C1-Esterase Inhibitor Protects Against Neointima Formation After Arterial Injury in Atherosclerosis-Prone Mice

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Background—Although activation of the complement system has been implicated in the progression of human atherosclerosis, its function during arterial remodeling after injury has not been investigated. Here, we examined the contribution of the complement cascade to neointima formation in apolipoprotein E–deficient mice using a C1-esterase inhibitor (C1-inhibitor).

Methods and Results—Apolipoprotein E–deficient mice fed an atherogenic diet were subjected to wire-induced endothelial denudation of the carotid artery and treated with C1-inhibitor (Berinert; 15 IU IV) or vehicle perioperatively and subsequently every 2 days. The effectiveness of C1-inhibitor treatment was confirmed by measurement of plasma C1-inhibitor activity. A significant reduction in serum triglyceride levels was observed in C1-inhibitor–treated mice, whereas cholesterol levels did not differ. After 3 weeks, neointimal area was significantly reduced in C1-inhibitor–treated mice versus controls, whereas medial area was unaltered. This was associated with a significant decrease in neointimal and medial macrophage and CD3+ T-cell content. Expression of C3 mRNA was significantly reduced in plaques of C1-inhibitor–treated mice 10 days after injury, as assessed by reverse-transcription polymerase chain reaction. The peak in serum C3 levels after injury was markedly downregulated by C1-inhibitor, as evidenced by ELISA. Immunohistochemistry revealed strong expression of C3 and C3c, which colocalized to plaque macrophages and was reduced in C1-inhibitor–treated mice. C1-inhibitor impaired monocyte arrest on activated endothelium and platelets under flow conditions in vitro and leukocyte recruitment to carotid arteries 1 day after injury in vivo.

Conclusions—C1-inhibitor limits neointimal plaque formation and inflammation. This may involve blockade of complement activation, inhibition of leukocyte recruitment, and reduced triglyceride levels, thus providing a multimodal approach to treat arterial disease. (Circulation. 2008;117:70-78.)

Key Words: atherosclerosis ■ inflammation ■ cell adhesion molecules ■ complement

Atherosclerosis is an inflammatory disease that is strongly affected by the action of monocytes/macrophages and T cells.1 Emerging evidence further suggests that the complement system plays a role in atherosclerosis, although its exact functions and mechanisms of action remain unclear.2,3 In atherosclerotic plaques, activation of the classic complement pathway has been observed. In addition, mRNA of complement components, including C3 activation products and membrane attack complex, are also detectable in diseased arteries. Membrane attack complex depositions colocalize with lipoproteins, which are known to be potent activators of the complement system in the aortic intima in rabbits at early stages of atherogenesis and in human atherosclerotic lesions,4 and they correlate with the severity of arterial damage.5,6

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However, complement regulatory proteins have not been found to be upregulated in human plaque tissue.7 Studies using complement-deficient animals have yielded apparently contradictory conclusions. In rabbits, C6 deficiency protects against diet-induced atherosclerosis, which supports the importance of the terminal pathway.8 In contrast, no differences in diet-induced lesion size were observed in C5-deficient apolipoprotein E–deficient (Apoe<sup>−/−</sup>) mice9 or in C3-deficient low-density lipoprotein (LDL) receptor–deficient (Ldlr<sup>−/−</sup>) mice.10 Recently, Persson et al11 have reported an increase in aortic lesion area in chow-fed C3-deficient Ldlr<sup>−/−</sup>Apoe<sup>−/−</sup> mice, but interpretations of their data were complicated by a
more atherogenic lipid profile and a reduction in body weight in C3-deficient mice.

