High levels and inflammatory effects of soluble CXC ligand 16 (CXCL16) in coronary artery disease: down-regulatory effects of statins

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Aims CXCL16 may be involved in inflammation and lipid metabolism, and we hypothesized a role for this chemokine in coronary artery disease (CAD).

Methods and results We performed clinical studies in CAD patients as well as experimental studies in cells with relevance to atherogenesis [i.e. endothelial cells, vascular smooth muscle cells (SMC), and peripheral blood mononuclear cells (PBMC)]. We also examined the ability of HMG-CoA reductase inhibitors (statins) to modulate CXCL16 levels both in vivo and in vitro. Our main findings were: (i) patients with stable (n = 40) and unstable (n = 40) angina had elevated plasma levels of CXCL16 compared with controls (n = 20); (ii) low-dose simvastatin (20 mg qd, n = 15) and high-dose atorvastatin (80 mg qd, n = 9) down-regulated plasma levels of CXCL16 during 6 months of therapy; (iii) in vitro, atorvastatin significantly decreased the interleukin (IL)-1β-mediated release of CXCL16 from PBMC and endothelial cells; (iv) attenuating effect of atorvastatin on the IL-1β-mediated release of CXCL16 in PBMC seems to involve post-transcriptional modulation as well as down-regulation of CXCL16 release through inhibition of the protease a disintegrin and metalloproteinase 10 (ADAM10); (v) soluble CXCL16 increased the release of IL-8, monocyte chemoattractant peptide 1, and matrix metalloproteinases in vascular SMC and increased the release of IL-8 and monocyte chemoattractant peptide 1 in PBMC, with particularly enhancing effects in cells from CAD patients.

Conclusion Our findings suggest that soluble CXCL16 could be linked to atherogenesis not only as a marker of inflammation, but also as a potential inflammatory mediator.

KEYWORDS Atherosclerosis; Chemokines; Coronary artery disease; Statins

1. Introduction

Chemokines such as monocyte chemoattractant protein-1 (MCP-1), interleukin 8 (IL-8), and fractalkine (CX3 ligand 1, CX3CL1) are thought to play an important pathogenic role in atherogenesis by activating and directing leukocytes into the atherosclerotic lesion.1–3 CXC ligand 16 (CXCL16), a newly discovered chemokine of the CXC family that is expressed in soluble and transmembrane forms, has through interaction with its receptor CXCR6 been found to guide migration of activated T cells into inflamed tissue.4,5 It is identical to scavenger receptor SR-PSOX, which mediates uptake of oxidized low-density lipoprotein (oxLDL),6 suggesting the involvement of CXCL16 in both inflammation and lipid metabolism.

Although these properties may suggest a role for CXCL16 in atherogenesis, its role in atherosclerotic disorders is debated. Thus, while enhanced CXCL16 and CXCR6 expression has been shown in atherosclerotic lesions from humans and apolipoprotein-E-deficient mice,7,8 conflicting data exist on the circulating CXCL16 levels in human atherosclerosis as both decreased9 and increased10 plasma concentrations have been reported in patients with coronary artery disease (CAD). Moreover, while CXCL16 has been shown to be up-regulated in atherosclerotic plaques by the pro-inflammatory cytokine interferon (IFN)-γ, potentially acting in a positive feedback loop to increase inflammation in the atherosclerotic lesion,6 a recent murine study

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suggests that targeted disruption of CXCL16 accelerates atherosclerosis.11

In the present study we attempted to further elucidate the possible role of CXCL16 in atherogenesis by performing clinical studies in CAD patients as well as experimental studies in cells with relevance to atherogenesis [i.e. endothelial cells, vascular smooth muscle cells (SMC), and peripheral blood mononuclear cells (PBMC)]. We also examined the ability of HMG-CoA reductase inhibitors (statins) to modulate CXCL16 levels both in vivo and in vitro.

