnett's test was applied as parametric test. A two-tailed probability value  $< 0.05$  was considered significant.

The study was approved by the local bioethical committee and all patients gave their informed consent.

## Results

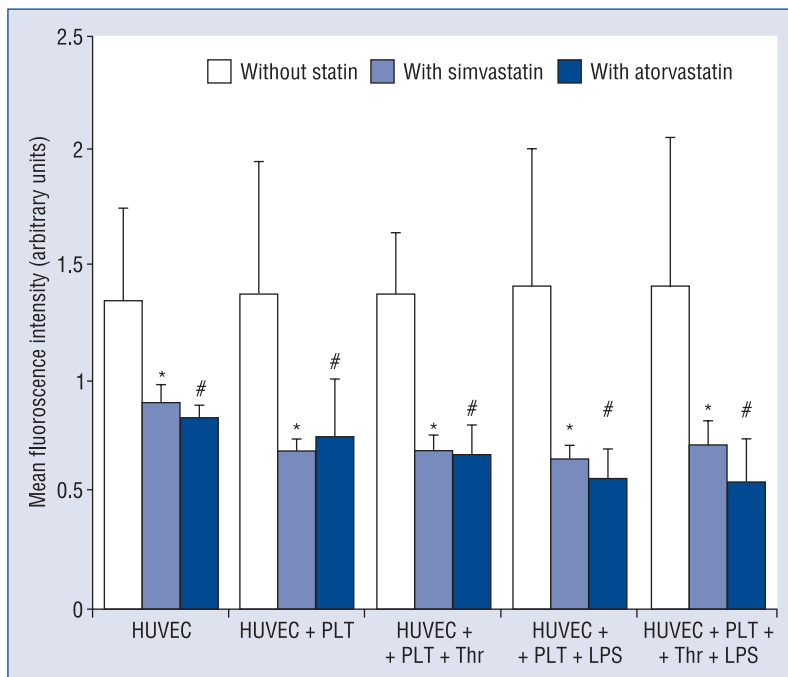
### Effects of simvastatin and atorvastatin on endothelial cell surface markers

HUVEC pre-incubation with simvastatin resulted in significantly decreased surface expression of VCAM-1 from  $1.37 \pm 0.57$  to  $0.69 \pm 0.05$  ( $p = 0.013$ ) after contact with resting platelets, and from  $1.26 \pm 0.4$  to  $0.69 \pm 0.07$  ( $p = 0.007$ ) after contact with thrombin-stimulated platelets. Pre-incubation with simvastatin significantly reduced surface expression of VCAM-1 from  $1.4 \pm 0.6$  to  $0.66 \pm 0.05$  ( $p = 0.014$ ) after contact with LPS-stimulated platelets, and from  $1.4 \pm 0.66$  to  $0.7 \pm 0.1$  ( $p = 0.03$ ) after contact with LPS- and thrombin-stimulated platelets (Fig. 1).

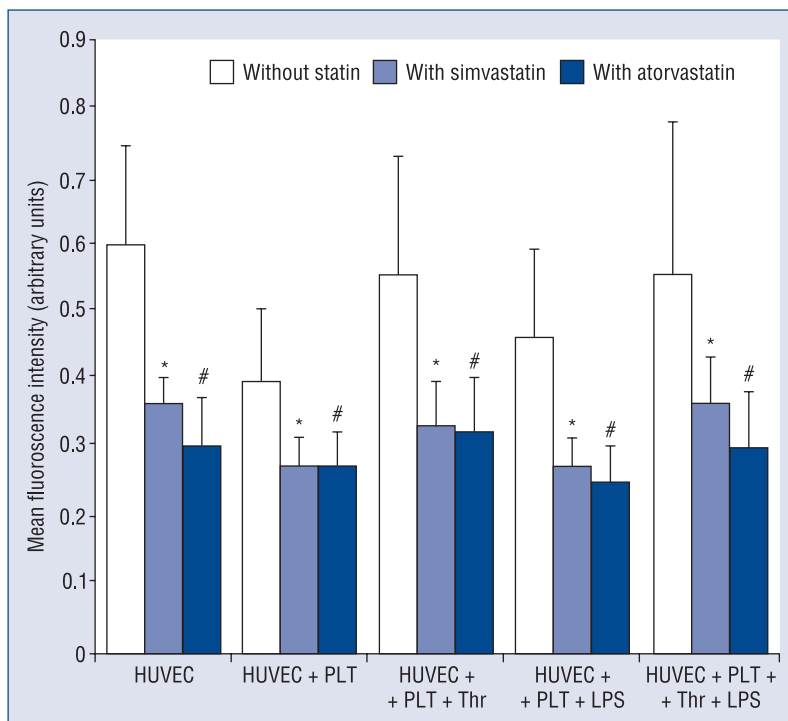
HUVEC pre-incubation with simvastatin resulted in significantly decreased surface expression of uPAR from  $0.4 \pm 0.1$  to  $0.27 \pm 0.04$  ( $p = 0.038$ ) after contact with platelets, and from  $0.56 \pm 0.17$  to  $0.33 \pm 0.06$  ( $p = 0.035$ ) after contact with thrombin-stimulated platelets. Pre-incubation with simvastatin significantly reduced surface expression of uPAR from  $0.46 \pm 0.13$  to  $0.27 \pm 0.04$  ( $p = 0.015$ ) after contact with LPS-stimulated platelets (Fig. 2).