C1-esterase inhibitor (C1-inhibitor) is a naturally occurring, unique serine protease inhibitor of C1r and C1s. C1-inhibitor also controls the mannose-binding lectin–associated proteases MASP-1 and MASP-2 within the lectin pathway, which play a role in the activation and proteolysis of the complement system. In hereditary angioedema, C1-inhibitor is available for clinical substitution therapy. Notably, treatment with C1-inhibitor has also been implied to be beneficial in a variety of other disease models, including sepsis, brain and myocardial ischemia-reperfusion injury, acute transplant rejection, traumatic shock, and vascular leakage syndromes after thermal injury, interleukin (IL)-2 therapy, or cardiopulmonary bypass. Underlying mechanisms have been attributed primarily to an inhibition of the complement and contact system, which results in a reduction in the generation of its biologically active products, such as C3a, C5a, C5b-9 membrane attack complex, and bradykinin. Effects of the C1-inhibitor resulted in the decreased release of various cytokines, including tumor necrosis factor, IL-10, IL-6, and IL-8, in sepsis. In addition to effects on the complement system, the C1-inhibitor has also been described to inhibit factors of the intrinsic coagulation system.

Leukocyte infiltration is crucially involved in the response to vascular injury. Exposure of extracellular matrix and adhesion of activated platelets after endothelial denudation contribute to the inflammatory cell recruitment and accumulation of smooth muscle cells (SMCs). The adhesion of circulating monocytes to sites of endothelial denudation and neointimal hyperplasia is under sequential control of adhesion molecules. Selectins are expressed by activated endothelial cells and SMCs (E-selectin and P-selectin), activated platelets (P-selectin), and peripheral leukocytes (L-selectin). After arterial injury, P-selectin plays a critical role in neointima formation by mediating the interaction of activated platelets with monocytes or progenitor cells and with the vessel wall, which leads to the delivery of platelet-derived chemokines, namely, CCL5. Recently, Cai et al have reported that C1-inhibitor can bind to both E- and P-selectin via a sialyl-LewisX tetrasaccharide presented on its N-linked glycan and can thus interfere with leukocyte–endothelial cell interactions in vitro and in vivo. In the present study, we tested the hypothesis that C1-inhibitor may inhibit neointimal hyperplasia after arterial injury by direct effects on the complement system and by its newly described protease-inhibitory functions and antiinflammatory properties.

Methods

For additional details, please see the online-only Data Supplement.

Mouse Model of Carotid Artery Injury

ApoE−/− mice fed an atherogenic diet were subjected to wire injury of the common carotid artery. Some mice received 15 IU of the human C1-esterase inhibitor Berinert P (Aventis Behring [now CSL Behring], King of Prussia, Pa) 30 minutes before and after injury and subsequently every other day (n=9), whereas others received vehicle.
Flow Adhesion Assay

Human umbilical vein endothelial cells (HUVECs), Jurkat T cells, and MonoMac 6 cells were maintained as described previously. Adhesion of leukocytes to activated HUVECs or platelets was analyzed in a parallel-wall flow chamber.

Measurement of Plasma C1-Inhibitor Activity and Serum Lipids

Functional C1-inhibitor activity in citrate plasma was analyzed with the Berichrom C1-inhibitor kit (Dade Behring, Marburg, Germany). Serum triglyceride, total cholesterol, high-density lipoprotein cholesterol, and LDL cholesterol fractions were measured by standard laboratory assays.

Complement C3 ELISA and Reverse-Transcription Polymerase Chain Reaction

Mouse C3 in serum was detected with an ELISA kit. Total RNA was isolated from acetone-fixed slides of carotid arteries and reverse-transcribed into cDNA, and reverse-transcription polymerase chain reaction analysis was performed.

Intravitral Microscopy

The arrest of rhodamine 6G–labeled leukocytes on carotid arteries 1 day after injury was analyzed by epifluorescence intravital microscopy.

Statistical Analysis

Data are presented as mean±SEM. Differences with \( P<0.05 \) were considered to be statistically significant.

All authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

C1-Inhibitor Reduces Neointimal Plaque Formation and Inflammation in Carotid Arteries

To investigate the effect of long-term C1-inhibitor treatment on accelerated plaque formation, we used a model of wire-induced neointimal hyperplasia in atherosclerosis-prone Apoe\(^{-/-}\) mice fed an atherogenic diet. Mice were injected intravenously 30 minutes before and after injury and subsequently every 2 days with the human C1-inhibitor Berinert P for 3 weeks. Compared with vehicle-treated mice (controls), C1-inhibitor treatment significantly decreased neointimal plaque size, whereas medial area was unaltered (Figure 1A and 1B). Naturally, the intima/media ratio was significantly reduced in C1-inhibitor–treated carotid arteries (0.77±0.12 in controls versus 0.36±0.07 in the C1-inhibitor–treated group, \( P<0.05 \)). We next investigated the cellular infiltrate in carotid artery sections after 3 weeks of treatment. The quantification of immunohistochemical stainings showed that the reduction in neointimal plaque size was associated with a significant decrease in the relative content of Mac-2\(^{+}\) macrophages (Figure 1C and 1D) and CD3\(^{+}\) T cells (Figure 1E) in the neointima and in the media of C1-inhibitor–treated mice versus controls. Conversely, the relative content of SMCs quantified by staining for \( \alpha \)-smooth muscle actin was significantly higher in the media of C1-inhibitor–treated mice (48.7±13.4% versus 17.6±2.7% in controls, \( P<0.005 \)) and was slightly increased in the neointima of C1-inhibitor–treated mice (12.1±2.8% versus 10.6±1.0% in controls, \( P=0.05 \)). The relative content of neutrophils was marginal but not significantly reduced in both neointima (4.8±1.6% versus 1.3±1.2% of total cell numbers) and media (1.9±0.8% versus 0.7±0.7% in controls, \( P=0.05 \)) of C1-inhibitor–treated mice. The number of neointimal apoptotic terminal dUTP nick end-labeling (TUNEL)–positive cells did not differ between control and C1-inhibitor–treated mice (12.0±3.1% versus 17.4±1.6% of total cell numbers, respectively, \( P=0.05 \)).

C1-Inhibitor Reduces Serum Triglycerides Without Altering Body Weight and Serum Cholesterol

Because changes in the lipid profile have been observed in complement-deficient animals, triglyceride and cholesterol levels were analyzed. Compared with controls, C1-inhibitor–treated mice displayed significantly reduced serum triglyceride levels (Figure 2A) and a slight reduction in total serum cholesterol levels, which, however, did not achieve significance (Figure 2B). Similar to cholesterol levels, no changes in LDL or high-density lipoprotein cholesterol fractions were observed in C1-inhibitor–treated mice compared with controls (Figure 2C and 2D). No difference in body weight was
observed between C1-inhibitor–treated mice and controls (Figure 2E). The effectiveness of C1-inhibitor treatment was assessed by measuring functional C1-inhibitor activity in plasma. This confirmed the effectiveness of dosing for treatment with C1-inhibitor (Figure 2F).

**Complement Expression in Atherosclerotic Carotid Arteries**

To explore the importance of locally activated complement components in the arterial wall, frozen sections were stained for C3, a central protein in the activation of the complement system, and for its final major proteolytic fragment, C3c, which indicates a recent activation. A significantly increased expression of complement C3 was seen in carotid arteries of control mice (Figure 3A and 3B). C3 was localized to the endothelium but also was found within the plaque adjacent to the elastic laminae and in areas with foam cell formation. Notably, staining for C3 was markedly reduced in C1-inhibitor–treated mice (Figure 3A and 3B). Double immunofluorescence staining with the macrophage marker Mac-2 revealed a strong colocalization of complement C3 with macrophages in the neointima of control Apoe<sup>−/−</sup> mice, foremost within the foam cell area (Figure 3C). Thus, the decrease in neointimal C3 deposition in C1-inhibitor–treated mice may be related to the reduced macrophage content.

Strong expression of complement C3c was detected in the endothelium and diffusely distributed in cellular and noncellular areas of the neointima, and similarly, its expression was markedly reduced in carotid arteries of C1-inhibitor–treated Apoe<sup>−/−</sup> mice (Figure 3D and 3E).

