Reduction in Fas/APO-1 plasma concentrations correlates with improvement in left ventricular function in patients with idiopathic dilated cardiomyopathy treated with pentoxifylline

Inflammatory cytokines have been related to the pathogenesis and progression of heart failure. Cytokines can modulate cardiovascular function through different mechanisms, such as depressing contractile function, promoting left ventricular remodelling, uncoupling myocardial β adrenergic receptors, and inducing apoptosis. Loss of myocytes caused by apoptosis or programmed cell death occurs in patients with heart failure and may contribute to progressive myocardial dysfunction. Fas is an apoptosis signalling surface receptor known to trigger programmed cell death in a variety of cell types, and increased plasma concentrations of soluble Fas receptors have been reported in patients with heart failure. Pentoxifylline is a xanthine derived agent known to inhibit the production of tumour necrosis factor α (TNFα). It was also found to inhibit apoptosis in different human cell types in vitro and in vivo. However, the effects of pentoxifylline on apoptosis signalling receptors in patients with heart failure has not been investigated. We have previously reported beneficial effects of pentoxifylline in patients with idiopathic dilated cardiomyopathy. Treatment with pentoxifylline resulted in a significant improvement in functional class and left ventricular function, and was associated with a reduction in TNFα plasma concentrations.

The aims of the present study were to evaluate: (1) the effects of pentoxifylline on Fas/APO-1 plasma concentrations in patients with idiopathic dilated cardiomyopathy; (2) the possible correlation between the changes in the cytokine or Fas concentrations and the changes in left ventricular ejection fraction following six months of treatment with pentoxifylline; and (3) to establish predictors of outcome.

In a single centre, prospective, double blind, randomised, placebo controlled trial, we included 49 patients with idiopathic dilated cardiomyopathy in New York Heart Association functional class II or III and left ventricular ejection fraction < 40% by radionuclide angiography. All patients received treatment with digoxin, diuretics, and angiotensin converting enzyme inhibitors. After baseline examinations were performed, patients were randomised to pentoxifylline 400 mg three times daily (n = 25) or a matching placebo (n = 24). Mean (SD) age of the study population was 52 (11) years and baseline mean left ventricular ejection fraction 23 (9)%. There were no significant baseline differences between groups. Eight patients died during the study period (six in the placebo group). Treatment with pentoxifylline was associated with a significant improvement in functional class, and left ventricular ejection fraction (21 (8)% to 35 (16)%, p = 0.0007). No significant changes in left ventricular dimensions or function were observed in the placebo group. Twelve of the patients treated with pentoxifylline showed an improvement of the ejection fraction > 10 absolute units, and were arbitrarily defined as improvers, whereas only two patients in the placebo group showed a similar degree of improvement (p = 0.008 between groups).

TNFα and Fas plasma concentrations were significantly higher in the study group compared to 20 healthy volunteers (7.4 (5) pg/ml vs 1.4 (1) pg/ml, p < 0.0001; and 6.7 (5) U/ml vs 0.8 (0.2) U/ml, p < 0.0001, respectively). Baseline concentrations of TNFα and Fas were similar in the placebo and treatment arms. Patients treated with pentoxifylline had a significant reduction in the TNFα (6.6 (5) pg/ml to 1.9 (1) pg/ml, p = 0.0005) and Fas plasma concentrations (8.0 (7) U/ml to 5.5 (6) U/ml, p = 0.004) after six months, with no significant changes in the placebo group. Despite a similar ejection fraction at baseline, non-improvers had larger left ventricular end diastolic (p = 0.06) and end systolic diameters (p = 0.05), and a higher E/A ratio (p = 0.03) compared to improvers. Baseline TNFα plasma concentration was significantly higher in the 12 patients that were defined as improvers in the pentoxifylline group compared to non-improvers (7.26 (3.2) pg/ml vs 3.06 (2.3) pg/ml respectively, p = 0.03). Similarly, there was also a trend towards higher baseline Fas concentrations among improvers (p = 0.09). No other significant differences in the baseline characteristics were noted between these two groups. Following six months of treatment with pentoxifylline, both improvers and non-improvers showed a significant reduction in TNFα concentrations, although there was a trend towards a more significant decline among the improvers (reduction of 5.2 (4) pg/ml v 2.0 (3) pg/ml in improvers and non-improvers respectively, p = 0.06). There was a significant correlation between baseline Fas/APO-1 plasma concentrations, and changes in ejection fraction after treatment (r = 0.47, p = 0.03), and a significant negative correlation between the changes in Fas plasma concentrations, and the change in ejection fraction at six months (r = -0.49, p = 0.03). No significant correlation was observed between baseline TNFα concentrations or changes in TNFα concentration, and changes in ejection fraction following treatment. Of all baseline variables analysed, only Fas/APO-1 concentration and E/A ratio emerged as independent predictors of improvement in the multivariate analysis (p = 0.02).