HUVEC pre-incubation with atorvastatin resulted in significantly decreased surface expression of VCAM-1 from  $1.37 \pm 0.57$  to  $0.7 \pm 0.2$  ( $p = 0.01$ ) after contact with platelets, and from  $1.26 \pm 0.4$  to  $0.68 \pm 0.12$  ( $p = 0.007$ ) after contact with thrombin-stimulated platelets. Pre-incubation with atorvastatin significantly reduced surface expression of VCAM-1 from  $1.4 \pm 0.6$  to  $0.57 \pm 0.12$  ( $p = 0.008$ ) after contact with LPS-stimulated platelets, and from  $1.4 \pm 0.66$  to  $0.55 \pm 0.18$  ( $p = 0.01$ ) after contact with LPS- and thrombin-stimulated platelets (Fig. 1).

HUVEC pre-incubation with atorvastatin resulted in significantly decreased surface expression



**Figure 1.** Surface expression of VCAM-1 on HUVEC with and without 24 h pre-incubation with simvastatin or atorvastatin; N = 7 experiments; PLT — platelets; Thr — thrombin; LPS — lipopolysaccharide; \*p < 0.05 against not pre-treated HUVEC; #p < 0.05 against not pre-treated HUVEC.



**Figure 2.** Surface expression of uPAR on HUVEC with and without 24 h pre-incubation with simvastatin or atorvastatin; N = 7 experiments; PLT — platelets; Thr — thrombin; LPS — lipopolysaccharide; \*p < 0.05 against not pre-treated HUVEC; #p < 0.05 against not pre-treated HUVEC.

of uPAR from  $0.4 \pm 0.1$  to  $0.27 \pm 0.04$  ( $p = 0.03$ ) after contact with platelets, and from  $0.56 \pm 0.17$  to  $0.32 \pm 0.079$  ( $p = 0.02$ ) after contact with thrombin-stimulated platelets. Pre-incubation with atorvastatin significantly reduced surface expression of uPAR from  $0.46 \pm 0.13$  to  $0.25 \pm 0.05$  ( $p = 0.007$ ) after contact with LPS-stimulated platelets, and from  $0.55 \pm 0.22$  to  $0.3 \pm 0.08$  ( $p = 0.03$ ) after contact with LPS- and thrombin-stimulated platelets (Fig. 2).