**Serum Complement C3 Levels and C3 mRNA Analysis in Plaques**

Produced by the liver but also in tissues at sites of inflammation, serum C3 serves as an acute-phase reactant. Given its C1-inhibitor–dependent expression in the neointima of carotid arteries after injury, we analyzed serum C3 levels at different time points after injury. Compared with levels in Apoe<sup>−/−</sup> mice without injury, a 2-fold elevation of serum C3 levels was evident at 1 and 4 days after injury (Figure 4A). In comparison, C3 levels were significantly decreased to levels of uninjured mice in C1-inhibitor–treated Apoe<sup>−/−</sup> mice at day 1 and 4. At day 10 and 21 after injury, C3 levels receded but remained significantly elevated compared with uninjured mice levels and were only slightly decreased by C1-inhibitor treatment in contrast to control Apoe<sup>−/−</sup> mice (Figure 4A). We next examined whether complement C3 mRNA expression was de novo in neointimal plaques. Total RNA was extracted from uninjured carotid arteries and at day 1 and 10 after injury from arteries of control and C1-inhibitor–treated
Moreover, the arrest of Jurkat T cells was reduced the adhesion of MonoMac 6 on activated platelets of endothelial denudation, monocyte arrest on surface-accumulation and activation of platelets. To mimic conditions arterial wall, the exposure of extracellular matrix triggers the ure 5A). After endothelial cell denudation injury of the reduced MonoMac 6 cell arrest on activated HUVECs (Fig-

controls, pretreatment with C1-inhibitor dose-dependently was not upregulated at 1 day after injury (1.07 ± 0.02-fold increase over uninjured arteries), and no differences were observed between control and C1-inhibitor–treated mice, C3 mRNA levels were increased significantly at 10 days after injury (2.74 ± 1.24-fold increase) and were reduced by C1-inhibitor treatment (Figure 4B).

C1-Inhibitor Inhibits the Adhesion of Monocytes In Vitro and In Vivo

C1-inhibitor has been described to interfere with leukocyte–endothelial cell interactions in vitro and in vivo. To investigate direct effects of the C1-inhibitor on leukocyte recruitment, we used a laminar flow adhesion assay. Monocytic MonoMac 6 cells were preincubated with C1-inhibitor at different concentrations and perfused over HUVEC monolayers activated with tumor necrosis factor-α. Compared with controls, pretreatment with C1-inhibitor dose-dependently reduced MonoMac 6 cell arrest on activated HUVECs (Figure 5A). After endothelial cell denudation injury of the arterial wall, the exposure of extracellular matrix triggers the accumulation and activation of platelets. To mimic conditions of endothelial denudation, monocyte arrest on surface-adherent human platelets activated with collagen G was analyzed. Pretreatment with C1-inhibitor dose-dependently reduced the adhesion of MonoMac 6 on activated platelets (Figure 5B). Moreover, the arrest of Jurkat T cells was dose-dependently reduced by treatment with C1-inhibitor (Figure 5C). To explore the importance of leukocyte recruitment to the injured vessel wall in vivo, intravital microscopy analyses were performed. Notably, the number of leukocytes adherent to carotid arteries 1 day after injury was significantly reduced in C1-inhibitor–treated mice compared with controls, which further extended and corroborated the present in vitro findings (Figure 5D and 5E). Because selectin-mediated interactions serve as a prerequisite for firm monocyte arrest, and the C1-inhibitor has been shown to directly interfere with P- and E-selectin functions, these results clearly indicate that C1-inhibitor acts at least in part by reducing inflammatory monocyte and T-cell recruitment in the context of vascular injury.

Discussion

The present data demonstrate that long-term treatment with human C1-inhibitor inhibits neointima formation after wire injury in atherosclerosis-prone ApoE−/− mice. The significant reduction in neointimal hyperplasia was associated with markedly diminished numbers of plaque macrophages and CD3+ T cells, complement C3 and C3c expression, and C3 mRNA in carotid arteries, as well as serum C3 levels. Additionally, C1-inhibitor directly suppressed leukocyte adhesion both in vitro and in vivo. In vitro, we found that C1-inhibitor treatment limited the adhesion of monocytes and T cells to activated HUVECs and limited monocyte arrest on human platelets under flow conditions. The in vivo relevance of the present findings was corroborated by a reduction in leukocyte adhesion to injured carotid arteries in C1-inhibi-
tor–treated mice. We thus provide evidence that the vasoprotective effects of C1-inhibitor add to the well-acknowledged inhibition of complement activation and that C1-inhibitor directly blocks inflammatory cell recruitment, in turn attenuating complement production.