2. Methods

2.1 Patients and controls

Patients with angiography in our coronary care unit were consecutively recruited into the study (Table 1). All patients with unstable angina (n = 40) had experienced ischemic chest pain at rest within the preceding 48 h (i.e. Braunwald's class IIIb), with but no evidence of myocardial necrosis by enzymatic criteria. Transient ST-T segment depression and/or T-wave inversion were present in all cases. All patients with stable angina (n = 40) had stable effort angina of > 6 months duration and a positive exercise test (Table 1). The diagnosis of CAD was confirmed in all patients by coronary angiography showing at least 1 vessel disease (> 50% narrowing of luminal diameter). Controls in the study were sex- and age-matched healthy individuals recruited from the same area of Norway (Table 1). The design and characteristics of the statin study have previously been described including 30 patients with previous myocardial infarction (MI) and without previous statin therapy in the previous month, ECG abnormalities invalidating ST-segment analyses, concomitant inflammatory diseases such as infections and autoimmune disorders, and liver or kidney disease. In both studies (statin and angina study), platelet-free ethylenediaminetetraacetic acid (EDTA) plasma were collected and stored as previously described.12 All parts of the study were approved by the local ethical committee and conducted according to the ethical guidelines outlined in the Declaration of Helsinki for use of human tissue and subjects. Informed written consent was obtained from all subjects.

2.2 Cell culture experiments

Freshly isolated PBMC, obtained from heparinized blood by Isopaque-Ficoll (Lymphoprep; Nycomed, Oslo, Norway) gradient centrifugation, were incubated in flat-bottomed 96-well trays (2 x 10⁶/mL; Costar, Cambridge, MA, USA), in medium alone [RPMI 1640 with 2 mmol/L L-glutamine and 25 mmol/L HEPES buffer (Invitrogen, Paisley, UK) supplemented with 10% of foetal calf serum (FCS; Sigma, St Louis, MO, USA)] or with different concentrations (ranging from 0.001 to 1.5 µg/mL) of human recombinant CXCL16 (chemokine domain, R&D System, Minneapolis, MN, USA), IL-1β (R&D System, 0.1 or 5 ng/mL), phospholipid myristate acetate (PMA; Sigma, 100 nM) with or without preincubation with atorvastatin (gifts from Pfizer) and/or mevalonate (Sigma) for 1 h before addition of IL-1β. Primary human umbilical vein endothelial cells (HUVEC) were obtained from umbilical cord veins by digestion with 0.1% collagenase A (Boehringer Mannheim GmbH, Mannheim, Germany) and cultured to confluence for 2-4 days.13 The media were then discarded, and HUVEC were stimulated with IL-1β (5 ng/mL) with or without preincubation with atorvastatin and/or mevalonate for 1 h before addition of IL-1β. Human aortic SMC were obtained from PromoCell GmbH (Heidelberg, Germany) and grown in SMG Growth Medium 2 with complete supplement mix (PromoCell). At 90% confluence, the culture was trypsinized and replated. The day prior to experiments, cells were seeded in 24-well plates (1.5 x 10⁶ cells/mL; Costar) and grown in the same medium. At experimental start, the cells were cultured in OptiMEM with Glutamax (Invitrogen) alone or with different concentrations of human recombinant CXCL16 (chemokine domain, ranging from 0.001 to 1.5 µg/mL). At different time points, cell-free supernatants, cell suspension (only PBMC), and cell pellets from PBMC, HUVEC, and vascular SMC were harvested and stored at -80 °C until analysis or used directly for flow cytometry analyses (PBMC suspension). The endotoxin levels of all stimulants and culture media were < 10 pg/mL (Limulus Amebocyte Assay; BioWhittaker, Walkersville, MD, USA).

2.3 Real-time quantitative reverse transcription-polymerase chain reaction

Total RNA was extracted from PBMC and HUVEC using RNaseasy columns (Qiagen, Hilden, Germany), subjected to DNase I treatment (RQI DNase; Promega, Madison, WI, USA), and stored in RNA storage solution (Ambion, Austin, TX, USA) at -80 °C. Primers were designed using the Primer Express software, version 2.0 (Applied Biosystems, Foster City, CA, USA) for CXCL16 [forward primer (FP): 5'-GGCCCCACCAAGAAGGTTTAC-3', reverse primer (RP): 5'-CTGAAAGATGCCCCTCTGAC-3'], CCRX6 (FP: 5'-ATGGGATGACTGGAGTGGTGTCACT-3', RP: 5'-TTGAAGAGGGGCTCCTAGGTA-3'), matrix metalloproteinase-1 (MMP-1) (FP: 5'-CTGGAGATGAGGATCGACTGC-3', RP: 5'-GCTGGGAAGGATTCTTCTTTG-3'), and MMP-2 (FP: 5'-ATGAGACTGGCTACGTGAG-3', RP: 5'-TCAGGGCAGAGCAGGAATTTT-3'). Quantification of mRNA was performed using the ABI Prism 7500 (Applied Biosystems).14 Gene expression of the housekeeping gene β-actin was used for normalization.