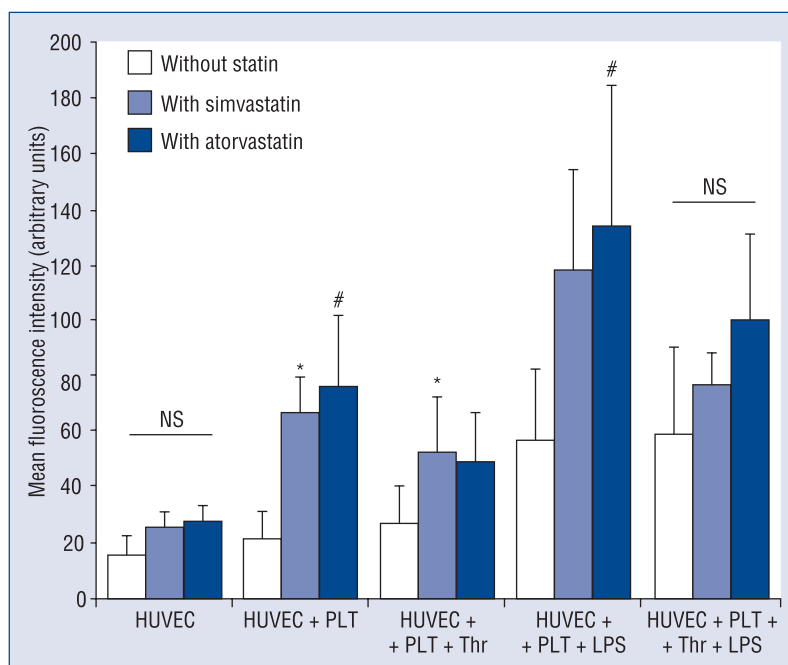
Regarding ICAM-1 expression on HUVEC, pre-incubation with simvastatin significantly increased surface expression of ICAM-1 from  $21.03 \pm 10.7$  to  $67.44 \pm 11.5$  ( $p = 0.0051$ ) after contact with platelets, and from  $26.5 \pm 14.2$  to  $52.32 \pm 20.25$  ( $p = 0.049$ ) after contact with thrombin-stimulated platelets (Fig. 3). Additionally, pre-incubation with atorvastatin significantly increased surface expression of ICAM-1 from  $21.03 \pm 10.7$  to  $76 \pm 25.8$  ( $p = 0.0018$ ) after contact with platelets, and from  $57.3 \pm 25.9$  to  $133.7 \pm 50.9$  ( $p = 0.02$ ) after contact with LPS-stimulated platelets (Fig. 3).

Simvastatin or atorvastatin had no significant effects on the surface expression of MT1-MMP on endothelial cells.

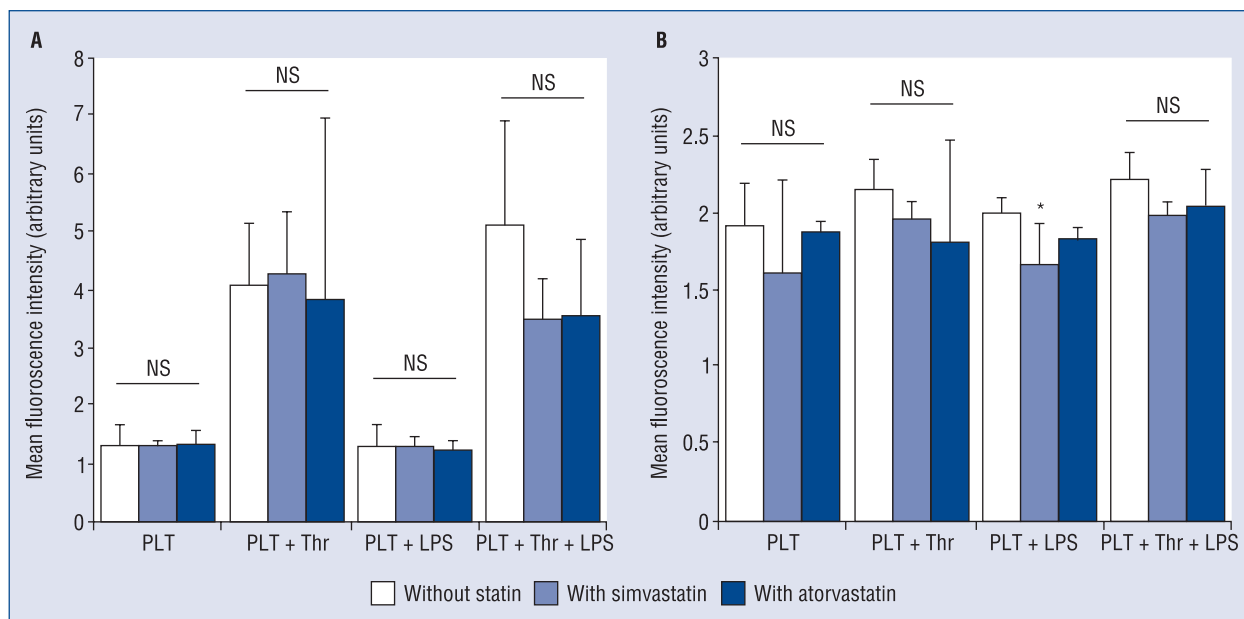
In a comparison between simvastatin and atorvastatin, we also observed no significant differences in their effects on human umbilical vein endothelial cell surface markers.

