Short Communication

Inflammatory Changes in Small Blood Vessels in the Endomyocardium of Cardiac Syndrome X in Female Patients with Increased C-Reactive Protein

(cardiac syndrome X / C-reactive protein / inflammation / mononuclear infiltrates / apoptosis)

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Abstract. The pathogenesis and pathohistological changes of CSX, a syndrome characterized by anginal chest pain and normal coronary arteries on coronary angiography, is poorly understood. The purpose of this study was to analyse morphological changes in small blood vessels of the CSX patients with increased CRP levels (above 5 mg/l). EMB was performed for diagnostic purposes in 31 female patients with CSX, and EMB specimens were histologically and immunohistochemically analysed. Increased CRP was found in 18 (58.1%) female patients with CSX. Signs of inflammation in the walls of small blood vessels were demonstrated in 13 (76%) and TUNEL-positive endothelial cells in 3 (17%) women with increased CRP. Morphological analysis of small blood vessels in EMB in CSX female patients with increased CRP levels revealed signs of inflammation and apoptosis of endothelial cells, indicating the role of inflammation in the pathogenesis of CSX.

Introduction

Cardiac syndrome X (CSX) is a syndrome characterized by anginal chest pain and normal coronary arteries on coronary angiography, to which either electrocardiographic changes or myocardial perfusion disorders indicating myocardial ischemia may be associated. The disease often affects postmenopausal female population (Kaski, 2004). Although the disease is not associated with a higher mortality, the life and treatment of such patients is more difficult (Kaski et al., 2004). So far, increased resistance of small blood vessels in the heart and reduced coronary microvascular dilatatory response have been reported as the causes of the disease (Kaski et al., 2004).

Recent findings have suggested that inflammation may be the cause of endothelial dysfunction and anginal chest pain in CSX (Arroyo-Espliguero et al., 2003; Cosin-Sales et al., 2003; Kaski, 2004). C-reactive protein (CRP), a marker of inflammation and a predictor of vascular events, has been associated with impaired endothelial function in coronary artery disease (CAD) patients, but also with coronary microvascular endothelial dysfunction in CSX patients (Arroyo-Espliguero et al., 2004; Jadhav et al., 2006). Beside CSX, other disorders such as diabetes mellitus, high blood pressure, smoking, and dyslipidaemia may be associated with endothelial dysfunction.

Emerging data also suggest that CRP is not merely an inflammatory marker but also a potential cause of endothelial activation in CSX, since it has been associated with enhanced levels of cellular adhesion molecules, increased endothelin-1 expression, and reduced bioavailability of nitric oxide. CRP levels have been reported to be elevated in CSX patients and to correlate with electrocardiogram markers of myocardial ischemia and clinical disease activity (Arroyo-Espliguero et al., 2003; Cosin-Sales et al., 2003; Jadhav et al., 2006).

The purpose of this study was to determine morphological changes in small blood vessels in the endomyocardial biopsy specimens of the CSX patients with increased CRP levels (above 5 mg/l).

Material and Methods

Endomyocardial biopsy (EMB) was performed for diagnostic purposes in 31 female patients with CSX (chest pain with normal coronary arteries on coronary angiography), aged 55 to 69 years, between 1992 and 1999. The concentration of serum CRP was determined in all patients. Informed consent for cardiac catheterization and endomyocardial biopsy was obtained from all

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Abbreviations: CRP – C-reactive protein, CSX – cardiac syndrome X, EMB – endomyocardial biopsy, TUNEL – terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling.

patients, and all procedures were conducted according to the principles of Declaration of Helsinki. Patients with diabetes, dyslipidaemia, and hypertension as well as smokers were excluded from the study.

Graduated series of histological specimens were prepared to evaluate morphological changes along the course of a certain blood vessel. The endomyocardial specimens were stained with haematoxylin-eosin.

The terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) (Apo Taq plus Peroxidase Kit ONCOR, Gaithersburg, MD) was used to demonstrate apoptotic nuclei following the manufacturer's instructions as described previously (Zorc et al., 2003).

Morphological evaluation was performed for any inflammatory changes, such as endothelial swelling and inflammatory cell infiltration. Mononuclear or polymorphonuclear and perivascular infiltration of blood vessel walls was demonstrated immunohistochemically with leukocyte common antigen (LCA) (Hussein et al., 2006). In the interstitium, the type of interstitial fibrosis was analysed. Apoptotic vascular nuclei were counted. Serum CRP levels were measured in all patients (Rolf Greiner Biochemica, Boehringer Mannheim, Ingelheim, Germany).

