Increased urinary 15-F₂-t-isoprostane concentrations in patients with non-ischaemic congestive heart failure: a marker of oxidative stress

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Objective: To investigate a novel marker of oxidative stress in patients with congestive heart failure (CHF).

Patients: 15 patients with mild CHF, 15 patients with severe CHF with acute exacerbation, and 15 control subjects.

Main outcome measures: Measurement of urinary 15-F₂-t-isoprostane, plasma brain natriuretic peptide (BNP), serum interleukin 6 (IL-6), and serum thrombomodulin concentrations. In patients with severe CHF, samples were taken at admission and 4, 7, and 14 days after admission.

Results: Urinary 15-F₂-t-isoprostane, plasma BNP, and serum IL-6 concentrations in patients with severe CHF were significantly higher than those in control subjects or in patients with mild CHF. However, concentrations of serum thrombomodulin, a marker of endothelial damage, were not different between patients with CHF and control subjects. In addition, urinary 15-F₂-t-isoprostane, plasma BNP, and serum IL-6 concentrations in patients with severe CHF gradually decreased in proportion to the severity of CHF during hospitalisation. Interestingly, urinary 15-F₂-t-isoprostane concentrations significantly correlated with plasma BNP concentrations and serum IL-6 concentrations, but not with serum thrombomodulin concentrations.

Conclusions: Urinary 15-F₂-t-isoprostane concentrations increased in proportion to the severity of CHF in patients. This may be caused by increased 15-F₂-t-isoprostane production. These findings suggest that urinary 15-F₂-t-isoprostane may be a marker of morbidity as well as oxidative stress in patients with CHF.

Congestive heart failure (CHF) leading to high mortality is a major health problem. Although many factors including mechanical stress, inflammation, and myocardial ischaemia are involved in the pathophysiology of heart failure, the precise mechanisms of heart failure have not been fully defined. An increasing body of evidence suggests that oxidative stress contributes to many cardiovascular diseases, including atherosclerosis, ischaemia/reperfusion injury, and hypertension. In addition, previous studies have suggested that oxidative stress mediated by reactive oxygen species has a role in the pathogenesis of heart failure.

F₂-t-isoprostanes are prostaglandin isomers synthesised in vivo, independently of the activity of cyclo-oxygenase, through the free radical catalysed peroxidation of arachidonic acid in biological membranes. Increased cellular production of F₂-isoprostanes has been described, and there is evidence of increased synthesis of 15-F₂-t-isoprostane during cell mediated and copper mediated lipoprotein oxidation. 15-F₂-t-isoprostane was shown to be a specific, chemically stable, quantitative marker of oxidative stress in vivo. Increased urinary excretion or plasma concentrations of 15-F₂-t-isoprostane have been observed in many conditions including smoking, diabetes, and cardiovascular diseases.

Both brain natriuretic peptide (BNP) and interleukin 6 (IL-6) are markers of morbidity and prognostic indicators in a variety of patients with left sided heart failure. In the present study, we measured the urinary concentrations of 15-F₂-t-isoprostane, as well as plasma concentrations of BNP and serum concentrations of IL-6 and thrombomodulin, in patients with CHF to investigate whether urinary 15-F₂-t-isoprostane, a biomarker of lipid peroxidation, is also a marker of morbidity in patients with CHF. We also investigated the correlation of 15-F₂-t-isoprostane with BNP and IL-6, other established biomarkers of CHF.

METHODS

Study patients

We studied 15 outpatients with mild CHF in New York Heart Association (NYHA) functional class I or II (eight men and seven women, median age 62 years, range 47–73 years) and 15 patients with severe CHF in NYHA functional class III or IV (seven men and eight women, median age 65 years, range 46–82 years) admitted to our hospital for acute exacerbation of CHF (table 1). The diagnosis of heart failure was confirmed in all patients by clinical findings and non-invasive assessment of cardiac function. Left ventricular ejection fraction was determined by echocardiographic evaluation. Patients with coronary artery disease were excluded by angiographic findings or clinical history. Patients with renal failure, infection, chronic inflammatory disease, and malignancy were excluded from this study. The control group consisted of 15 healthy volunteers (seven men and eight women, median age 63 years, range 45–74 years) without cardiovascular disease. This study was approved by our institutional human investigations committee, and written informed consent was obtained from all patients and volunteers before participation.