### Endothelial cell mediated and direct effects of simvastatin and atorvastatin on platelets

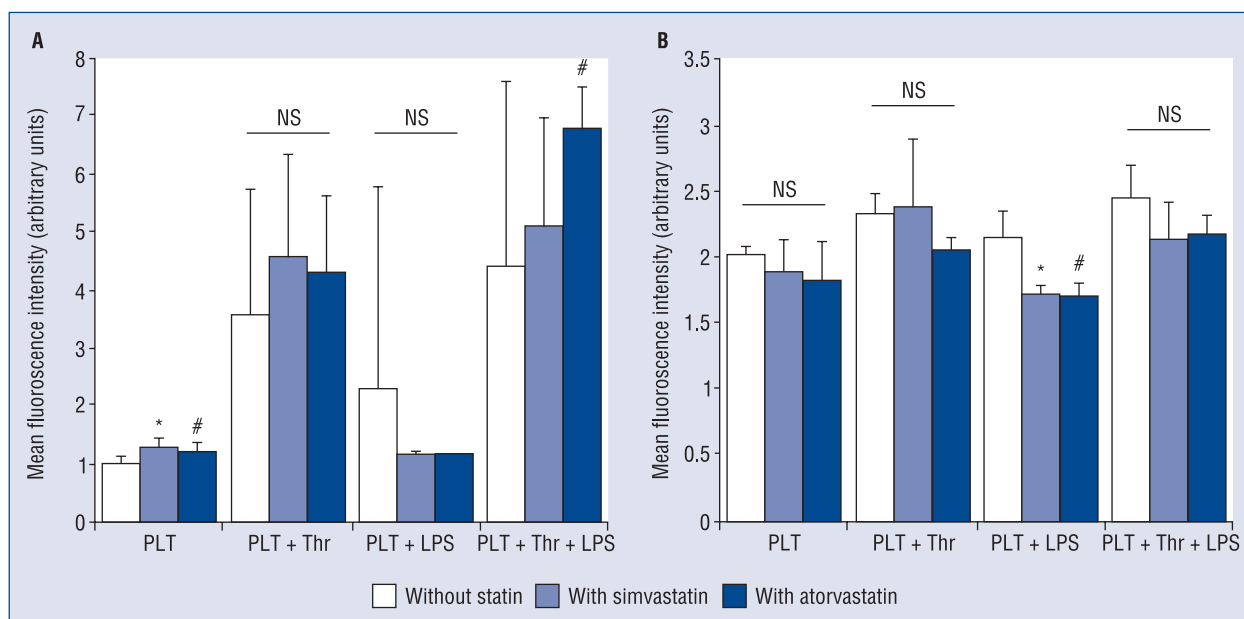
Platelet surface expression of CD62P did not differ significantly between platelets in direct contact with simvastatin or atorvastatin pre-incubated endothelial cells compared to platelets incubated with untreated endothelial cells (Fig. 4). Surface expression of CD40L on platelets after one hour's direct contact with HUVECs, pre-incubated with simvastatin, and stimulated with LPS, significantly decreased from  $1.99 \pm 0.09$  to  $1.66 \pm 0.26$  ( $p = 0.0038$ ) (Fig. 4). To discriminate between those effects mediated by pre-treated endothelial cells and the possible direct effects of simvastatin or atorvastatin on platelets, platelets were directly incubated with either simvastatin or atorvastatin solely for 1 h. When this was done, simvastatin incubation significantly increased CD62P expression on unstimulated platelets from  $0.99 \pm 0.1$  to  $1.31 \pm 0.13$  ( $p = 0.0012$ ), and atorvastatin incubation significantly increased CD62P expression on unstimulated platelets from  $0.99 \pm 0.1$  to  $1.21 \pm 0.12$  ( $p = 0.01$ ), and when stimulated with LPS and thrombin from  $4.43 \pm 3.14$  to  $6.78 \pm 0.7$  ( $p = 0.0047$ ) (Fig. 5). Surface expression of CD40L on platelets under stimulation with LPS was significantly reduced by simvastatin from  $2.14 \pm 0.19$



**Figure 3.** Surface expression of ICAM-1 on HUVEC with and without 24 h pre-incubation with simvastatin or atorvastatin; N = 7 experiments; PLT — platelets; Thr — thrombin; LPS — lipopolysaccharide; NS — not significant; \* $p < 0.05$  against not pre-treated HUVEC; # $p < 0.05$  against not pre-treated HUVEC.



