Brief Rapid Communications

Elevated Levels of 8-iso-Prostaglandin F$_{2\alpha}$ in Pericardial Fluid of Patients With Heart Failure

A Potential Role for In Vivo Oxidant Stress in Ventricular Dilatation and Progression to Heart Failure

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Background—It has been suggested that oxidant stress may play a role in the pathophysiology of heart failure. However, no definitive information is available because most previous approaches used to measure oxidant stress are nonspecific, inaccurate, and unreliable.

Methods and Results—To evaluate oxidant stress in the heart, we measured pericardial fluid levels of 8-iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$), a specific and quantitative marker of oxidant stress in vivo, in a series of 51 consecutive patients with ischemic and/or valvular heart disease referred for cardiac surgery. Pericardial levels of 8-iso-PGF$_{2\alpha}$ were correlated with the functional severity of heart failure (NYHA classification) and with echocardiographic indices of ventricular dilatation measured by independent physicians. Pericardial levels of 8-iso-PGF$_{2\alpha}$ were significantly increased in patients with symptomatic heart failure compared with asymptomatic patients and gradually increased with the functional severity of heart failure ($P=0.003$). In addition, pericardial levels of 8-iso-PGF$_{2\alpha}$ were significantly correlated with left ventricular end-diastolic and end-systolic diameters ($P=0.008$ and .026, respectively).

Conclusions—Pericardial levels of 8-iso-PGF$_{2\alpha}$ increase with the functional severity of heart failure and are associated with ventricular dilatation. These data suggest an important role for in vivo oxidant stress on ventricular remodeling and the progression to heart failure. (Circulation. 1998;97:1536-1539.)

Key Words: heart failure • free radicals • pericardium

Heart failure is a serious health problem.$^1$ Identification of pathophysiological pathways that lead to heart failure is necessary to design specific therapeutic strategies that will hinder the progression of the disease. Oxidant stress, an imbalance between oxidant production and antioxidant defenses in favor of the former, leads to tissue injury and is thought to contribute to the pathophysiology of heart failure in humans, particularly heart failure due to coronary artery disease.$^{2,3}$ However, no definitive information is available as to the importance of this process in vivo given that most traditional methods previously used to assess oxidant stress are nonspecific, inaccurate, and unreliable.$^4$

Oxidative modification of arachidonic acid leads to the formation of free radical–catalyzed products called F$_{2\alpha}$-isoprostanes.$^5$ One of these compounds, 8-iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$), has recently been shown to be a specific, chemically stable, quantitative marker of oxidant stress in vivo.$^6$ Therefore, to assess the importance of oxidant stress in the progression to heart failure, we measured, using a specific validated immunoassay,$^7$ pericardial fluid levels of 8-iso-PGF$_{2\alpha}$ in a series of consecutive patients with ischemic and/or valvular heart disease referred for cardiac surgery.

Methods

Patient Selection and Characteristics

This study included 51 consecutive patients (36 men and 15 women; age range, 39 to 78 years) with ischemic heart disease ($n=19$), valvular heart disease ($n=27$), or both ($n=5$). Valvular heart disease comprised 17 cases of mitral regurgitation, 7 mitral stenosis, 13 aortic stenosis, 9 aortic insufficiency, and 1 tricuspid regurgitation. Ten patients had an association of two or several types of valvular disease. There were no symptoms, signs, or history of pericardial disease. No patient had undergone previous cardiac surgery. The patients were addressed for coronary artery bypass surgery, valve replacement, or valve repair. Among other parameters, age, smoking status, diabetes, aspirin treatment, use of antioxidants, and episodes of ischemia-reperfusion were documented in each patient because these parameters have previously been reported to influence plasma or urinary 8-iso-PGF$_{2\alpha}$ levels.$^6,7$ Eight patients presented with recent acute coronary syndromes (6 had unstable angina and 2 had myocardial infarction) at least 4 days before surgery. Eighteen patients were taking aspirin. Nine patients had diabetes mellitus. Only 3 patients were current smokers. The remaining patients were nonsmokers ($n=16$) or had abstained from cigarette smoking for periods ranging from 7 months to 32 years ($n=31$). Only 2 patients were taking allopurinol, a medication with potential antioxidant properties. No patient received antioxidants for cardiac protection.

The functional severity of heart failure was assessed before surgery by use of the NYHA classification, and the patients were
All patients had detectable pericardial fluid levels of 8-iso-PGF<sub>2a</sub>, significantly increased with the functional severity of heart failure assessed using the NYHA classification.