The reduction in Fas concentrations observed in this study suggests a possible inhibition of apoptosis by pentoxifylline. The finding that baseline concentrations of TNFα and Fas/APO-1 concentrations were higher in patients with a more favourable response to treatment is important to help us to understand the mechanism of action of pentoxifylline in patients with heart failure. The lack of statistical correlation between the changes in cytokine concentrations and the changes in left ventricular function might be explained by the fact that in the pentoxifylline group TNFα dropped even in patients that did not improve the ejection fraction. Finally, although current evidence supports the proposal that apoptosis occurs in heart failure, it is still unclear to what extent it contributes to the progression of myocardial dysfunction. Hiroti and colleagues, using a gene knockout mouse model, recently showed that apoptosis plays a critical role in the transition between compensatory cardiac hypertrophy and heart failure during aortic pressure overload. Therefore, the use of agents that can block apoptotic pathways could be a useful strategy in the treatment of patients with heart failure. The results of our study suggest that attenuation of apoptosis together with a reduction in TNFα concentrations may be beneficial.
the mechanism of improvement of left ventricular function in patients with ischaemic dilated cardiomyopathy treated with pentoxyfylline.

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Increased formation of F3-isoprostanes in patients with severe heart failure

During the last decade, considerable interest has been focused on the potential role of oxygen free radical generation in the pathophysiology of chronic congestive heart failure. Oxidative stress has been demonstrated in different animal models of congestive heart failure: volume overload, pressure overload, myocardial infarction, and adriamycin-induced cardiomyopathy. In contrast with animal studies, human studies are less convincing, given that most of the traditional methods used to assess oxidative stress in the clinical setting are non-specific or inaccurate.1

A novel family of prostaglandin F3 isomers, called F3-isoprostanes, produced in vivo by a free radical peroxidation of arachidonic acid, has recently been described.3 Isoprostaglan-  
din F3 type III, formerly known as 8-isoprostaglandin F3, is a biochemically stable F3-isoprostane, formed by direct free radical peroxidation of arachidonic acid of cell membranes or circulating low density lipoprotein.1 Its quantification in tissues and biological fluids has suggested to be a reliable measure of oxidative injury in vivo. Indeed, urinary excretion of this compound has been well characterised and is currently used as an index of lipid peroxidation in human diseases. A recent study has shown that concentrations of isoprostaglandin F3 type III were increased in pericardial fluid of patients with symptomatic heart failure.4 However, urinary measurements may be of more clinical use for follow up studies. The purpose of the present study was to investigate urinary isoprostaglandin F3 type III formation as an index of lipid peroxidation in patients suffering from severe heart failure.

Twenty five consecutive patients suffering from heart failure were included between April and July 1999. Patients were referred before heart transplantation or for a left ventricular failure episode. Patients were matched with 25 healthy volunteers (22 men, 3 women, median age 56 years (range 25–73 years), 11 smokers). The criterion for admission into the study was a left ventricular ejection fraction < 45%. Criteria for exclusion were severe valve disease and congenital heart disease. Two subgroups of patients were identified: seven patients with chronic heart failure secondary to ischaemic heart disease—that is, documented myocardial infarction and/or angiographically demonstrated coronary artery disease; and 18 patients with idiopathic dilated cardiomyopathy, with angiographically normal coronary arteries. The clinical characteristics of the patients are depicted in table 1.

This study conformed to the principles outlined in the declaration of Helsinki.