2.4 Western blotting

Western blotting was performed as described previously,15 with equal amounts of protein being separated from each sample by SDS-PAGE (10%) before being transferred to polyvinylidene fluoride (PVDF) membranes. Filters were incubated with mouse antibody against a disintegrin and metalloprotease 10 (human ADAM10, Chemicon/Millipore, Billerica, MA, USA) or with rabbit

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Data are presented as mean ± SD. Statins, hydroxymethylglutaryl coenzyme A reductase inhibitors.
antibody against human ADAM17 (Calbiochem, Gibbstown, NJ, USA) followed by species-specific horseradish peroxidase-coupled secondary antibodies (Cell Signaling, Beverly, MA, USA). All forms of a disintegrin and metalloproteinase 10 (ADAM10) and a disintegrin and metalloproteinase 17 (ADAM17) were normalized against β-tubulin (mouse anti-β-tubulin; Sigma). The immune complexes were visualized with the use of Supersignal West Pico (Pierce, Rockford, IL, USA) and exposed films were detected by using Kodak 440 CF imaging station (Boston, MA, USA). The software Total Laboratory v.110 (Phoretx, Newcastle, UK) was used for quantification.

2.5 Flow cytometry

Staining was performed on cryopreserved PBMC (CXCR6) or PBMC suspension (CXCL16, atorvastatin in vitro experiments) using fluoroscein isothiocyanate-conjugated anti-CD3 and peridinin chlorophyll protein-conjugated anti-CD14 from Becton-Dickinson (San Diego, CA, USA), and phycoerythrin-conjugated anti-CXCR6 and allophycocyanin-conjugated anti-CXCL16 from R&D Systems. Isotype controls were used as appropriate. Flow cytometry was performed using a FACSCalibur instrument with CellQuest software (Becton Dickinson). List mode files were collected for 50 000 cells from each sample.

2.6 Measurement of matrix metalloproteinases

MMP levels in vascular SMC supernatants were measured by multiplex suspension array technology using the BioPlex (Bio-Rad, Hercules, CA, USA). MMP-1, MMP-2, MMP-3, and MMP-7 multiplexable beads were purchased from R&D Systems. The quantification was accomplished by using the BioPlex Manager Software (BioRad).

2.7 Total matrix metalloproteinase activity

Total MMP activity in SMC supernatants was measured by a fluorogenic peptide substrate (R&D systems) used to assess broad-range MMP activity (MMP-1, -2, -7, -8, -9, -12 and -13 can cleave the peptide) using the protocol recommended by the manufacturer. Briefly, the MMP substrate was diluted in TCN buffer (50 mM Tris-HCl, 150 mM NaCl, 10 mM CaCl$_2$; pH 7.5) and added to the supernatants before incubation at 37°C. After 120 min the total MMP activity was determined on a fluorimeter (FLX 800 Microplate Fluorescence Reader, Bio-Tek Instruments, Winooski, VT, USA).

2.8 Enzyme immunoassay

Concentration of CXCL16, IL-8, IL-10, tumour necrosis factor α (TNFα), and MCP-1 were measured by enzyme immunoassays (EIA) (R&D Systems).

2.9 Statistical analysis

For comparisons of two groups of individuals, the Mann–Whitney U test was used. When comparing three groups of individuals, the non-parametric Kruskal–Wallis test was used. If a significant difference was found, Mann–Whitney U test was used to calculate the difference between each pair of groups. For comparisons within the same individuals, Wilcoxon’s matched-pair test was used. In the in vitro studies of vascular SMC and HUVEC, Mann–Whitney U test was used. Probability values of $P < 0.05$ (two-sided) were considered statistically significant.

3. Results

3.1 CXC ligand 16 levels in angina patients and healthy controls

Both stable and unstable angina patients had raised plasma levels of CXCL16 comparing healthy controls, although the increase in unstable angina did not reach statistical significance ($P = 0.09$) (Figure 1). Some of the patients had additional risk factors that potentially could promote inflammatory responses (i.e. diabetes and hypertension), but the same pattern of CXCL16 levels was seen even if these patients were excluded from the study.

