New ideas - Experimental

Anti-endothelial cell antibodies are associated with peripheral arterial disease and markers of endothelial dysfunction and inflammation

Cesar Varela*1, Joaquin de Haro, Silvia Bleda, Leticia Esparza, Ignacio Lopez de Maturana, Francisco Acín

Angiology and Vascular Surgery Department, Hospital Universitario de Getafe, Madrid, Spain

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Abstract

Our aim is to describe the effect of circulating anti-endothelial cell antibodies on the endothelial dysfunction, inflammation and early structural changes of the vascular wall that surround peripheral arterial disease. For this purpose, an observational translational controlled study was carried out. We included 32 patients with symptomatic peripheral arterial disease and 16 healthy control individuals with no previous autoimmune disease. We assessed the flow-mediated arterial dilatation as a marker of endothelial function, the carotid intima–media thickness and the plasma levels of C-reactive protein in all the subjects. Circulating anti-endothelial cell antibodies were detected with indirect immunofluorescence. We found a higher prevalence of these autoantibodies in patients than in controls (40% vs. 6%; P=0.01). Flow-mediated arterial dilatation was lower in subjects with anti-endothelial cell antibodies [3.10% (0–5.05%) vs. 12.54% (6.74–18.40%); P=0.01]. Carotid intima–media thickness [1.04 (0.78–1.17) vs. 0.72 (0.54–1.02) mm; P=0.01] and C-reactive protein level [10.00 (3.50–14.80) vs. 3.00 (3.00–6.95) mg/l; P=0.01] were higher in subjects seropositive for these autoantibodies. We concluded that circulating anti-endothelial cell antibodies could be associated with peripheral arterial disease in individuals with no previous autoimmune disease; however, further prospective studies are required to establish a causal relationship.

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Keywords: Anti-endothelial cell antibodies; Endothelial dysfunction; Inflammation; Peripheral arterial disease

1. Introduction

Currently, there is a wide variety of data pointing to a possible autoimmune origin for atherosclerosis. In this context, anti-endothelial cell antibodies are a heterogeneous group of circulating antibodies targeting endothelial cells and detected in different vasculitic, inflammatory or autoimmune situations whose common denominator is endothelial damage [1]. These autoantibodies have also been detected in coronary artery disease, associated with unstable angina, clinical recurrence and restenosis after percutaneous transluminal coronary angioplasty [2]. Anti-endothelial cell antibodies target a wide variety of self-antigens in the extracellular matrix and endothelial membrane [3–5].

These autoantibodies have various physiopathological effects arising from scarcely known mechanisms, such as induction of apoptosis [6], cytotoxicity [7], increase in leukocyte adhesion and cytokine secretion [8, 9], activation of coagulation and thrombosis.

Our aim here is to describe the effect of anti-endothelial cell antibodies on the endothelial dysfunction, inflammation and structural changes in the vascular wall that surround peripheral arterial disease in order to provide new insights in the field of the etiological pathophysiology of atherosclerosis.

2. Methods

An observational translational controlled study was conducted that included 32 patients with symptomatic peripheral arterial disease of the lower limbs: 23 with Fontaine stage II (intermittent claudication; group A), and nine with Fontaine stage III–IV (ischemic rest pain and/or focal tissue necrosis; group B), after hemodynamic confirmation of the disease (Doppler scans and treadmill exercise testing) and/or from imaging tests (echo Doppler scans, arteriography or magnetic angioresonance). No patient included had been previously revascularized or was presenting with active or infected tissue necrosis lesions. The control group included 16 healthy subjects with normal results on vascular examination and no cardiovascular risk factors who were not in receipt of pharmacological treatment and/or from imaging tests (echo Doppler scans, arteriography or magnetic angioresonance).

E-mail address: varelot@hotmail.com (C. Varela).

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2.4. Statistical analysis

Differences between groups were considered statistically significant at \( P < 0.05 \) in a two-tailed test. The normality of continuous variables was analyzed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The association between categorical variables was studied using the \( \chi^2 \)-test and the Fisher’s exact test when required. The association between continuous variables was analyzed using the Mann–Whitney U-test. Continuous variables are expressed as the median [interquartile range (p25 – p75)], and the categorical variables as percentages.

3. Results

All the anti-endothelial cell antibodies detected were IgG isotype, and all the serum samples reacted against proteinase 3, collagenase and \( \beta_2 \)-glycoprotein I antigens. We found a higher prevalence of these autoantibodies in patients with peripheral arterial disease than in the control subjects [13 (40%) vs. 1 (6%); \( P < 0.01 \)], but their frequency was similar between group A and group B [9 (39%) vs. 4 (44%); \( P = 0.70 \)]. There was a higher prevalence of ‘high autoimmune activity’ in patients with claudication (group A) than in patients with critical limb ischemia (group B), although no statistically significant differences were observed [six (26%) vs. one (11%); \( P = 0.64 \)]. The characteristics of the included subjects are presented in Table 1.

3.1. Relationship between circulating anti-endothelial cell antibodies and flow-mediated arterial dilatation

Flow-mediated arterial dilatation was lower in subjects with circulating anti-endothelial cell antibodies. When we analyzed the flow-mediated arterial dilatation according to stratification by autoimmune activity, we observed that subjects with ‘high autoimmune activity’ presented lower values than subjects with ‘low autoimmune activity’ (Fig. 1). Flow-mediated arterial dilatation was lower in patients with peripheral arterial disease than in healthy subjects [5.07% (0.70 – 10.26%) vs. 16.42% (10.25 – 20.17%); \( P = 0.01 \)]. Patients with claudication (group A) tended to show lower values than patients with critical limb ischemia (group B) [4.60% (0–7.50%) vs. 11.62% (2.00 – 19.00%); \( P = 0.06 \)].
3.2. Relationship between circulating anti-endothelial cell antibodies and C-reactive protein levels

We found higher levels of C-reactive protein in subjects with circulating anti-endothelial cell antibodies and in ‘high autoimmune activity’ subjects (Fig. 2). Levels of this inflammatory marker were also higher in patients with peripheral arterial disease than in control subjects [8.30 (4.22–10.50) vs. 3.00 (3.00–3.00) mg/l; \( P < 0.01 \)], and higher in patients from group B when compared with group A [11.85 (8.47–22.95) vs. 6.30 (3.35–9.91) mg/l; \( P = 0.01 \)].