The complement system is an essential component of the immune response and plays a role in acute and chronic vascular inflammation. Its excessive or uncontrolled activation entails a considerable risk of harming the host by directly or indirectly mediating inflammatory tissue destruction. Complement activation can be initiated via 3 different pathways. C1-inhibitor controls both the classic and mannose-binding lectin pathways by covalent complex formation with activated C1r, C1s, and MASPs. The alternative pathway differs in that it is constantly activated in the fluid phase. C1-inhibitor can also inhibit the alternative pathway by binding to C3b and interfering with factor B binding to C3b. Complement components and their transcripts can be detected in diseased vessels and atherosclerotic plaques. Different mechanisms may contribute to complement activation in the arterial wall during atherogenesis. Although cholesterol lipid fractions isolated from human aortic lesions can activate the alternative pathway, C-reactive protein binding to oxidized lipoproteins or modified LDL exhibits classic complement-activating properties. Downstream of C1, C3a, C5a, or C5b-9 can induce leukocyte adhesion, and C3a and C5a are involved in enhancement of
vascular permeability, vasoconstriction, chemotaxis, and activation of inflammatory cells. Moreover, receptors for C3a and C5a have recently been found to be expressed in human coronary artery plaques. Beyond the direct effects on C1 activity, the C1-inhibitor can subsequently result in reduced generation of biologically active end products, such as the anaphylatoxins C3a and C5a, as well as C5b-9 membrane attack complex, all of which can induce tissue injury.

Because it is the central acute-phase component of complement activation, we focused on serum levels of C3 and its local expression in the vascular wall. Interestingly, an association with C3 levels has been implied for human coronary artery disease. Serum C3 levels, which were markedly elevated between day 1 and 4 but later declined, were most markedly reduced (to uninjured levels) by treatment with C1-inhibitor, which indicates a systemic inhibition of complement activation. Although immunohistochemistry revealed the presence of C3 and C3c in neointimal plaques of Apoe/H/H mice in association with recovered endothelium and of C3 in colocalization with recruited macrophages after injury, neointimal C3 expression and deposition of C3c were both markedly reduced in C1-inhibitor–treated mice. Moreover, mRNA levels of C3 were reduced in C1-inhibitor–treated versus control Apoe/H/H mice 10 days after injury.

Figure 5. C1-inhibitor inhibits leukocyte arrest on HUVECs and platelets in vitro and in vivo. MonoMac 6 cells (A) or Jurkat T cells (C) left untreated or pretreated with human C1-inhibitor 100 or 200 μL/mL were perfused over tumor necrosis factor-α–activated HUVECs (A, B) or collagen G–activated human platelet monolayers (C) under flow conditions. The number of firmly adherent cells was determined after 2 minutes of accumulation in multiple fields (n=3 to 5). D and E, Intravital microscopy was performed to analyze leukocyte adhesion to carotid arteries 1 day after injury in vivo. D, Representative images are shown that depict rhodamine 6G–labeled leukocytes adhering to carotid arteries (original magnification ×20). E, Quantification of adhered leukocytes in multiple fields (>6 fields per assay) was performed (n=4). *P<0.01, C1-inhibitor vs control; #P<0.05, C1-inhibitor 100 vs C1-inhibitor 200. C1-Inh indicates C1-inhibitor.