3.2 Effect of statin therapy on plasma levels of CXC ligand 16

Experimental and some clinical data indicate that statins may confer cardiovascular benefits in addition to the lipid lowering activity at least partly by modulating the inflammatory arm of atherosclerosis.$^{17}$ We therefore next examined the effect of simvastatin (20 mg qd, $n = 15$) and atorvastatin (80 mg qd, $n = 9$) on plasma levels of CXCL16 in CAD patients (see Methods). As expected, 6 months treatment with statins significantly ($P < 0.05$ in both treatment groups) reduced plasma levels of total cholesterol (atorvastatin: $6.3 \pm 1.2$ mmol/L vs. $3.9 \pm 0.2$ mmol/L and simvastatin: $6.0 \pm 1.1$ mmol/L vs. $4.0 \pm 0.6$ mmol/L), LDL-cholesterol (atorvastatin: $4.1 \pm 1.2$ mmol/L vs. $1.5 \pm 0.2$ mmol/L and simvastatin: $4.0 \pm 0.9$ mmol/L vs. $1.9 \pm 0.6$ mmol/L), and triglycerides (atorvastatin: $1.7 \pm 0.1$ mmol/L vs. $1.2 \pm 0.1$ mmol/L and simvastatin: $1.7 \pm 0.4$ mmol/L vs. $1.3 \pm 0.4$ mmol/L). These changes were accompanied by a marked decrease in plasma levels of CXCL16 in both the low-dose simvastatin and in the high-dose atorvastatin group (Figure 2), representing conventional and aggressive statin therapy, respectively. However, we found no
significant correlation between the decrease in lipid parameters and the decrease in CXCL16 (P > 0.1 for all lipid parameters), suggesting that the decrease in CXCL16 is not secondary to a decrease in lipid levels.

3.3 Effect of atorvastatin on CXC ligand 16 release in peripheral blood mononuclear cells and human umbilical vein endothelial cells

To further examine whether statins exert the CXCL16-related effects at the cellular or at a systemic levels through lowering of plasma concentration of cholesterol, we tested the ability of atorvastatin to modulate the expression and release of CXCL16 in PBMC from healthy controls (n = 8) and endothelial cells, both cell types known to be important cellular sources of CXCL16.10,18 To mimic the in vivo situation in CAD, the cells were co-stimulated with IL-1β, an inflammatory cytokine known to be up-regulated in CAD.16 As shown in Figure 3A, IL-1β markedly enhanced the release of CXCL16 from PBMC reaching a maximum after culturing for 72 h, and notably, this effect was significantly attenuated by atorvastatin. A similar pattern with suppressive effect of atorvastatin on the IL-1β-mediated release of CXCL16 was also seen in HUVEC after culturing for 20 h, although the amount of CXCL16 release was in general much lower in HUVEC than in PBMC (Figure 3B). In both cell types, the suppressive effect of atorvastatin on the IL-1β-mediated CXCL16 release was significantly attenuated by the addition of mevalonate (Figure 3A and B). However, the atorvastatin-mediated effect was not totally abolished, potentially suggesting additional mechanisms for the suppressive effect of atorvastatin than interference with the mevalonate pathway. In contrast to these effects at the protein level, atorvastatin had no significant effect on the IL-1 β-mediated increase in mRNA levels of CXCL16 in either PBMC or HUVEC (data not shown), suggesting that atorvastatin down-regulates CXCL16 release through post-transcriptional mechanisms. Finally, decreased release of CXCL16 could potentially reflect accumulation of CXCL16 within the cells or at the cell surface. However, the attenuating effect of atorvastatin on the release of CXCL16 in IL-1β-activated PBMC was accompanied by a corresponding down-regulatory effect of atorvastatin on CXCL16 levels in PBMC pellets as well as on membrane expression of CXCL16 (mean fluorescence intensity) as assessed by EIA and flow cytometry, respectively, although the decrease in membrane-bound CXCL16 did not reach statistical significance (Figure 3C).

