Effect of Chronic Heart Failure on Nuclear Factor Kappa B in Peripheral Leukocytes

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Nuclear factor kappa B (NF-κB) is a ubiquitous transcription factor activated by various stimuli that are implicated in the progression of chronic heart failure. Therefore, we examined the activation of NF-κB in peripheral leukocytes, the only nucleated cell population noninvasively accessible in patients with heart failure. In patients with stable heart failure with no obvious other reason for NF-κB activation, NF-κB was significantly activated. ©2004 by Excerpta Medica, Inc.

In past years, several reports have documented increased concentrations of cytokines, such as tumor necrosis factor, interleukin-1β, and interleukin-6, in the plasma as well as the myocardium of patients with advanced heart failure (HF). 1,2 Most of these cytokines share the downstream activation of the transcription factor nuclear factor κB (NF-κB) or have NF-κB response elements in their promoter. NF-κB is a crucial transcription factor of immune and stress responses. Over 150 stimuli are known to activate NF-κB. In contrast, by activation of >150 target genes, 3 NF-κB is involved in various cardiac pathophysiological processes, for example, ischemic preconditioning, 4 unstable angina pectoris, 5 atherogenesis, 6 cardiac adhesion molecules, and cardiac allograft rejection. 7 Therefore, NF-κB could be of central importance for immunologic and stress responses in HF. In the inactive state, NF-κB resides in the cytoplasm and is bound to its inhibitor IκB in many cell types, including cardiac myocytes and inflammatory cells. Upon stimulation, IκB and NF-κB dissociate and NF-κB is translocated in the nucleus. 8 Therefore, nuclear translocation is a marker of NF-κB activation. Because leukocytes are the only noninvasively accessible nucleated cell population in humans, we used them to study NF-κB activation in patients with HF.

Methods of white cell isolation have been described elsewhere. 9 Briefly, 4 ml of blood was drawn into tube containing ethylenediaminetetraacetic acid (EDTA). Two milliliters were incubated with or without lipopolysaccharide (100 ng/ml) for 45 minutes, a stimulator of NF-κB. White cells were collected after centrifugation with >3 ml Ficoll Plaqe (Amersham, Upsala, Sweden).

After Dounce homogenization, white cells were lysed for 10 minutes on ice in a solution containing 10 mmol/L Hepes (pH 7.6), 10 mmol/L potassium chloride, 1.5 mmol/L MgCl₂, 0.5% Nonidet-40, 1 mmol/L dithiothreitol, and 0.5 mmol/L phenylmethylsulfonl fluoride. Nuclei were precipitated by centrifugation at 800 g for 30 seconds, supernatants saved as cytosolic extracts, and the nuclei resuspended in a solution of 20 mmol/L Hepes, 1.5 mmol/L MgCl₂, 420 mmol/L potassium chloride, 0.2 mmol/L EDTA, 1 mmol/L dithiothreitol, and 0.5 mmol/L phenylmethylsulfonl fluoride. The mixture was incubated on ice for 30 minutes, the supernatant collected as nuclear extract after centrifugation for 15 minutes at 13,000 g, and an equal amount of glycerol buffer added (20 mmol/L Hepes, 100 mmol/L potassium chloride, 0.2 mmol/L EDTA, 20% glycerol).

p65 Activity of nuclear extracts was measured with an enzyme-linked immunosorbent assay (ELISA) (Active Motif, Rixensart, Belgium) according to the manufacturer’s protocol. Values were normalized to a provided positive control. All measurements were done in duplicate. The p65 ELISA has been previously validated. 9

The study was approved by the ethics committee of the University of Würzburg and conformed to the Declaration of Helsinki. Blood samples of 26 patients with stable HF and 13 controls were collected. Inclusion criteria included an ejection fraction <50% as estimated by echocardiography. Control patients were healthy blood donors with an average age of 56 ± 1 year. Patients with inappropriate NF-κB activation upon lipopolysaccharide stimulation were excluded from further analysis.

All replicate data are expressed as mean and SEM. Absolute differences among 2 groups were compared using a t test. Statistical significance was achieved when 2-tailed p values were <0.05. Statistical analyses were carried out using the StatView statistics program (Abacus Concepts, Inc., Berkley, California).

Patients were included if they had stable HF as characterized by an ejection fraction of <50% as estimated by echocardiography. Patients with conditions known to induce NF-κB were excluded. This includes acute infections, rheumatoid arthritis, acute myocardial infarction, unstable angina pectoris, or cancer. Control patients were matching healthy volunteers on no medications. Detailed characteristics (ejection fraction, C-reactive protein, medications, and so forth) are listed in Table 1. Patients had different origins of HF, including idiopathic dilated car-

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The American Journal of Cardiology Vol. 94 September 1, 2004

0002-9149/04/$–see front matter doi:10.1016/j.amjcard.2004.05.041
NF-κB was significantly activated in patients with stable HF (Figure 1) compared with healthy volunteers (3.00 ± 0.29 vs 5.09 ± 0.58, percentage of control provided by the manufacturer, p = 0.01). The low activation levels of NF-κB in the control group were not due to a lack of functional NF-κB, because stimulation ex vivo with lipopolysaccharides almost doubled the amount of NF-κB (not stimulated vs stimulated with lipopolysaccharides, 3.00 ± 0.29 vs 4.58 ± 0.53, p = 0.02). However, lipopolysaccharides activated NF-κB in patients with HF even further (not stimulated vs stimulated with endotoxin, 5.09 ± 0.58 vs 16.57 ± 4.28, p = 0.01), NF-κB activation was negatively correlated with the New York Heart Association functional class (r = −0.46, p = 0.03; see Figure 1).