Significantly increased numbers of SMCs in the media of C1-inhibitor–treated mice compared with control mice 3 weeks after injury might reflect a more stable plaque phenotype. In addition, no differences were observed in the number of apoptotic cells in control versus C1-inhibitor–treated mice. Mononuclear cell infiltration has emerged as a driving force for neointimal plaque growth and is controlled by expression of adhesion receptors, such as P-selectin or vascular cell adhesion molecule-1, on activated endothelium. P-selectin is detectable on the endothelial surface at all stages of atherosclerotic lesion development in humans and in Apoe/H/H mice and can be induced by atherogenic factors, eg, oxidized LDL or inflammatory cytokines. Found in storage granules of resting platelets and in endothelial Weibel-Palade bodies, P-selectin is rapidly translocated to the surface of stimulated cells, where it participates in the initial steps of capture and rolling of circulating leukocytes by interacting with carbohydrate ligands. In addition, E-selectin, which functionally overlaps with P-selectin, is upregulated under proinflammatory conditions in mice fed a high-fat diet. In the context of endothelial denudation,
P-selectin is expressed on platelets deposited on the provisional matrix at the site of injury but is also present on luminally exposed neointimal SMCs and contributes to initial leukocyte/monocyte recruitment and ultimately to neointimal hyperplasia. Notably, C1-inhibitor has been found to directly inhibit leukocyte rolling on purified P-selectin or E-selectin in vitro, and it blocks leukocyte infiltration after cytokine challenge in a peritonitis model in vivo. The activity of C1-inhibitor in limiting leukocyte adhesion is not related to its protease inhibitory function, because an N-deglycosylated form that lacked the reactive center was as potent as the native form. This is in line with the interpretation that inhibition results from interactions of sialyl-Lewis on C1-inhibitor with selectins. The significant reduction of triglycerides in C1-inhibitor–treated mice in the present study is an interesting finding and may play a role in the reduction of plaque inflammation after injury in Apoe−/− mice. Although no direct evidence has yet been provided that C1-inhibitor can interfere directly with triglyceride metabolism, a link between complement C3 and lipid metabolism is implied by elevated C3 concentrations in association with obesity and elevated fasting and postprandial triglyceride levels. This effect may be due to acylation stimulating protein (ASP), which is a known cleavage product from C3. ASP is identical to C3ad, which is generated through interaction of C3 with factor B and the enzyme adipsin, resulting in cleavage of the parent protein, complement C3, to generate C3a, followed by desargininization of the carboxyl terminus to generate ASP. Expressed in adipose tissue, ASP is known to increase fat storage by increasing triglyceride synthesis and decreasing intracellular lypolysis. A reduction in C3 levels may thus lead to decreased production of ASP and decreased triglyceride synthesis. Because little is known about plasma C3 levels, ASP, and factor B in rodents and their correlation with triglyceride synthesis and lipid metabolism, future studies are warranted to better understand the specific contributions of a systemic complement response in relation to adipose tissue and its effects on local processes involved in neointimal hyperplasia and atherosclerosis.

Treatment with C1-inhibitor has been proposed to be beneficial in a variety of acute diseases, including vascular leakage syndromes, sepsis, hyperacute transplant rejection, and ischemia–reperfusion injury. Here, we report the first use of C1-inhibitor for long-term treatment in mice. The dosage used in vivo was based on previous studies in mice. Although those studies used C1-inhibitor for shorter treatment periods, we used it for 3 weeks. In humans, C1-inhibitor half-life was calculated to be ∼35 hours, and administration of C1-inhibitor (40 IU/kg) to patients during bypass surgery after myocardial infarction has been shown to be beneficial, without adverse effects or complications. In a porcine model, C1-inhibitor at 40 IU/kg was effective and had no associated adverse effects, but used at a higher dose (100 and 200 IU/kg), it provoked severe side effects and coagulation disorders. In mice, however, treatment with the C1-inhibitor (at 15 IU per mouse, which corresponds to ∼400 IU/kg) was well-tolerated, and no adverse effects, infections, or bleeding disorders were observed.

The present study demonstrates that the transient inhibition of C3 activation results in a marked decrease of acute vascular inflammation and neointima formation in atherosclerosis-prone mice. Similarly, transient complement blockade with the complement receptor–related gene y-1g, a recombinant protein of the mouse membrane complement inhibitor Crry, provided protection against vein-graft thickening in Apoe-3-Leiden mice. Given these findings, studies in C3-deficient mice yielded apparently inconsistent conclusions. The complement system has been implicated in the progression of human atherosclerosis, but the importance of C3 and C3-related products in systemic lipid metabolism remains unclear. Genetic deficiency in C3 has been shown to increase lesion size and to impair lesion maturation beyond the foam cell stage in ldlr−/− mice without altering serum lipoprotein/cholesterol profiles. The authors of that study therefore concluded that local effects of complement within the lesions led to the differences observed in ldlr−/− C3−/− mice and that an intact complement system was required for the progression of atheromas from foam cell– and lipid-rich lesions to those with high SMC and collagen content.