3.6 Atorvastatin attenuates ADAM10 in peripheral blood mononuclear cell

While atorvastatin down-regulated the release as well as the intracellular and membrane-bound levels of CXCL16 in IL-1β-activated PBMC (Figure 3C), the decrease in intracellular (P < 0.05) and membrane-bound (P = 0.1) CXCL16 was more modest than the decrease in CXCL16 levels in PBMC supernatants (P < 0.01), suggesting an atorvastatin-mediated inhibition of CXCL16 shedding as an additional mechanism. Transmembrane CXCL16 is shed from the cell surface by the activity of ADAM10 and ADAM17,5,19 and we therefore next examined the ability of atorvastatin to modulate these proteases in IL-1β-stimulated PBMC (n = 4, healthy controls) after culturing for 20 h. While atorvastatin had no effect on the ADAM10 precursor, it significantly down-regulated the IL-1β-induced increase in the highly processed form of ADAM10, as assessed by Western blot analyses (Figure 4A and B). In contrast, while PMA, a direct activator of protein kinase C (PKC), potently induced the expression of the highly processed form of ADAM10, this induction was not modulated by atorvastatin (Figure 4C and D), suggesting that the atorvastatin-mediated inhibition of ADAM10 involves signalling pathway up-streams or independent on PKC activation. Moreover, in line with the lack of effect of atorvastatin on PKC-mediated
ADAM10 expression, the ability of atorvastatin to suppress the PMA-stimulated release of CXCL16 was rather modest and not significant (<15% reduction), further indirectly supporting that inhibition of ADAM10 could contribute to the down-regulatory effects of atorvastatin on CXCL16 levels in IL-1β-activated PBMC. As for ADAM17, we found low expression in both IL-1β and PMA-activated PBMC with no effect of atorvastatin (data not shown).

3.7 Effect of CXC ligand 16 on the secretory potential of vascular smooth muscle cells

CXCL16 receptor, CXCR6, has been confirmed to be expressed in SMC in human atherosclerotic lesions, and in contrast to HUVEC, we found that vascular SMC contained significant amount of CXCR6 transcripts. To study any potential pathogenic consequences of the raised CXCL16 levels in CAD, we therefore examined the effect of CXCL16 on the release of the pro-atherogenic chemokines MCP-1 and IL-8 in vascular SMC. We found that the soluble form of CXCL16 increased the release of both IL-8 and MCP-1, reaching maximal effect at a concentration of 1 μg/mL after culturing for 20 h (Figure 5A and B), showing some enhancing effects also at a concentration of 0.1 μg/mL. In contrast, CXCL16 had no effects on the release of IL-10 and TNFα in these cells (data not shown). In addition to inflammation, vascular SMC could promote matrix degradation through secretion of MMPs, and notably, we found that CXCL16 significantly induced the release of MMP-1 and MMP-2 into SMC supernatants after culturing for 20 h as shown by multiplex technology (Figure 5C and D). The expression of MMPs can be regulated at multiple levels including transcriptional and post-transcriptional modification, and interestingly, while the CXCL16-induced release of MMP-2 was accompanied by a corresponding increase in mRNA level, this was not seen for MMP-1, suggesting that the CXCL16-enhancing effect on the collagenase (MMP-1) is operating at a different level than the enhancing effect on the gelatinase (MMP-2). Additionally, while CXCL16-activated SMC provided large amount of MMP-1 and MMP-2, the protein levels of MMP-3 and MMP-7 in CXCL16-stimulated SMC were in general very low or undetectable (data not shown). Finally, as depicted in Figure 4G, CXCL16 also increased MMP bioactivity in SMC supernatants, further supporting that the CXCL16-mediated enhancement of MMP levels in SMC is really and biologically significant, at least partly involving enhanced expression of MMP-1 and MMP-2.

3.8 Effect of CXC ligand 16 on cytokine release in peripheral blood mononuclear cells from coronary artery disease patients and healthy controls

CXCR6 is strongly expressed on T cells within the PBMC population, and to further elucidate a potential role of CXCL16 in atherogenesis, we examined the effect of