**Figure 4.** Surface expression of CD62P (A) and CD40L (B) on platelets after 1 h of direct contact with HUVECs, with and without pre-incubation with simvastatin or atorvastatin; N = 7 experiments; PLT — platelets; Thr — thrombin; LPS — lipopolysaccharide; NS — not significant; \*p < 0.05 vs non pre-treated platelets.



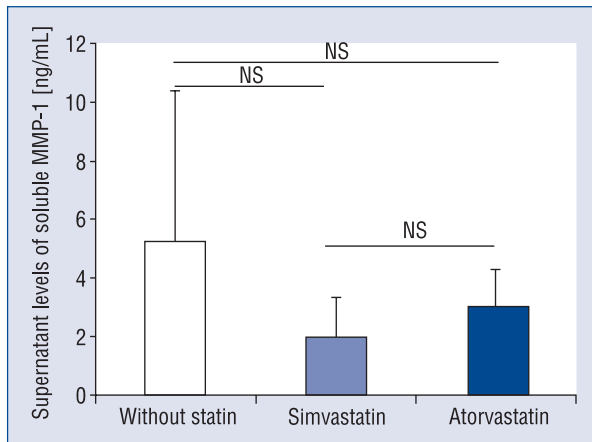
**Figure 5.** Direct effects of simvastatin or atorvastatin on surface expression of CD62P (A) and CD40L (B) on platelets. Platelets were directly incubated with simvastatin or atorvastatin for 1 h and stimulated with lipopolysaccharide (LPS) and/or thrombin (Thr); N = 7 experiments; PLT — platelets; NS — not significant; \*p < 0.05 vs non pre-treated platelets; #p < 0.05 vs non pre-treated platelets.

to  $1.72 \pm 0.06$  ( $p = 0.0002$ ) and by atorvastatin from  $2.14 \pm 0.19$  to  $1.69 \pm 0.11$  ( $p < 0.0001$ ) (Fig. 5).

In a comparison between simvastatin and atorvastatin, we observed no significant differences in their individual effects on platelet surface markers.

**Effects of simvastatin and atorvastatin on HUVEC supernatant levels of soluble MMP-1**

Upon direct HUVEC stimulation with interleukin  $1\beta$ , supernatant levels of MMP-1 were lower on HUVEC pre-treated with simvastatin ( $1.97 \pm 1.38$



**Figure 6.** Supernatant levels of soluble MMP-1 upon stimulation with interleukin  $1\beta$  on HUVEC with and without pre-incubation with simvastatin or atorvastatin; NS — not significant.

ng/mL;  $p = 0.39$ ) and with atorvastatin ( $3.06 \pm 1.23$  ng/mL;  $p = 0.55$ ) compared to untreated HUVEC ( $5.22 \pm 5.15$  ng/mL), although these differences were not statistically significant (Fig. 6).

## Discussion

Many clinical studies, as well as data from recent experimental studies, have revealed that statins have additional effects beyond their serum cholesterol-lowering properties and that statins modify various biological processes in the vessel wall [12, 28–31, 37–43]. In the present study, simvastatin and atorvastatin reduced the expression of proatherogenic activity and progression markers on platelets and endothelial cells under pro-inflammatory conditions in an *in vitro* endothelial cell model. Simvastatin and atorvastatin both significantly reduced the expression of VCAM-1 and uPAR on endothelial cells after direct contact with activated platelets and surface expression of CD40L on platelets. These results underpin the extensive atheroprotective effects of statins, which may, in addition to their lipid-lowering potential, contribute to the inhibition of atherosclerosis progression.