**Measurement of 8-iso-PGF<sub>2a</sub>**

Undiluted samples of pericardial fluid were collected immediately after incision in tubes containing 1 mmol/L 4-hydroxy-TEMPO. Time from incision to collection of pericardial fluid varied from 22 minutes to 130 minutes. Samples were immediately centrifuged at 3000g for 10 minutes and stored at -80°C. We analyzed the samples after incision in tubes containing 1 mmol/L 4-hydroxy-TEMPO. We analyzed the samples for their 8-iso-PGF<sub>2a</sub> content by direct assay using a validated enzyme immunoassay. In brief, the standard or the biological sample, 8-iso-PGF<sub>2a</sub>, coupled to acetylcholine esterase from the electric eel, and specific antiserums were added (50 µL each) at appropriate dilutions. All measurements were done in duplicate.

**Statistical Analysis**

Values are expressed as mean±SEM. Data were compared by use of a one-way ANOVA. Simple regression analysis was performed to analyze the relation between 8-iso-PGF<sub>2a</sub> and echocardiographic indices of ventricular dilatation and function. A value of P<.05 was considered to be statistically significant.

**Results**

**Elevated Pericardial Levels of 8-iso-PGF<sub>2a</sub> in Symptomatic Heart Failure**

All patients had detectable pericardial fluid levels of 8-iso-PGF<sub>2a</sub> ranging from 2 to 70 pg/mL. Time from incision to collection of pericardial fluid (52.2±5.6 minutes) had no influence on 8-iso-PGF<sub>2a</sub> levels. Levels of 8-iso-PGF<sub>2a</sub> in symptomatic patients (NYHA class 2 and 3, n=41) were significantly higher than those in asymptomatic patients (NYHA 1, n=10) (27.0±2.5 versus 11.1±1.6 pg/mL, respectively; P=.0037). Pericardial levels of 8-iso-PGF<sub>2a</sub> significantly increased with the functional severity of heart failure (11.1±1.6 pg/mL in NYHA 1 patients, 20.8±2.9 pg/mL in NYHA 2 patients, and 33.4±3.7 pg/mL in NYHA 3 patients; P=.0003) (Fig 1). Patients treated with aspirin also presented with elevated levels of 8-iso-PGF<sub>2a</sub> that correlated with NYHA status. These values were not different from those of patients not taking aspirin. Pericardial levels of 8-iso-PGF<sub>2a</sub> were not influenced by age, sex, smoking status, diabetes, medical treatment, or recent history of acute coronary syndromes.

**Correlation Between Pericardial Levels of 8-iso-PGF<sub>2a</sub> and Ventricular Dilatation**

Echocardiographic evaluation was performed by independent cardiologists just before surgery in 27 unselected patients. Data on LVEDD were available for all these patients. Data on LVESD and left ventricular fractional shortening were available for 23 of these patients. Pericardial levels of 8-iso-PGF<sub>2a</sub> were significantly correlated with LVEDD (r=.5, P=.008) (Fig 2, top) and LVESD (r=.46, P=.026) (Fig 2, bottom). There was no correlation between pericardial levels of 8-iso-PGF<sub>2a</sub> and left ventricular fractional shortening (P=.45).

**Figure 1.** Pericardial levels of 8-iso-PGF<sub>2a</sub> in patients with ischemic and/or valvular heart disease referred for cardiac surgery. Pericardial levels of 8-iso-PGF<sub>2a</sub> significantly increased with the functional severity of heart failure assessed using the NYHA classification.

**Figure 2.** Top, Relation between pericardial levels of 8-iso-PGF<sub>2a</sub> and LVEDD. Pericardial levels of 8-iso-PGF<sub>2a</sub> correlated significantly with LVEDD (y=0.304x+45.2, n=27, r=.5, P=.008). Bottom, Relation between pericardial levels of 8-iso-PGF<sub>2a</sub> and LVESD. Pericardial levels of 8-iso-PGF<sub>2a</sub> correlated significantly with LVESD (y=0.188x+28.2, n=23, r=.46, P=.026).
Discussion

It has been suggested that oxidant stress may play a role in the pathophysiology of heart failure in animals and humans.\(^2\) However, traditional approaches used to evaluate the importance of oxidant stress in vivo, including the analysis of reactive aldehydes, lipid hydroperoxides, conjugated dienes, and exhaled pentane, suffer from major limitations due to the inaccuracy of these methods in estimating the actual rate of lipid peroxidation in vivo.\(^3\)\(^,\)\(^4\) The most widely used index of lipid peroxidation is the measurement of malondialdehyde by the thiobarbituric acid–reacting substances assay. When this assay was used as an index of lipid peroxidation in vivo, conflicting results were obtained. Indeed, this assay suffers from lack of specificity and overestimates actual malondialdehyde levels by >10-fold,\(^1\) rendering the assay inaccurate as an indicator of oxidant stress in vivo.

