Ongoing Myocardial Damage in Chronic Heart Failure is Related to Activated Tumor Necrosis Factor and Fas/Fas Ligand System

Koichi Setsuta, MD; Yoshihiko Seino, MD*; Takeshi Ogawa, MD**; Toshiaki Ohtsuka, MD*; Kohji Seimiya, MD*; Teruo Takano, MD*

Background Elevated concentrations of cardiac troponin T and heart-type fatty acid-binding protein (H-FABP) identify patients with chronic heart failure (CHF) and ongoing myocardial damage (OMD) who are at increased risk for future cardiac events. Cardiomyocyte necrosis and/or apoptosis via activated tumor necrosis factor (TNF) and the Fas/Fas ligand (FasL) system may be related to the development of OMD.

Methods and Results The serum concentrations of H-FABP, a sensitive marker of membrane damage of cardiomyocytes, soluble Fas (sFas) and TNF- \( \beta \) were measured in 38 patients with CHF. The concentrations of H-FABP, TNF- \( \beta \) and s-Fas in patients with New York Heart Association (NYHA) III + IV were all significantly higher than in those patients in NYHA II. H-FABP; III + IV 9.3±5.9 vs II 5.1±1.8 ng/ml, p=0.003, TNF- \( \beta \); III + IV 10.5±3.8 vs II 8.0±2.7 pg/ml, p=0.02, sFas; III + IV 3.36±1.37 vs II 2.58±0.84 ng/ml, p=0.03). Increased concentrations of H-FABP significantly correlated with the concentrations of TNF- \( \beta \) \( (r=0.57, p=0.0001) \) and sFas \( (r=0.69, p<0.0001) \), independent of renal function.

Conclusion OMD detected by H-FABP, a marker of membrane damage, is related to activated TNF and the Fas/Fas system, which suggests a pathophysiological role of cardiomyocyte necrosis and/or apoptosis in patients with worsening heart failure. \( (\text{Circ J} \ 2004; 68: 747 – 750) \)

Key Words: Apoptosis; Fas/Fas ligand; Fatty acid-binding protein; Heart failure; Tumor necrosis factor

Progressive deterioration of ventricular function is frequently observed in patients with chronic heart failure (CHF) and recent reports, including ours, have shown that elevated concentrations of cardiac troponin T and heart-type fatty acid-binding protein (H-FABP), a low molecular protein that is abundant in the cytosol of cardiomyocytes, identify patients with ongoing myocardial damage (OMD) who are at increased risk for future cardiac events. The process of OMD is still unclear, but is possibly related to necrosis and/or apoptosis of cardiomyocytes. Several studies have demonstrated that activated tumor necrosis factor (TNF) and the Fas/Fas ligand (FasL) system play a significant role in the apoptosis of cardiomyocytes. The Fas molecule (Fas) is a transmembrane protein and a member of the TNF receptor family which can initiate apoptosis. The soluble form of Fas (sFas) is produced by alternative splicing of the transcript and is present in circulating blood. Increased serum concentrations of sFas are considered to reflect activation of the Fas/FasL system in vivo. TNF- \( \beta \) is a proinflammatory cytokine with a crucial role in cell death, including apoptosis of cardiomyocytes. Furthermore, the serum concentrations of sFas and H-FABP, which we have reported as a sensitive cytosolic marker of OMD, are increased in accordance with the severity of CHF. However, there are few studies investigating the relationships between OMD and TNF and the Fas/FasL system in patients with CHF, so the aim of the present study was to discover their pathophysiological linkage in patients with CHF.

Methods

Patients We studied 38 consecutive patients with CHF (23 men, 15 women, age 66±12 years). The severity of CHF was New York Heart Association (NYHA) functional class II in 23, NYHA III in 13 and IV in 2 patients. The etiology of CHF was previous myocardial infarction in 12, hypertensive heart disease in 11, dilated cardiomyopathy in 9, valvular heart disease in 3, congenital heart disease in 2 patients, and hypertrophic cardiomyopathy in 1 patient. Exclusion criteria were recent (within 3 months) history of ischemic heart disease and myocarditis, active pulmonary and liver disease, autoimmune disorder, infection, malignant disease, muscle disorder (creatine kinase (CK) ≥200 IU/L) and renal insufficiency (serum creatinine ≥2.5 mg/dl). Informed consent was obtained from all patients.

Measurements of Biochemical Markers Blood samples were obtained in the morning from the CHF patients while they were in a stable condition after an overnight fast. Samples were promptly centrifuged, and the plasma and sera were stored below –30°C. sFas (Medical & Biological Laboratories Co, Ltd, Nagoya, Japan; detection limit: 0.5 ng/ml), TNF- \( \beta \) (Japan Immunoreseach...
Assessment of $^{99m}$Tc Sestamibi SPECT Image

Thirteen patients with non-ischemic CHF underwent $^{99m}$Tc sestamibi SPECT. The SPECT images were visually assessed by 2 radiologists and a cardiologist, who were unaware of the biochemical data, using a 17-segment, 5-point (0, normal perfusion; 1, mild reduction—not definitely abnormal; 2, moderately reduction—definitely abnormal; 3, severe reduction; 4, absent uptake) scoring system according to the recommendation of the American Society of Nuclear Cardiology. The summed scores (SS) were calculated by summing the scores of all the segments.

Statistical Analysis

Values are expressed as mean±SD. Comparison of the mean concentrations of H-FABP, TNF-α and sFas between NYHA II and NYHA III + IV patients were performed by unpaired t-test. Linear regression analyses between the H-FABP concentrations and those of TNF-α and sFas were performed. Statistical significance was established at p<0.05.