The benefit of statin therapy appears to exceed the cholesterol-lowering effect by blocking HMG-CoA reductase, possibly by protective effects on endothelial nitric oxide bioactivity [29] and atherosclerotic plaque stabilization [28, 40, 41]. In addition, a recent study has shown that statins can suppress inflammatory response independently of HMG-CoA reductase inhibition by means of binding directly to a novel regulatory site of the  $\beta_2$  inte-

grin via inhibiting leukocyte function antigen-1 [37]. The mechanism of anti-inflammatory properties of statins was further described by Yoshida et al. [38], who demonstrated that cerivastatin reduced monocyte adhesion to vascular endothelium by decreasing expression of integrins and actin polymerization through inactivation of RhoA.

In 1990, Berk et al. [44] highlighted the role of inflammation in coronary artery disease with an elevation of C-reactive protein (CRP). In the CARE trial, statins significantly decreased plasma hs-CRP levels over a five-year period in patients who did not experience recurrent coronary events. This emphasizes the direct anti-inflammatory potential of statins [39]. These studies indicate that statins are effective in decreasing systemic and vascular inflammation. The CD40-CD40L signalling pathway plays a key role in the modulation of inflammatory responses between vascular cells and platelets, involving adhesion molecules, pro-inflammatory cytokines and chemokines. Statins have been proven to decrease CD40 expression and CD40-related activation of vascular cells [40, 41]. Mosheimer et al. [43] showed that activated platelets induce COX-2 expression in HUVEC, and that this effect can be reversed by pre-incubation of platelets with atorvastatin; this effect is due to the down-regulation of CD40L on activated platelets by atorvastatin. It is worth noting that in the present study we were able to support these previous findings: simvastatin and atorvastatin induced a significant reduction of CD40L expression on platelets, whereas no significant individual differences between both statins regarding CD40L platelet surface expression could be observed. The simvastatin and atorvastatin *in vitro* concentration applied in the present study was  $1 \mu\text{mol/L}$ , which is within the range of effective serum concentrations seen in clinical practice [42, 45].

A limited number of randomized controlled clinical trials comparing the use of atorvastatin to that of simvastatin have shown that atorvastatin is more effective in reducing total cholesterol levels and LDL-cholesterol levels, as well as the rate of CV events [46–48]. Jacobson et al. [49] found in a retrospective study that the risk of the first CV event was significantly lower among patients in whom atorvastatin, rather than simvastatin, had been newly initiated. uPAR is present at increased levels in atherosclerosis, and correlates directly with CV disease severity [50].

In our study, atorvastatin and simvastatin both significantly reduced the expression of VCAM-1 and uPAR on endothelial cells and CD40L surface expression on platelets. However, we observed no

significant differences between the statins regarding individual effects on human umbilical vein endothelial cell and platelet surface markers. In the ARMYDA-ACS study, Patti et al. [51] demonstrated an increased expression of ICAM-1 and VCAM-1 after preloading with high dose atorvastatin and after percutaneous coronary intervention. A possible explanation for the increased expression of ICAM-1 may be the periprocedural use of glycoprotein IIb/IIIa inhibitors. Our study also showed a significantly increased expression of ICAM-1 on endothelial cells preincubated with statins and after stimulation with activated platelets. However, we cannot provide detailed mechanistic insights as to why statins increased the expression of ICAM-1 on endothelial cells, but significantly decreased VCAM-1 effects. Future studies will be needed to further explore the possible mechanism which supports the influence of statins on CV diseases.

### Conclusions

We demonstrated that simvastatin and atorvastatin both extenuate the expression of proatherogenic markers on endothelial cells and platelets under pro-inflammatory conditions. These effects may explain the ability of statins to reduce CV events and progression of atherosclerosis in addition to their cholesterol-lowering properties.

### Acknowledgements

The authors do not report any conflict of interest regarding this work.