![Figure 4](https://academic.oup.com/cardiovascres/article-abstract/79/1/195/269594/791165268594?dl=1&pguest=1)
CXCL16 (chemokine domain) on the release of IL-8 and MCP-1 in PBMC from seven healthy controls and seven patients with stable angina. As shown in Figure 6A and B, CXCL16 (1 μg/mL) induced a marked increase in IL-8 (~20-fold) and MCP-1 (~10-fold) levels in PBMC from angina patients reaching maximum after culturing for 72 h. In contrast, the effect of CXCL16 in PBMC from healthy controls was rather modest, and as for IL-8, not significant (Figure 6A and B). Furthermore, while CXCL16 had no effects on TNFα release (data not shown), it induced a moderate and significant increase in IL-10 levels with the same pattern in angina patients and healthy controls (Figure 6C). Thus, while CXCL16 induced a modest increase of inflammatory (MCP-1) and anti-inflammatory (IL-10)
CXCL16 in angina patients involves factors down-streams of CXCR6. Furthermore, when CXCL16 was combined with low dosage of IL-1β (0.1 ng/mL), enhancing effects of CXCL16 on MCP-1, and also to some degree on IL-8, was seen at lower concentrations of CXCL16 (i.e. 0.01 μg/mL), but again, this was not accompanied by any changes in CXCR6 expression (flow cytometry).

4. Discussion

In the present study, we showed that increased plasma levels of CXCL16 in CAD patients are independent of co-morbidity such as diabetes and hypertension. Moreover, while most of the previous studies have focused on the effects of the membrane-bound form of CXCL16, we showed that soluble CXCL16 induces inflammatory responses in vascular SMC and PBMC, with particularly prominent effects in PBMC from CAD patients. These findings suggest that soluble CXCL16 could be linked to atherogenesis not only as a marker of inflammation, but also as a potential inflammatory mediator.

CXCL16 exist in a membrane-bound and soluble form, and inflammatory cytokines like the combination of TNF-α and IFNγ have previously been found to enhance the release of soluble CXCL16 from endothelial cells and SMC.5 Herein we showed that IL-1β has similar properties on its own promoting CXCL16 release in endothelial cells and particularly in PBMC. Based on the link between inflammatory mediators and the cleavage of the membrane-bound to the soluble form of CXCL16, it has been suggested that soluble CXCL16 could serve as a reliable marker of inflammation.23 Indeed, raised levels of soluble CXCL16 have been reported in some inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematosus.24–26 However, conflicting data exist on plasma levels of CXCL16 in CAD. Thus, while Sheikine et al.9 found decreased CXCL16 levels in both stable and unstable angina, Lehrke et al.10 reported increased CXCL16 levels in a large population of CAD patients, particularly in those with unstable disease. In the present study we reported increased plasma levels of CXCL16 in CAD patients. Although we found no differences between stable and unstable angina, potentially reflecting factor such as a lesser degree of disease instability in the present study population, our finding further support the concept that CAD patients are characterized by raised plasma levels of CXCL16.

It seems that the membrane-bound and soluble form of CXCL16 possesses completely different biological functions,5,23 and much focus has been drawn against the function of the transmembrane form. Thus, enhanced expression of CXCL16 on endothelial cells, vascular SMC, and macrophages within the vessel wall has been shown to promote binding and adhesion of lymphocytes, oxLDL particles, and bacteria,23 potentially contributing to vascular inflammation. However, soluble CXCL16 may also promote inflammatory responses, acting as a classical chemotactic towards various lymphocyte subsets.23 Moreover, there are also some reports suggesting that soluble CXCL16 may have functions beyond that of leukocyte recruitment. Hence, soluble CXCL16 has been shown to promote SMC proliferation.27 Herein we report that CXCL16-activated vascular SMC release inflammatory chemokines and MMPs, suggesting that CXCL16 may transform these cells from a
contractile to a proliferative/secretory phenotype which is a hallmark of the vascular remodelling characterizing atherosclerosis. Moreover, while CXCL16 induced a modest increase of inflammatory and anti-inflammatory cytokines in PBMC from healthy individuals, the net effect of CXCL16 in angina patients seems to be markedly inflammatory, further underscoring the relevance of CXCL16-mediated inflammation in atherosclerosis. One might argue that the highest concentration of CXCL16 used in the in vitro experiments (1 μg/mL) is not relevant to the in vivo situation. However, it is not inconceivable that similar concentrations may be found in the inflammatory microenvironment within an atherosclerotic plaque, consisting of several types of CXCL16 secreting cells (e.g. macrophages, SMC, and endothelial cells). The combination of CXCL16 with several other inflammatory cytokines operating within the atherosclerotic lesion may further enhance its inflammatory potential as also suggested by our finding of inflammatory effects of CXCL16 at lower concentration when combined with low dosage of IL-1β.