The described NF-κB activation in patients with stable HF was independent of the presence of renal failure (no renal failure vs renal failure, 5.26 ± 0.65 vs 4.92 ± 1.68, p = NS) or diabetes mellitus (no diabetes mellitus vs diabetes mellitus, 5.49 ± 0.67 vs 3.68 ± 1.35, p = NS). This is in accordance with a study investigating activation of NF-κB in unstable angina.3

The cytokines implicated in mediating myocardial depression in systemic sepsis, such as tumor necrosis factor, interleukin-1β, and interleukin 6, as well as inducible nitric oxide synthase, induce a number of sustained effects on cardiac myocyte phenotype and function, and are activated in HF. Interestingly, the transcription factor NF-κB not only induces the genes described above but also regulates their encoding proteins, suggesting NF-κB as an important, central factor in the activation and function of immunity in the failing heart. A pivotal role of NF-κB is now further supported by the present study, which demonstrates selective and marked activation of NF-κB in leukocytes of patients with stable HF independent of diabetes mellitus or chronic renal failure.

The observed NF-κB activation was most likely sustained because only patients with chronic and stable HF were studied. Moreover, sustained NF-κB activation in the myocardium of animals and humans with chronic HF has been recently described.9,10

When compared with sham operated animals, NF-κB activation was about 2.5-fold higher in rats 10 weeks after myocardial infarction, the chronic phase of left ventricular remodeling. Interestingly, larger infarct sizes and a higher degree of dilation were both significantly correlated with less NF-κB activation. This is in accordance with the results presented here, showing higher New York Heart Association class associated with less NF-κB activation. In addition, human failing hearts explanted at the time of heart transplantation exhibited marked nuclear translocation of NF-κB compared with control hearts irrespective of origin.9

In the present study, we extended these observations: in HF NF-κB is not only activated locally in the heart but also systemically in peripheral leukocytes. Thus, NF-κB may have an important role in the pathogenesis of HF.

### TABLE 1 Clinical Characteristics of Patients (n = 24)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic etiology</td>
<td>63%</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>66.6 ± 2.5</td>
</tr>
<tr>
<td>Men:women</td>
<td>21:3</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>0.81 ± 0.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.32 ± 0.1</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.1 ± 0.3</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>New York Heart Association class</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitor</td>
<td>21 (88%)</td>
</tr>
<tr>
<td>β blocker</td>
<td>21 (88%)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>21 (88%)</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>5 (21%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (17%)</td>
</tr>
</tbody>
</table>

![Figure 1](image-url)
Acknowledgment: We thank Christian Mainhardt for help recruiting healthy volunteers.


**Proportion of Patients in a Congestive Heart Failure Care Management Program Meeting Criteria for Cardiac Resynchronization Therapy**

Albert Yuh-Jer Shen, MD, XunZhang Wang, MD, Jonathan Doris, MD, and Naing Moore, MD

Among 1,138 patients in our congestive heart failure care management database, 37 (3.2%) met guideline criteria for cardiac resynchronization therapy. Advancing age was a predictor of prolonged QRS duration in this population, but gender, ejection fraction (EF) and cause of heart failure were not. There was a trend toward an inverse correlation between QRS duration and EF among patients with EF ≤35%. ©2004 by Excerpta Medica, Inc.

**Current United States practice guidelines** suggest consideration for cardiac resynchronization therapy (CRT) in patients who meet the following criteria: medically refractory New York Heart Association class III or IV congestive heart failure (CHF) with idiopathic dilated or ischemic cardiomyopathy, QRS interval ≥130 ms, left ventricular (LV) end-diastolic diameter ≥55 mm, and ejection fraction (EF) ≤35%. Although the prevalence of intraventricular conduction delay has been estimated at 20% to 50% of all patients with cardiac systolic dysfunction, it is unclear what proportion of patients with CHF meets all the criteria for CRT.

The CHF care management program at Kaiser Permanente Medical Center enrolls patients recently diagnosed with symptomatic heart failure. Patients are referred to the program from either the in- or outpatient setting. The patient’s symptom status and medication list are closely followed by the care manager and discussed with the patient’s physician as needed. Dosages of medications, including vasodilators, β blockers, digitalis, spironolactone, and diuretics are titrated to optimal or maximally tolerable levels. Patients are discharged from the program if they meet 1 of the following criteria: voluntary withdrawal, hemodialysis (these patients are enrolled in a separate care management program), heart transplantation, inability to maintain regular contact, transfer to hospice or skilled nursing facility, or death.

This study protocol was approved by our institutional review board. We examined our CHF care management program database and collected the following on all patients enrolled as of December 2001: age, gender, EF, ischemic versus nonischemic cause of CHF, atrial rhythm, and QRS duration on their most recent 12-lead electrocardiogram. All but 2 patients in the program had a numerical estimation of their EF by 2-dimensional echocardiography. Many also had undergone contrast or radionuclide ventriculography. In cases where >1 EF value was available, the most recent value was used. Echocardiographic EF was obtained by visual estimation or the modified Simpson’s rule. In cases where a range was given, the median value was taken. Cause of systolic dysfunction was determined by clinical history, noninvasive testing, and coronary angiography, when clinically appropriate.

We identified all patients with EF ≤35% and who were in sinus rhythm, because most trials of CRT excluded those in atrial fibrillation or flutter. Patients with ventricular paced rhythm were excluded from further analysis. The computerized medical records of patients with QRS ≥130 ms were reviewed for the following: New York Heart Association class, cause

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