### References

1. Jahn J, Dalhoff K, Katus HA. Coronary artery disease: An inflammatory or infectious process. *Basic Res Cardiol*, 2000; 95 (suppl. 1): I59–I64.
2. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*, 2002; 105: 1135–1143.
3. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*, 2000; 342: 836–843.
4. Zerneck A, Weber C. Inflammatory mediators in atherosclerotic vascular disease. *Basic Res Cardiol*, 2005; 100: 93–101.
5. Zwaka TP, Hombach V, Torzewski J. C-reactive protein-mediated low density lipoprotein uptake by macrophages: Implications for atherosclerosis. *Circulation*, 2001; 103: 1194–1197.
6. Ruggeri ZM. Platelets in atherothrombosis. *Nat Med*, 2002; 8: 1227–1234.
7. Lusis AJ. Atherosclerosis. *Nature*, 2000; 407: 233–241.
8. Amirkhosravi A, Alexander M, May K et al. The importance of platelets in the expression of monocyte tissue factor antigen measured by a new whole blood flow cytometric assay. *Thromb Haemost*, 1996; 75: 87–95.
9. Gotz AK, Zahler S, Stumpf P, Welsch U, Becker BF. Intracoronary formation and retention of micro aggregates of leukocytes and platelets contribute to posts ischemic myocardial dysfunction. *Basic Res Cardiol*, 2005; 100: 413–421.
10. Kälsch T, Elmas E, Nguyen XD et al. Enhanced coagulation activation by in vitro lipopolysaccharide challenge in patients with ventricular fibrillation complicating acute myocardial infarction. *J Cardiovasc Electrophysiol*, 2005; 16: 858–863.
11. Kälsch T, Nguyen XD, Elmas E et al. Coagulation activation and expression of CD40 ligand on platelets upon in vitro lipopolysaccharide-challenge in patients with unstable angina. *Int J Cardiol*, 2006; 111: 217–223.
12. Steiner S, Speidl WS, Pleiner J et al. Simvastatin blunts endotoxin-induced tissue factor in vivo. *Circulation*, 2005; 111: 1841–1846.
13. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. *Cardiovasc Res*, 2004; 61: 498–511.
14. Henn V, Slupsky JR, Grafe M et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*, 1998; 391: 591–594.
15. Schonbeck U, Mach F, Libby P. CD154 (CD40 ligand). *Int J Biochem Cell Biol*, 2000; 32: 687–693.
16. Reed GL. Platelet secretory mechanisms. *Semin Thromb Hemost*, 2004; 30: 441–450.
17. Mach F, Schonbeck U, Sukhova GK, Atkinson E, Libby P. Reduction of atherosclerosis in mice by inhibition of CD40 signaling. *Nature*, 1998; 394: 200–203.
18. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa b activation in endothelial cells. *J Biol Chem*, 2001; 276: 7614–7620.
19. Davies MJ, Gordon JL, Gearing AJ et al. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol*, 1993; 171: 223–229.
20. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol*, 2007; 27: 2292–2301.
21. Kitagawa K, Matsumoto M, Sasaki T et al. Involvement of ICAM-1 in the progression of atherosclerosis in APOE-knockout mice. *Atherosclerosis*, 2002; 160: 305–310.
22. Lutgens E, Gorelik L, Daemen MJ et al. Requirement for CD154 in the progression of atherosclerosis. *Nat Med*, 1999; 5: 1313–1316.
23. Pepper MS. Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. *Arterioscler Thromb Vasc Biol*, 2001; 21: 1104–1117.
24. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*, 1994; 94: 2493–2503.
25. Preissner KT, Kanse SM, May AE. Urokinase receptor: A molecular organizer in cellular communication. *Curr Opin Cell Biol*, 2000; 12: 621–628.
26. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*, 2000; 101: 207–213.
27. Endo A. The discovery and development of HMG-CoA reductase inhibitors. *J Lipid Res*, 1992; 33: 1569–1582.
28. Bellosta S, Bernini F, Ferri N et al. Direct vascular effects of HMG-CoA reductase inhibitors. *Atherosclerosis*, 1998; 137 (suppl.): S101–S109.