The contribution of complement activation to lesion formation might thus depend on distinct mechanisms underlying various models of chronic (eg, diet-induced) versus acute (eg, injury-induced or graft-related) vascular damage, which could possibly explain the different effects of complement inhibition or deficiency. Alternatively, genetic deletion may be compensated by a counterregulation of other relevant inflammatory components.

As a potential limitation of the present study, it may not be possible to readily translate our data from a mouse model of accelerated lesion formation to human conditions of arterial injury, eg, after stent placement in the context of primary atherosclerosis. One should take into account that we used genetically altered mice fed a high-fat diet to promote lesion formation, which entails levels of hypercholesterolemia that exceed those seen in most patients. Moreover, local mechanisms of stent-induced injury, arterial anatomy, and remodeling may differ in humans.

In conclusion, after arterial injury in atherosclerosis-prone mice, C1-inhibitor not only blocks complement activation and deposition in the arterial wall, it also appears to limit neointimal plaque formation and inflammation by lowering triglyceride levels and through direct inhibitory effects on leukocyte recruitment in the denuded and recovering artery. The present study suggests that C1-inhibitor is a multifaceted antiinflammatory protein that exerts its effects through a variety of mechanisms, including both protease inhibition and several different noncovalent interactions that are unrelated to protease inhibition. This may thus provide a multimodal approach for the treatment of arterial disease and remodeling.

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Disclosures
None.

References


Human C1-esterase inhibitor (C1-inhibitor) is available in clinical practice for substitution therapy of hereditary angioedema. Many observations suggest that C1-inhibitor is a multifaceted antiinflammatory protein that exerts its effects through a variety of mechanisms. Notably, treatment with C1-inhibitor has also been implied to be beneficial in a variety of disease models, including sepsis, ischemia-reperfusion injury, acute transplant rejection, traumatic shock, and vascular leakage syndromes after interleukin-2 therapy or cardiopulmonary bypass. The expected beneficial effect has been attributed primarily to an inhibition of the complement and contact system and several different noncovalent interactions that are unrelated to protease inhibition. The direct interactions of C1-inhibitor with selectins result in suppression of leukocyte rolling and transmigration across the endothelial surface. Furthermore, some data suggest a beneficial effect in human inflammatory disease. In the present study, C1-inhibitor successfully limited neointimal hyperplasia and inflammation after arterial injury by direct effects on the complement system but also by directly blocking the leukocyte–endothelial cell interaction. These data provide a rationale for the potential use of C1-inhibitor in clinical practice for conditions in which endothelial activation and complement and contact system activation promote pathogenesis and disease progression. A very recent study has shown that low C1-inhibitor levels are associated with and are predictive of early restenosis after carotid endarterectomy in humans. Therefore, it is conceivable that C1-inhibitor may be useful in the prevention of restenosis in patients with atherosclerosis after arterial interventions such as percutaneous coronary angioplasty or stent implantation.
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In the article by Shagdarsuren et al, “C1-Esterase Inhibitor Protects Against Neointima Formation After Arterial Injury in Atherosclerosis-Prone Mice,” that was posted online on December 10, 2007 (DOI: 10.1161/CIRCULATIONAHA.107.715649), a few errors occurred.

1. On the title page, a hyphen needs to be inserted in the name of the third author, Yassin Djalali-Talab.

2. In the last sentence of the Introduction, page 71, the final “s” needs to be removed from the term “complement systems.”

3. In the legend of Figure 5, the text “**P/H11021 0.005,” needs to be removed because double asterisks do not appear in the figure.

These errors have been corrected in the final print version of the article (Circulation. 2008;117:70–78).

The authors regret these errors.

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