Table 1 Clinical characteristics of 25 patients with severe heart failure

| Age (years) (median, range) | 57 (27–75) |
| Body mass index (kg/m²) | 22.3 |
| Smokers | 12/25 |
| Disease duration (months) (median, range) | 12 (0–96) |
| Aetiology of heart failure | 28% |
| Ischaemic cardiomyopathy | 72% |
| NYHA functional class | 40% |
| II | 36% |
| III | 24% |
| IV | 25 (11) |
| LVEF (%) | 56.8 (4) |
| LVEDD (mm) | 66.8 (4) |
| VO2 max (% predicted values), n = 14 | 67 (18) |
| Medication | 92% |
| ACE inhibitors | 88% |
| Loop diuretics | 72% |
| Digoxin | 48% |
| β blockers (carvedilol) | 40% |
| Aspirin | 28% |
| Oral anticoagulant treatment | 4% |

Continuous variables were expressed as means with standard deviations in parentheses. Analysis of variance (Kruskal-Wallis method, followed by Mann-Whitney U test) was used for statistical comparisons. Regression analyses were performed using the Spearman rank correlation test. Values of p < 0.05 were considered significant.

Urinary excretion of isoprostaglandin F3 type III was significantly higher (p < 0.0001) in patients suffering from severe heart failure compared with age and sex matched controls: 259 (191) versus 110 (36) pmol/mmol creatinine (fig 1). Concentrations of isoprostaglandin F3 type III in New York Heart Association (NYHA) class IV patients were significantly higher (p < 0.05) than those in NYHA class II and III: 426 (296) versus 199 (83) and 214 (139) pmol/mmol, respectively (fig 1). Urinary excretion of 11-dehydro-thromboxane B1, was significantly higher (p < 0.001) in patients compared with controls: 317 (242) versus 119 (64) pmol/mmol. However, no significant differences were found between the different functional classes.

Figure 1 Urinary concentration of isoprostaglandin F3 type III in controls (n = 25), and patients in NYHA classes II (n = 10), III (n = 9), and IV (n = 6). The line drawn through the middle of the boxes represents the median. The top and bottom of the boxes are the 75th and 25th centiles. The top and bottom of the T bars are the 90th and 10th centiles. * p < 0.05 versus class II and III.
Urinary concentrations of isoprostaglandin F$_2\alpha$ type III were not correlated to left ventricular end diastolic diameter or to left ventricular ejection fraction. A difference between ischaemic and idiopathic dilated cardiomyopathy subgroups was not observed. There was a non-significant trend towards a correlation between isoprostaglandin F$_2\alpha$ type III concentrations and V$_{O2,max}$. A significant correlation was found between isoprostaglandin F$_2\alpha$ type III and 11-dehydro-thromboxane B$_2$, urinary concentrations ($r = 0.64, p < 0.001$), suggesting a link between lipid peroxidation and platelet activation. No significant differences in isoprostaglandin F$_2\alpha$ type III urinary concentrations were found between patients on aspirin versus those not on aspirin (241 (118) vs 271 (230) pmol/mmol, respectively), or between patients on carvedilol versus those not on carvedilol (256 (140) vs 260 (220) pmol/mmol, respectively).

In our study, a significant increase in isoprostaglandin F$_2\alpha$ type III concentrations was found in the urine samples from patients with severe heart failure compared with controls, independent of cardiac failure aetiology. Consistent with a previous study on pericardial dial concentrations of isoprostaglandin F$_2\alpha$ type III, we found a correlation between the function of the heart and the urinary concentration. Furthermore, there was a trend towards a correlation between isoprostaglandin F$_2\alpha$ type III concentrations and V$_{O2,max}$. These results suggest that oxidative stress is correlated with the progression of heart failure and with the deterioration of functional capacity. However, it remains undetermined whether lipid peroxidation plays a pathophysiological role in the evolution of cardiac failure, or if isoprostaglandin F$_2\alpha$ type III only reflects tissue damage in patients with heart failure. It has been suggested that free radical generation may play a role in both contractile dysfunction and structural damage to the myocardium. Furthermore, isoprostaglandin F$_2\alpha$ type III has been shown to be a potent vasoconstrictor in animal and human vascular beds. As a consequence, isoprostaglandin F$_2\alpha$ type III may contribute to the increased peripheral and coronary vascular resistance observed in cardiac failure and thus contribute to the functional deterioration.