F\(_2\)-isoprostanes are a family of prostaglandin F\(_{2\alpha}\)-isomers formed in situ on phospholipids by the peroxidation of arachidonic acid, catalyzed by free radicals.\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\) They are presumably released into biological fluids through a phospholipase-mediated mechanism and are excreted in the urine.\(^1\)\(^0\) One of these compounds, 8-iso-PGF\(_{2\alpha}\), has recently been shown to be a specific, quantitative index of oxidant stress in vivo.\(^1\)\(^1\) In this study, we found a significant increase in pericardial fluid levels of 8-iso-PGF\(_{2\alpha}\) in patients with symptomatic heart failure. Although we did not identify the cell type(s) responsible for this increased production, virtually all cell types present in the heart, including myocardial and pericardial cells, might be implicated. Consistent with studies showing that PGF\(_{2\alpha}\)-isomers are formed from mainly noncyclooxygenase oxidative transformation of arachidonic acid,\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\) the subgroup of patients treated with aspirin also presented with elevated levels of 8-iso-PGF\(_{2\alpha}\). Although elevations in plasma or urinary F\(_2\)-isoprostane levels have been reported in association with several cardiovascular risk factors,\(^6\)\(^-\)\(^1\)\(^1\) no individual subgroup with elevations in pericardial 8-iso-PGF\(_{2\alpha}\) levels could be identified in our patient population, considering sex, age, smoking status, or diabetes.\(^6\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^1\)\(^2\)\(^,\)\(^1\)\(^3\)\(^,\)\(^1\)\(^4\)\(^,\)\(^1\)\(^5\)\(^,\)\(^1\)\(^6\)\(^,\)\(^1\)\(^7\)\(^,\)\(^1\)\(^8\)\(^,\)\(^1\)\(^9\)\(^,\)\(^2\)\(^0\)\(^,\)\(^2\)\(^1\)\(^,\)\(^2\)\(^2\)\(^,\)\(^2\)\(^3\) However, the increased formation of pericardial 8-iso-PGF\(_{2\alpha}\) levels could be identified in our patient role in the progression from asymptomatic to symptomatic failure as assessed by NYHA status. Therefore, our findings were associated with increased functional severity of heart failure.\(^2\)\(^4\)\(^,\)\(^2\)\(^5\)\(^,\)\(^2\)\(^6\)\(^,\)\(^2\)\(^7\) suggesting that oxidant stress in the heart may play a significant role in the progression from asymptomatic to symptomatic heart failure and in the progressive deterioration of functional capacity.

Apart from being an index of oxidant stress, 8-iso-PGF\(_{2\alpha}\) generated during this process may exert potent vasoconstrictor activity in vivo.\(^1\)\(^8\)\(^-\)\(^2\)\(^1\) We propose that this mechanism, acting locally, may be responsible, at least in part, for the limited functional capacity in patients with increased 8-iso-PGF\(_{2\alpha}\) levels. This may occur through a decrease in subendocardial blood flow and an alteration in diastolic function, for example. This hypothesis should be verified in future studies.

Another interesting finding in the present study points to a potential effect of oxidant stress on ventricular remodeling. Pericardial fluid levels of 8-iso-PGF\(_{2\alpha}\) were significantly correlated with echocardiographic indices of ventricular enlargement, LVEDD, and LVESD. This observation is important because several studies have shown that these and other echocardiographic parameters of ventricular dilatation are strong determinants of prognosis in symptomatic or asymptomatic patients with or without overt cardiovascular disease.\(^2\)\(^8\)\(^-\)\(^2\)\(^7\)

Little is known about the relation between oxidant stress and ventricular remodeling. In our patients, acute ischemia or ischemia-reperfusion are not likely to explain the persistent increase in 8-iso-PGF\(_{2\alpha}\) several days after the clinical syndrome, although the clearance of this compound in pericardial fluid is totally unknown. A more likely hypothesis involves the role of mechanical factors. It has recently been shown that overstretching of the myocardium leads to enhanced generation of reactive oxygen species, with increased expression of Fas and induction of apoptosis.\(^2\)\(^8\) This in turn would lead to rarefaction and slippage of myocytes, resulting in an even more pronounced ventricular dilatation. This pathophysiological mechanism might account for the significant correlation between pericardial 8-iso-PGF\(_{2\alpha}\) and ventricular dilatation observed in the present study and in part for the occurrence of apoptosis in the dilated human heart.\(^2\)\(^9\)\(^-\)\(^3\)\(^1\) However, it cannot be ruled out that 8-iso-PGF\(_{2\alpha}\) is solely a marker for increased myocardial damage in our patients with heart failure.

Study Limitations

This study included patients with ischemic or valvular heart diseases in NYHA classes 1 to 3. Whether our findings will extend to patients with other types of heart disease (hypertensive or dilated cardiomyopathy, for example) or to patients with more severe heart failure (NYHA class 4) merits further investigation.

Conclusions

In conclusion, this is the first study showing that ventricular dilatation and symptomatic heart failure are associated with an increase in pericardial fluid levels of 8-iso-PGF\(_{2\alpha}\), which is likely to reflect oxidant stress in the heart. Our data open the way for dose finding with antioxidant drugs and evaluation of their potential therapeutic effects on both ventricular remodeling and the progression to heart failure.

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