29. Hernandez-Perera O, Perez-Sala D, Navarro-Antolin J et al. Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. *J Clin Invest*, 1998; 101: 2711–2719.
30. Treasure CB, Klein JL, Weintraub WS et al. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *N Engl J Med*, 1995; 332: 481–487.
31. Rezaie-Majd A, Prager GW, Bucek RA et al. Simvastatin reduces the expression of adhesion molecules in circulating monocytes from hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol*, 2003; 23: 397–403.
32. Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest*, 1973; 52: 2745–2756.
33. Gimbrone MA, Jr. Culture of vascular endothelium. *Prog Hemost Thromb*, 1976; 3: 1–28.
34. Gawaz M, Neumann FJ, Dickfeld T et al. Activated platelets induce monocyte chemotactic protein-1 secretion and surface expression of intercellular adhesion molecule-1 on endothelial cells. *Circulation*, 1998; 98: 1164–1171.
35. Dickfeld T, Lengyel E, May AE et al. Transient interaction of activated platelets with endothelial cells induces expression of monocyte-chemoattractant protein-1 via a p38 mitogen-activated protein kinase mediated pathway. Implications for atherogenesis. *Cardiovasc Res*, 2001; 49: 189–199.
36. May AE, Kälsch T, Massberg S, Herouy Y, Schmidt R, Gawaz M. Engagement of glycoprotein IIb/IIIa (alphaIIb)beta3 on platelets upregulates CD40L and triggers CD40L-dependent matrix degradation by endothelial cells. *Circulation*, 2002; 106: 2111–2117.
37. Weitz-Schmidt G, Welzenbach K, Brinkmann V et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med*, 2001; 7: 687–692.
38. Yoshida M, Sawada T, Ishii H et al. HMG-CoA reductase inhibitor modulates monocyte-endothelial cell interaction under physiological flow conditions in vitro: Involvement of Rho GTPase-dependent mechanism. *Arterioscler Thromb Vasc Biol*, 2001; 21: 1165–1171.
39. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation*, 1999; 100: 230–235.
40. Mulhaupt F, Matter CM, Kwak BR et al. Statins (HMG-CoA reductase inhibitors) reduce CD40 expression in human vascular cells. *Cardiovasc Res*, 2003; 59: 755–766.
41. Wagner AH, Gebauer M, Guldenzoph B, Hecker M. 3-hydroxy-3-methylglutaryl coenzyme A reductase-independent inhibition of CD40 expression by atorvastatin in human endothelial cells. *Arterioscler Thromb Vasc Biol*, 2002; 22: 1784–1789.
42. Veillard NR, Braunersreuther V, Arnaud C et al. Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. *Atherosclerosis*, 2006; 188: 51–58.
43. Mosheimer BA, Kaneider NC, Feistritz C et al. Cd40-ligand-dependent induction of COX-2 gene expression in endothelial cells by activated platelets: Inhibitory effects of atorvastatin. *Blood Coagul Fibrinol*, 2005; 16: 105–110.
44. Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in "active" coronary artery disease. *Am J Cardiol*, 1990; 65: 168–172.
45. Osman L, Amrani M, Ilsley C, Yacoub MH, Smolenski RT. Atorvastatin accelerates extracellular nucleotide degradation in human endothelial cells. *Mol Cell Biochem*, 2008; 308: 209–217.
46. Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). *Am J Cardiol*, 1998; 81: 582–587.
47. Jones PH, Davidson MH, Stein EA et al. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR<sup>®</sup> trial). *Am J Cardiol*, 2003; 92: 152–160.
48. Gentile S, Turco S, Guarino G et al. Comparative efficacy study of atorvastatin vs simvastatin, pravastatin, lovastatin and placebo in type 2 diabetic patients with hypercholesterolaemia. *Diabetes Obes Metab*, 2000; 2: 355–362.
49. Jacobson TA, Wertz DA, Hoy T, Kuznik A, Grochulski D, Cziriky M. Comparison of cardiovascular event rates in patients without cardiovascular disease in whom atorvastatin or simvastatin was newly initiated. *Mayo Clin Proc*, 2008; 83: 1316–1325.
50. Lacroix R, Sabatier F, Mialhe A et al. Activation of plasminogen into plasmin at the surface of endothelial microparticles: A mechanism that modulates angiogenic properties of endothelial progenitor cells *in vitro*. *Blood*, 2007; 110: 2432–2439.
51. Patti G, Chello M, Gatto L et al. Short-term atorvastatin preload reduces levels of adhesion molecules in patients with acute coronary syndrome undergoing percutaneous coronary intervention. Results from the ARMYDA-ACS CAMs (Atorvastatin for Reduction of Myocardial Damage during Angioplasty-Cell Adhesion Molecules) substudy. *J Cardiovasc Med (Hagerstown)*, 2010; 11: 795–800.