3.3. Relationship between circulating anti-endothelial cell antibodies and intima–media thickness

Intima–media thickness was higher in subjects with circulating anti-endothelial cell antibodies and in ‘high autoimmune activity’ subjects (Fig. 3). This marker of subclinical disease was also higher in patients with peripheral arterial disease than in healthy subjects [1.00 (0.80–1.15) vs. 0.61 (0.51–0.66) mm; \( P < 0.01 \)]. There were no statistical differences in intima–media thickness between group A and group B [1.00 (0.76–1.11) vs. 1.05 (0.91–1.53) mm; \( P = 0.10 \)].

4. Discussion

In this study, we have observed an association between the presence of circulating anti-endothelial cell antibodies and peripheral arterial disease. This association should not be explained by the coexistence of an autoimmune disease, as any condition that could have had an impact on the immune system of the analyzed subjects was excluded.

Anti-endothelial cell antibodies may cause endothelial damage through direct (apoptosis and cytotoxicity) and indirect (inflammation) mechanisms [1, 7]. In this context, some published data have suggested that these autoantibodies might cause endothelial cell apoptosis via the nitric oxide pathway [11, 12]. These findings are of great interest, considering that endothelial dysfunction, which behaves like a primary pathogenic atherosclerotic
event, has been attributed to a reduction in nitric oxide bioactivity.

In previous studies, we observed a correlation between the plasma nitrite concentration and endothelial dysfunction measured by flow-mediated arterial dilatation [13], and in the current sample, subjects with circulating anti-endothelial cell antibodies presented lower values of this endothelial function marker. Moreover, those subjects with a high titer of these autoantibodies also showed a lower flow-mediated arterial dilatation. These data suggest that autoimmunity could be closely associated with the endothelial dysfunction present from the initial stages of peripheral arterial disease.

On the other hand, these autoantibodies could activate endothelial cells through a nuclear factor kB dependent mechanism, inducing an endothelial inflammatory phenotype [8, 9]. In this context, we have found higher levels of C-reactive protein in subjects with circulating anti-endothelial cell antibodies and in ‘high autoimmune activity’ subjects. We know that this plasma protein is able to alter nitric oxide bioavailability by interacting with nitric oxide synthase, an enzyme that, at the same time, is dysfunctional in an oxygen free radical-rich environment. Thus, inflammation could maintain the endothelial dysfunction generated in the first stages of peripheral arterial disease through a vicious circle supported by redox disequilibrium [13].

The relationship found between circulating anti-endothelial cell antibodies and C-reactive protein levels suggests that autoimmunity could play an important role in the genesis of the chronic inflammation observed in patients with peripheral arterial disease. According to published data, it is possible that this inflammatory process associated with autoantibodies might act by perpetuating the endothelial dysfunction and causing endothelial damage.

In this study, all the samples analyzed showed reactivity against β2-glycoprotein I. Antibodies against this antigen might be able to trigger an endothelial signaling pathway comparable to that used by the interleukin-1 receptor/Toll-like receptor superfamily [14]. Toll-like receptors are a key component of the innate immune response against infection. Molecular mimicry of these glycoproteins with microbial products could justify a Toll-like receptor-dependent inflammatory response when anti-β2-glycoprotein I antibodies are present.

One of the manifestations of early morphological effects in atherosclerosis is an increase in the carotid intima-media thickness. High values of this measurement are markers of subclinical disease and influence the prevalence and prognosis of atherosclerosis [15]. In our sample, intima-media thickness behaved as an immune response marker, as high values were associated with the presence of circulating anti-endothelial cell antibodies.

We also found an association between the autoantibody titer and intima-media thickness. In this way, a hypothesis could be made about the potential relationship of circulating anti-endothelial cell antibodies with the progression and prognosis of peripheral arterial disease, as its pathogenic mechanisms could justify the instability of the atheromatous plaque. This hypothesis is coherent with previous findings in coronary patients, in whom an association was observed between circulating anti-endothelial cell
antibodies and unstable angina and re-stenosis after percutaneous transluminal coronary angioplasty [2].

The immune response that occurs in atherosclerosis is complex and cannot be reduced to the role played by a single autoantibody. However, in our sample, the presence of circulating anti-endothelial cell antibodies was associated with peripheral arterial disease in individuals with no previous autoimmune disease. Moreover, we found an association of this autoantibody with markers of endothelial dysfunction, early macrovascular arterial wall damage and inflammation. In this sense, the pathogenic mechanisms associated with anti-endothelial cell antibodies make it biologically feasible to assume that autoimmunity could play an important role in the genesis and the development of atherosclerosis. The observational translational design of this study allowed us to obtain valid results with the sample size used, which could be applicable in the developing our understanding of the etiopathogenesis of atherosclerosis.

5. Conclusions

In conclusion, circulating anti-endothelial cell antibodies could be associated with peripheral arterial disease in individuals with no previous autoimmune disease. However, further prospective studies are required to establish a causal relationship.

References