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Original Article

Morphological changes evaluation of left atrial appendage in patients with ischaemic heart disease

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ABSTRACT

Background: Since the majority of morphological changes evaluation of myocardium in ischaemic heart disease was in animal model, we detected the importance to evaluate such changes in human patients to gain insights into the targets of cellular damage and to reconcile or refine those experiments.

Methods: Tissue sections from left atrial appendage of the heart were carefully dissected from seventy five patients underwent conventional coronary artery bypass grafting at the cardiothoracic surgical department, Manchester Royal Infirmary. Tissue was fixed, sectioned, stained and six random sections were photographed and the images were assessed and quantified using Image Analyser Pro-Plus software, version 4.1. Arterioles, venules, intermediate sized vessels, and capillaries were directly counted within the highlighted area of myocardium under LM. Ultra-thin sections were imaged in a Tecnai 12 Biotwin transmission electron microscope at a magnification of ×4200 and photographed by a camera with a black and white film to quantify different structures of myocardium.

Results: The arteriole wall to lumen ratio was significantly increased in ischaemic heart disease patients 18.57 ± 2.89 compared to controls 8.3 ± 1.57, ($P < 0.01$). The regression analysis between vascular density and cardiomyocyte size demonstrated a significant inverse correlation between transverse cardiomyocyte diameter and arteriole, capillary and total vessel density ($P < 0.01$, 0.04, 0.02), respectively. Lumen area of the distal myocardial capillary was significantly reduced in IHD patients compared to controls ($P < 0.01$).

Conclusion: These results elucidate the morphological changes in the myocardial microvasculature of patients with ischaemic heart disease and its pathological magnitudes.

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At a glance commentary

Scientific background of the subject

The majority of studies have evaluated the morphological changes of myocardium in ischaemic heart disease in animal model. It is therefore of considerable importance to study the morphological changes of myocardium from patients to reconcile or refine experimental animal models of ischaemic heart disease.

What this study adds to the field

Several morphological changes were observed in the myocardial microvasculature and cardiomyocyte of ischaemic heart disease patients. Such changes elucidate the pathological magnitude of the disease.

ischaemic heart disease (IHD) is one of the commonest chronic diseases with high morbidity and mortality, (115.8/100,000 people) 12.6% of total disease death [1]. Only few pathomorphological studies define the changes occurring in the myocardium of patients with IHD are available in the literature [2–6]. Most of these studies have either used samples from autopsy cases, infarcted left ventricle or in myocardium from animal models. To the best of our knowledge none of the studies have used human tissue to quantify the capillary changes in IHD. It is therefore of considerable importance to define morphological changes of myocardium from patients with IHD to gain insights into the targets of cellular damage in patients, and to reconcile or refine experimental animal models of IHD. In the present study we have studied the Left Atrial Appendage (LAA) from patients with IHD.

The LAA or left auricle is a small projection from the upper border of left atrium curving around the root of pulmonary trunk to the front [7]. It is a long, tubular, hooked structure of different shapes and sizes [8]. It is trabeculated with muscular ridges called pectinate muscles running parallel to each other giving a comb like appearance. Its blood supply comes from left coronary artery running in atrio-ventricular sulcus [9]. Because of its higher distensibility as compared to left atrium proper, it may augment hemodynamic function by modulating relationship between left atrial pressure and volume [9,10]. It is an important source for the release of atrial natriuretic peptides (ANP) [10,11]. It may play a role in mediating thirst during hypovolemia [12].

The objectives of the current study were to employ light (LM) and electron microscopy (EM) to quantify the changes of myocardial microvasculature and cardiomyocytes in the myocardial tissue and to relate pathology to clinical variables in patients with IHD.

Materials and methods

Patients: seventy five patients underwent conventional coronary artery bypass grafting (CABG) with cardiopulmonary bypass, mitral valve replacement (MVR) or aortic valve replacement (AVR), or concomitant MVR and AVR with CABG at the cardiothoracic surgical department, Manchester Royal Infirmary were involved. Before surgery, all patients underwent cardiac catheterization and coronary angiography. This study was approved by the local Ethics Committee, and all patients gave their written informed consent before participation. Patients were classified into two clinical groups:

- Patients undergoing CABG alone (IHD group) (n = 53).
- Patients undergoing cardiac valve replacement with normal coronary angiography (control group) (n = 22).

Tissue processing for pathomorphology: Tissue sections from left atrial appendage of the heart were carefully dissected, divided into small sections and fixed in 2.5% glutaraldehyde in 1% cacodylate buffer (pH 7.4).

They were then secondarily fixed in 1% Osmium tetroxide. After that embedded in epon blocks and sectioned using Reichert mechanical advance ultra-microtome. O.7 μm sections were obtained for light microscopy and 0.08–0.09 μm sections for electron microscopy. Semi-thin sections were stained with 1% Toluidine blue while ultra-thin sections were stained with Methanolic Uranyl acetate and Lead citrate, the metallic and slightly radioactive dyes.

For LM 6 random areas from each section were visualized and photographed using Kodak image software. Sections were photographed at 2 magnifications (×20 and ×40). The ×20 lens was used to photograph most of the parameters while the ×40 was used to photograph the venules and the arterioles. Images were assessed and quantified using Image Analyser Pro-Plus software, version 4.1, with a standard grid bar to calibrate the images.

Arterioles, venules, intermediate sized vessels, and capillaries were directly counted within the highlighted area of myocardium under LM. From each section at least 6 random images were analyzed enabling the assessment of mean density (no./mm^2) for each subtype of blood vessel. Wall to lumen ratio (WLR) was also calculated by dividing the wall area by the corresponding luminal area of each arteriole or venule.

The transverse diameters of cardiomyocytes were measured. Cardiomyocytes with central nuclei were selected to ensure uniform selection. Approximately 100 cardiomyocytes were selected from each section. Independent assessment for the first few samples was carried out by expert pathologist to insure reading accuracy.

Ultra-thin sections were imaged in a Tecnai 12 Biotwin transmission EM at a magnification of ×4200 and photographed by a camera with a black and white film.

By EM following parameters were quantified:

- Luminal area (μm^2) (×4200) was measured by tracing the inner endothelial membrane.
- Endothelial outer membrane area (μm^2) (×4200) was measured by tracing along the outer endothelial membrane.
- Endothelial area (μm^2) (×4200) was calculated by subtracting the luminal area from endothelial outer membrane area.