Recently, Aslanian and Charo reported that CXCL16-deficient LDL receptor-/- mice were associated with accelerated atherosclerosis. This finding may seem conflicting with the present study suggesting pro-atherogenic effects of CXCL16. However, as the membrane-bound and soluble form of CXCL16 seem to have different biological function, enhanced atherosclerosis in CXCL16 knock-out mice may not necessarily argue against a pro-atherogenic effect of soluble CXCL16. Also, substantial evidence indicates that CXCL16 may have constitutive functions such as promotion of cell survival and normal leukocyte recruitment. Thus, while too much of CXCL16 may be harmful, too little may not necessarily be beneficial. Interestingly, it has recently been shown that blocking of CXCL16 actions by anti-sera limits the progression of glomerulonephritis, supporting the role of CXCL16 as a target for anti-inflammatory therapy. Moreover, a recent study by Galkina et al. reported that CXCR6-deficient (i.e. lacking the CXCL16 receptor) ApoE-/- mice showed attenuated atherosclerosis, accompanied by a decreased percentage of CXCR6+ T cells within the aortas, indicating a pro-atherogenic role of CXCL16/CXCR6 interaction. Nevertheless, future studies will have to more precisely define the inflammatory and constitutive functions of CXCL16 as well as clarify the different effects of the membrane-bound as opposed to the soluble form of CXCL16 in inflammation.

In the present study we showed that statin therapy significantly reduces plasma levels of CXCL16 in CAD patients with no differences between low-dose simvastatin and high-dose atorvastatin therapy. Lack of correlations between cholesterol-lowering and reduction in CXCL16 levels suggest that this down-regulation at least partly may be unrelated to lipid lowering. Our finding in the in vitro experiments showing the ability of atorvastatin to attenuate the release of CXCL16 in IL-1-activated endothelial cells and PBMC further supports such a notion. We found that along with the atorvastatin-mediated decrease in CXCL16 release there was a moderate decrease in intracellular and membrane-bound levels of CXCL16, but not in the mRNA level of CXCL16, suggesting that atorvastatin inhibits CXCL16 at the post-transcriptional level. However, the decrease in intracellular and membrane-bound CXCL16 was more modest than the decrease in CXCL16 release, suggesting an atorvastatin-mediated inhibition of CXCL16 shedding as an additional mechanism. ADAM10 has been found to be a major regulator of CXCL16 shedding from its membrane-bound form and indeed, we found that atorvastatin attenuated the expression of the highly processed form of ADAM10 in IL-1-activated PBMC, potentially contributing to its inhibitory effect on CXCL16 release in these cells. The finding that the modest and non-significant effects of atorvastatin on the PMA-mediated CXCL16 release was accompanied by the inability of atorvastatin to inhibit the PMA-mediated increase in ADAM10 may indirectly further support such a notion. Our data on PMA-activated PBMC may seem in contrast with previous reports showing no effects on ADAM10 and inducing effects on ADAM17. The reason for these discrepancies is at present not clear, but could at least partly reflect differences between primary kidney fibroblast cell lines and human monocytic cell lines as compared with freshly isolated PBMC from human individuals as used in the present study.

Landsberger et al. recently reported that cerivastatin down-regulated the expression of membrane-bound intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 in TNFα-activated HUVEC by increasing their shedding which may seem conflicting our findings in the present study. The reason for these discrepancies on the effect of statins on protein shedding may at least partly reflect different effects of statins in relation to dosage and co-stimuli, and could also reflect differences between the type of statin used. Moreover, the effect of statins may also vary between different inflammatory mediators. Thus, we have recently shown that atorvastatin attenuate the release of the CXC chemokine growth-related oncogene-α in IL-1-activated HUVEC by inhibiting its secretion.

In the present study, we showed that CAD patients are characterized by raised plasma levels of CXCL16 potentially reflecting persistent inflammation in these patients. Our in vitro experiments indicate that soluble CXCL16 is not only a marker, but also a mediator of inflammation with particularly enhancing effects in CAD patients. These findings may suggest a pro-atherogenic role of CXCL16 in CAD.

Conflict of interest: none declared.

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