Urinary 11-dehydro-thromboxane B$_2$—an index of thromboxane synthesis in vivo—was increased in heart failure patients, suggesting enhanced platelet activation. This finding is in accordance with those of a recent study, in which both soluble and platelet bound P-selecting concentrations were higher in patients with heart failure compared with controls. The correlation observed between 11-dehydro-thromboxane B$_2$ and isoprostaglandin F$_2\alpha$ type III excretion is consistent with a link between enhanced lipid peroxidation and platelet activation in heart failure.

An important advantage of isoprostaglandin F$_2\alpha$ type III measurement in urine over other methods is that it is non-invasive, reproducible, and may be repeated over time. Several therapeutic approaches including carvedilol, amiodarone, and vitamin E have recently been shown to protect cardiac myocytes against oxidative injury in vitro. The results of the present study provide a rationale for clinical trials based on measurements of F$_2\alpha$-isoprostanes to assess the antioxidant properties of these drugs in heart failure. Indeed, such an approach has recently been described in hypercholesterolaemia in diabetes. In both studies, vitamin E supplementation induced dose dependent reductions in F$_2\alpha$-isoprostanes concentrations. The present study shows that severe heart failure is associated with an increase in urine concentrations of isoprostaglandin F$_2\alpha$ type III, an index of lipid peroxidation. Urinary measurement of F$_2\alpha$-isoprostanes may provide a non-invasive biochemical end point for antioxidant therapeutics investigations in heart failure.

Commentary

Isoprostaglandin F$_2\alpha$, also known as isoprostas taglandin F$_2\alpha$ type III, is a member of a group of prostanooids called F$_2\alpha$-isoprostanes. These are produced as a result of cell membrane lipid peroxidation mediated by reactive oxygen species (free radical) chemistry. This small study reports that urinary excretion of isoprostaglandin F$_2\alpha$ can be used to assess the extent of oxidative stress in heart failure patients and that urinary concentrations correlate with the functional severity of disease. The technique may have potential for development as a non-invasive quantitative tool for monitoring response to treatment in patients with heart failure.

Failure of plasma brain natriuretic peptide to identify left ventricular systolic dysfunction in the community

Heart failure is a major public health problem. The clinical diagnosis is imprecise and a simple test of left ventricular function would greatly improve diagnostic accuracy. Measurement of brain natriuretic peptide (BNP) has been shown in some studies to be a potential marker of left ventricular dysfunction. Heart failure is more common in the elderly and is often treated in the community where a simple test would be of considerable benefit. We therefore measured plasma BNP values and compared them with echocardiographic measures of left ventricular function in a large, community based population.

Eight general practices of a total list size of 65 820 were surveyed. All patients currently prescribed loop diuretics were invited to take part in the study. The study included the majority of patients with the clinical syndrome of heart failure would be treated with them. Of the 1425 patients asked to participate, informed consent, echocardiograms, and measurement of plasma BNP concentration was 47 pg/ml (32–79 pg/ml) compared to 60 pg/ml (38–97 pg/ml) in those with an LVEF of < 40%, median rank sum test; p < 0.001. The plasma BNP
concentrations for both groups are presented as a scatterplot in fig 1B. A receiver operator characteristic (ROC) curve was constructed (SAS statistical software) to assess the sensitivity and specificity of BNP to detect left ventricular systolic dysfunc- tion. The area under the curve, which provides a measure of the overall diagnostic accuracy of the test, was 0.587, indicating poor sensitivity and specificity.

This suggests that the accuracy of BNP concentrations to provide a measure of left ventricular function and help diagnose heart failure is not as good as previously reported. The reason for the differences between our results and those of others is not clear. We believe our BNP assay is robust and the echocardiograms were performed by a small number of experienced operators and analysed in a standardised way. It is possible that our patients were different to those included in other studies. We deliberately chose patients who general practitioners thought might have heart failure and had accordingly treated them with diuretics. The diagnostic difficulties are clearly more important in the community. Furthermore the age of the patients included in this study was much higher than in others, but also the prevalence of heart failure is much higher in the elderly. We do not believe the previous prescription of diuretics would have adversely affected our results, as there is no evidence diuretics change BNP concentrations or have significant effects on echocardiographic measures of ejection fraction. The results of this study suggest that BNP concentrations may not be as accurate an aid to the diagnosis of heart failure as previously stated.

DJH is supported by the National Heart Research fund.

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