Results

H-FABP, TNF-α and sFas Concentrations in CHF Patients

The serum concentrations of H-FABP, TNF-α and sFas in the patients with NYHA class II or III + IV are shown in Table 1. H-FABP, TNF-α and sFas concentrations were all significantly higher in the NYHA III + IV patients than in the NYHA II patients.

Relationship of H-FABP to SS in Patients With Non-Ischemic CHF

A significant correlation was found between H-FABP and SS (Fig 1).

Correlation of H-FABP With TNF-α and sFas Concentrations

Table 2 shows the correlations between H-FABP and TNF-α, sFas and the other clinical parameters. There were significant correlations between the H-FABP concentrations and those of TNF-α (Fig 2A), sFas (Fig 2C), ANP, CK-MB, and NE. The serum concentration of H-FABP is increased by renal clearance,18 so we investigated the correlation of the H-FABP concentration with those of TNF-α and sFas according to various cut-off levels of Cr (Table 3). H-FABP was still significantly correlated with TNF-α (Fig 2B), and sFas (Fig 2D) in CHF patients withCr...
<1.5 mg/dl. Even in patients with Cr <1.2 mg/dl (normal renal function), a significant and high correlation of H-FABP with TNF-\( \alpha \) and sFas was confirmed.

**Discussion**

**Relationship of H-FABP With OMD in Patients With CHF**

We have previously reported that elevated concentrations of H-FABP reflected the severity of CHF and the presence of OMD more discerningly than the concentration of cardiac troponin T, a myofibril marker and in addition, an elevated concentration of H-FABP predicted a poor prognosis in patients with CHF. In the present study, H-FABP again correlated with ANP, BNP and NE. Furthermore, the concentration of H-FABP correlated with the extent of hypoperfusion on sestamibi SPECT, which suggests that the concentration of H-FABP is related to the extent of myocardial damage. The results of the present study did not show a correlation between H-FABP and CK, CK-MB or LVEF. CK and CK-MB are less specific markers for the detection of myocardial damage and we have previously reported the lack of significant correlations of H-FABP with troponin T with CK and CK-MB. Sato et al showed similar concentrations of CK in patients with dilated cardiomyopathy with and without detectable troponin T: LVEF is an important index of left ventricular (LV) systolic function, but our previous report also showed no significant correlation of H-FABP and troponin T with LVEF. Another report has revealed the lack of a significant difference in LVEF between CHF patients with and without elevated concentrations of troponin I. Therefore, it appears that there is not a close relationship between OMD and LVEF.

**Activation of TNF and Fas/FasL System in CHF**

The precise mechanism of OMD still remains unclear, although several mechanisms have been suggested. One is cardiomyocyte necrosis and/or apoptosis induced via activation of TNF and the Fas/FasL system. The Fas/FasL system is an important inducer of apoptosis. Serum sFas concentrations correlate with the level of Fas expression in tissue thus the increased serum concentration of sFas is considered to reflect activation of the Fas/FasL system. Recent studies have suggested that deterioration of cardiac function in CHF is associated with cardiomyocyte apoptosis via the activated Fas/FasL system.

TNF-\( \alpha \) also plays an important role in cardiomyocyte death, including via apoptosis, through several mechanisms. Toore-Amione et al showed that concentration of myocardial TNF-\( \alpha \) was increased in patients with dilated cardiomyopathy or ischemic heart disease, and that the plasma concentrations of TNF-\( \alpha \) in patients with an increased myocardial concentration of TNF-\( \alpha \) were significantly elevated compared with normal subjects. Therefore,
elevated concentrations of circulating TNF-α are considered to reflect increased myocardial TNF-α expression. Relationships of H-FABP With TNF-α and sFas

The present study has shown that the concentrations of H-FABP, TNF-α and sFas were all elevated in accordance with the severity of CHF, and further, that the H-FABP concentration was significantly correlated with that of both TNF-α and sFas. However, the serum concentration of H-FABP is influenced by renal clearance, so we investigated whether inactivation of TNF and the Fas/FasL system would suppress OMD and improve the prognosis in patients with CHF. It needs to be determined whether inactivation of TNF and the Fas/FasL system in a subgroup analysis of patients with normal renal function. Thus, we presume that OMD is related to the activation of TNF and the Fas/FasL system in patients with chronic heart failure. A recent study has suggested that TNF-α plays a role in the enhanced systemic and local production of NO.

Study Limitation and Therapeutic Implication

The present study is the first to present evidence that OMD is related to activation of TNF and the Fas/FasL system in patients with CHF. However, cardiomyocytes are not necessarily the only site of activation of these factors in CHF. Meldrum et al showed that TNF was released from both the heart and kidney after cardiopulmonary bypass. Although we confirmed a significant relationship between an increased concentration of H-FABP and activation of TNF and the Fas/FasL system in a subgroup analysis according to renal function, the influence of subtle renal insult associated with compensatory mechanisms may not have been completely ruled out.

Elevated concentrations of cardiac troponin T and H-FABP identify CHF patients with OMD who are at increased risk for future cardiac events. However, there is not yet definitive treatment that will suppress OMD. Skudicky et al showed that patients with idiopathic dilated cardiomyopathy treated with pentoxifylline, a phosphodiesterase inhibitor with an immunomodulatory effect and a significant therapeutic effect in experimental autoimmune myocarditis showed a significant decline in the Fas/APO-1 concentrations and improvement in LV function. Bozkurt et al reported that treatment with etanercept, a TNF antagonist, led to a significant dose-dependent improvement in LV function and remodeling in patients with advanced heart failure. It needs to be determined whether inactivation of TNF and the Fas/FasL system would suppress OMD and improve the prognosis in patients with CHF.

References