Results

Thirty-one female patients were enrolled in the study: 18 of the 31 (58.1%) female patients with increased serum CRP levels (above 5 mg/l), and 13 of the 31 (41.9%) female patients without increased serum CRP levels. Signs of vasculitis with endothelial oedema, mononuclear infiltration (Fig. 1) and polymorphonuclear infiltration of blood vessel walls or in perivascular areas were found in 13 out of 18 patients (76%) with increased serum CRP levels, whereas in the remaining 5 out of 18 patients (24%) with increased serum CRP levels, perivascular fibrosis without morphological signs of inflammation was found. Apoptosis in vascular cells was present in three patients with elevated CRP (17%).

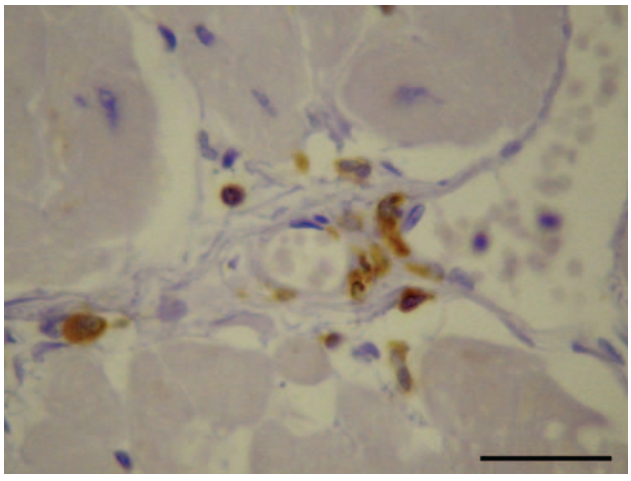

Fig. 1. Expression of leukocyte common antigen (LCA) in mononuclear cells in the vessel wall of a small blood vessel in a female patient with CSX (total magnification 630 ×, bar is 30 μm)

In the endomyocardial biopsy specimens of 13 CSX patients without increased CRP levels (41.9%) only hyalinization of the arteriolar subendothelium was found, whereas no morphological changes typical of inflammation of small blood vessels and no apoptosis in the wall of small blood vessels were observed.

Discussion

In the present study we report on inflammatory changes in small blood vessels in CSX patients with increased CRP, which is a marker of inflammation (acute phase protein).

In EMB specimens of CSX patients, mononuclear or polymorphonuclear infiltration and oedema of the endothelium were demonstrated in 13 of the 18 women (76%) with increased CRP, whereas perivascular fibrosis without inflammatory infiltrates were found in the remaining five (24%) women with increased CRP. Reactive fibrosis is a reaction to inflammation and is primarily perivascular (Swynghedauw, 1999). Thus, we believe that perivascular fibrosis might be the consequence of inflammation. These changes may be the sign announcing the development of atherosclerosis (Ross, 1999), which is manifested as hyalinization of small blood vessels as the disease progresses (Galbavy, 2001). This finding was demonstrated in our 13 CSX patients without increased CRP levels.

Inflammation causes endothelial dysfunction and increased secretion of vascular adhesion molecules, proinflammatory cytokines, and growth factors that may be responsible for the development of morphological changes in small blood vessels (Yamamoto et al., 2002; Lin et al., 2003; Kaski, 2004). Moreover, increased activation and dysfunction of the endothelial cells of small blood vessels may result in increased release of the vasoconstrictor endothelin that decreases the microvascular dilatatory response of small blood vessels (Yamamoto et al., 2002).

Immunohistochemical markers of apoptosis, i.e. TUNEL-positive endothelial cells were found in three of the 18 women (17%) with increased CRP. We speculate that apoptosis is involved in the process of endothelial dysfunction. Apoptosis of endothelial cells we found proves that part of cells in the vascular wall were so badly damaged that they actually died (Yamamoto et al., 2002; Lin et al., 2003; Kaski, 2004). As a result of these changes, reparation occurs, and within the vascular wall this is manifested first as transition to fibrosis, and later as hyalinization that was present in our 13 CSX patients without increased CRP levels. Similar morphological changes in CSX were reported by Yamamoto and coworkers (2002). They demonstrated cardiomyocyte

hypertrophy and apoptosis, replacement fibrosis, and capillary endothelial swelling in EMB specimens of patients with angina and normal coronary angiography.

Our results indicate that the inflammatory response of the vascular wall in patients with elevated serum CRP levels is the primary response to the noxious factor (Yamamoto et al., 2002; Kaski, 2004). This is followed by the release of inflammatory mediators and cytokines from the damaged endothelium.

In conclusion, morphological analysis of small blood vessels in EMB in CSX female patients with increased CRP levels revealed the signs of inflammation (mononuclear or polymorphonuclear infiltration and oedema of the endothelium), perivascular fibrosis, and apoptosis of endothelial cells, indicating the role of inflammation in the pathogenesis of CSX.

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