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ORIGINAL ARTICLE

Correlations between MTP and ROS Levels of Peripheral Blood Lymphocytes and Readmission in Patients with Chronic Heart Failure

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Background Peripheral blood lymphocytes exhibit changes that parallel those in failing cardiomyocytes. We hypothesised that mitochondrial transmembrane potential (MTP) and reactive oxygen species (ROS) levels of lymphocytes are associated with serum NT-proBNP and short-term prognosis in patients with chronic heart failure (CHF).

Methods Fifty-four CHF patients and 30 controls were enrolled in this prospective study. Mitochondrial transmembrane potential and ROS levels of lymphocytes were evaluated by flow cytometry and reported as the JC-1 fluorescence ratio and DCF fluorescence intensity, respectively. Serum NT-proBNP levels and biochemical parameters were also examined. All the participants received follow-up to evaluate clinical end-points after eight months.

Results Chronic heart failure patients had higher levels of DCF fluorescence intensity of lymphocytes and serum NT-proBNP, as well as lower levels of JC-1 fluorescence ratios compared with those of controls (all P<0.05). A closer relationship was found between Lg(DCF fluorescence intensity of lymphocytes) or JC-1 fluorescence ratio of lymphocytes and Lg(NT-proBNP) (both $P<0.05$) in CHF patients. During the eight-month follow-up period, 14 CHF patients (25.9%) were readmitted for severe HF, but none died. A logistic regression analysis showed that both ROS level and MTP of the lymphocytes were independent predictors (B=7.03, $P=0.006$; B= - 0.32, $P=0.029$, respectively) of readmission of CHF patients.

Conclusions In CHF patients at low risk, MTP and ROS levels of the lymphocytes showed a significant change that is associated with serum NT-proBNP and patient readmission.

Keywords Mitochondrial transmembrane potential • Reactive oxygen species • N-terminal-probrain natriuretic peptide • Chronic heart failure • Readmission

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Introduction

Chronic heart failure (CHF) patients have substantial functional limitations, frequent recurrent hospitalisation, and a five-year mortality of 50%, despite optimal use of current therapies [1–3]. Chronic heart failure has a complex pathophysiology and may involve multiple organs and systems, but the role of the immune system in CHF remains undefined.

Increasing evidence has shown that peripheral blood lymphocytes undergo changes similar to failing cardiomyocytes in heart failure. Previous studies showed that the properties of β-adrenergic receptor signalling, G protein-coupled receptor kinase (GRK2) expression and activity in lymphocytes mirror changes in failing cardiomyocytes [4,5]. Clinical investigations have also demonstrated that peripheral blood lymphocyte counts have long been associated with the prognosis of heart failure (HF) [6–8]. Lymphocytopaenia in CHF patients may be the result of programmed lymphocyte death caused by excessive sympathetic activation and increased oxidative stress/pro-inflammatory status [9].

The disruption of mitochondrial transmembrane potential (MTP), the driving force of cellular adenosine triphosphate formation and an important determinant of cellular energy status and physiological activity, is an obligate step in cell-death programs [10]. However, it remains unclear whether the loss of MTP is an initiator or an effect of apoptosis, or whether it is actually necessary for apoptosis induction to occur. Moreover, it has been observed that lymphocytes can have decreased MTP before the appearance of any morphological signs of apoptosis in response to dexamethasone stimulation [11].

The majority of intracellular reactive oxygen species (ROS) are generated as by-products of mitochondrial bioenergetics. Excessive release of ROS can induce nonspecific damage to cellular membranes, proteins, and DNA, particularly targeting proximal mitochondrial components. Although its role in the disorder of lymphocytes in HF remains unclear, ROS-induced mitochondrial damage predominantly affects energy production and contributes to sterile inflammation and cardiomyocyte death [12,13].

Several studies have shown that mitochondrial depolarisation is associated with advanced HF [9,14], however, the relationship between lymphocyte mitochondrial depolarisation status and ROS levels and prognosis in CHF patients has not yet been investigated. To gain further insight into the role of lymphocytes, we conducted a prospective observational study to investigate MTP and ROS levels in CHF patients with standardised management based on specific guidelines and to analyse their relationship with serum NT-proBNP level and short-term prediction value.

Methods

Study Population

Fifty-four systolic CHF patients, who had been hospitalised for heart failure consecutively from January 2014 to June 2014 and on guideline-recommended oral medication for at least three months after discharge, were prospectively enrolled in the outpatient department of the First Affiliated Hospital of Xi'an Jiaotong University. Baseline characteristics were defined at the time of blood collection. Inclusion criteria were further defined by typical signs and symptoms, a history of being hospitalised for HF and a left ventricular ejection fraction (LVEF) $\leq$ 45%. Patients were excluded from the study if they presented with the following clinical conditions: active infection, acute heart failure, malignancy, autoimmune disease, serious liver, kidney, blood disease, or chronic obstructive pulmonary disease. Patients who had been hospitalised for heart failure within three months before enrolment were also excluded. Thirty volunteers from outpatients, who were free of CHF and all conditions in the exclusion criteria, donated blood samples after overnight fasting as reference samples.

This protocol was approved by the ethics committee of the First Affiliated Hospital of Xi'an Jiaotong University (Shaanxi 710061, China) and was performed in accordance with the guidelines of the Helsinki Declaration. All patients provided written informed consent.

Clinical Measurements

The CHF patients meeting the inclusion criteria and the controls (without HF) were evaluated at the beginning of the study to obtain demographic data, past medical history, NYHA class, electrocardiogram, and echocardiography. The primary endpoints were all-cause and cardiovascular mortality and readmission for severe HF.

Blood Sampling

Ten mL blood was harvested from the median cubital vein of CHF patients and controls. Half of the blood was drawn into pro-coagulation tubes, and serum was separated within two hours of collection and stored in a frozen state at -70 °C fridge before testing for NT-proBNP levels and other routine biochemical function tests. NT-proBNP levels were detected using an electrochemiluminescence immunoassay (Roche, Mannheim, Germany). The remaining blood samples were EDTA-anti-coagulated and assayed immediately for peripheral blood mononuclear cell (PBMC) preparation and various fluorescence staining procedures.

PBMC Preparation and Flow Cytometry

All steps of PBMC preparation were performed at room temperature. The blood was mixed 1:1 with pre-warmed (room temperature) 1 × PBS (w/o Ca^{2+} and Mg^{2+}). The mixture was layered onto a Lymphoprep (LTS1077, Haoyang Biological Manufacture, Tianjin, China) and centrifuged at 400 × *g* for 30 min (no acceleration, no brake). The ring-shape interphase (PBMCs) was collected with a Pasteur pipette into a new 15 mL tube. This sample was diluted to 10 mL with 1 × PBS and centrifuged at 250 × *g* for 10 min. Following a further wash step in 1 × PBS (250 × *g*, 5 min), the cells were resuspended in 1 mL 1 × PBS to approximately 1 × 10^6 cells/mL. The PBMC viability was confirmed to be more than 95% by trypan blue exclusion.