Blood and urine collection

Samples from patients with severe CHF with acute exacerbation of CHF were collected at admission (on day 1), and 4, 7,
and 14 days after admission. Blood samples were collected after a 12 hour fast. All the subjects were supine and a 21 gauge needle was inserted into a large antecubital vein. A 5 ml sample of whole blood for plasma separation was drawn into a plastic tube containing 7.5 mg Na₂-ethylenediaminetetra-acetic acid. Plasma and serum were separated by prompt centrifugation of the blood samples at 1800 g at 4°C for 20 minutes. Each patient or volunteer collected urine overnight, immediately before blood sampling. The samples were immediately frozen and stored at −80°C.

**Measurement of 15-F₂t-isoprostane**

The method for measurement of 15-F₂t-isoprostane has been described previously. Briefly, urine (1 ml) was acidified (pH 3) with HCl and diluted with the same volume of water (pH 3). They were extracted using C₁₈ (CE) cartridges (International Sorbent Technology, Mid-Glamorgan, UK). These cartridges were preconditioned with 2 ml methanol and 2 ml water (pH 3). The solvent programme included washes with 10 ml each of water (pH 3) and CH₃CN-water (15:85, vol/vol). The elution solvent was composed of 4 ml of hexane-ethyl acetate-propan-2-ol (30:65:5, vol/vol). Eluates were then applied to NH₂ cartridges (International Sorbent Technology) preconditioned with hexane (5 ml). The NH₂ cartridges were sequentially washed with 5 ml of hexane-ethyl acetate (30:70, vol/vol) and 5 ml acetoniitrile. 15-F₂t-isoprostane was eluted from the NH₂ cartridges with 5 ml of ethyl acetate-methanol-acetic acid (10:85:5, vol/vol). The dried samples were reconstituted in enzyme immunoassay (EIA) buffer (1 ml) supplied by Cayman Chemical Co (Ann Arbor, Michigan, USA). Urinary 15-F₂t-isoprostane concentrations were determined using an EIA kit (Cayman Chemical Co). The sensitivity of the 15-F₂t-isoprostane assay was 12.3 pg/ml. All urinary measurements were corrected for recovery and creatinine excretion.

**Measurements of BNP, IL-6, and thrombomodulin**

The concentrations of plasma BNP and serum IL-6 were determined by radioimmunoassay and enzyme linked immunosorbent assay (ELISA) (SRL Inc, Tokyo, Japan), respectively. The sensitivity of the assays for BNP and IL-6 was 2.0 pg/ml and 1.0 pg/ml, respectively. Serum concentrations of thrombomodulin were determined using the one step sandwich EIA kit (Fuji Chemical Industries Ltd, Takaoka, Japan). The sensitivity of the assays for thrombomodulin was 1.1 ng/ml.

**RESULTS**

**Urinary 15-F₂t-isoprostane concentrations**

Figure 1 shows the urinary 15-F₂t-isoprostane concentrations in patients with congestive heart failure (CHF). Scatter plots show the urinary 15-F₂t-isoprostane concentrations in control subjects (n=15), in patients with mild CHF in New York Heart Association (NYHA) functional class I or II (n=15), and in patients with severe CHF in NYHA functional class III or IV (n=15). The data on day 1 are urinary 15-F₂t-isop prostane concentrations in patients with severe CHF at admission. Bars show median value.

**Statistical analysis**

Data are expressed as median (range). The data were analysed by non-parametric Kruskal-Wallis methods to avoid assumptions about the distribution of the measured variables. Subsequent pairwise comparisons were made with the Mann-Whitney U test. The differences between baseline and post-treatment values were analysed with the Wilcoxon signed rank test. Moreover, the association of eicosanoid measurements with other biochemical parameters was assessed by the Spearman rank correlation test. A probability value of p < 0.05 was considered significant.

**Plasma BNP concentrations**

Plasma BNP concentrations in patients with mild CHF (45–8–150) pg/ml) were significantly higher than those in control subjects (p < 0.001). In addition, plasma BNP concentrations in patients with severe CHF (p < 0.001) were significantly increased compared with those in control subjects (p < 0.001) or in patients with mild CHF (p < 0.001).
Correlations between urinary 15-F_{2t}-isoprostane during hospitalisation. Severe CHF did not change in proportion to the severity of CHF. Serum thrombomodulin concentrations in patients with mild CHF (2.2 (1.0–5.0) pg/ml) were significantly higher than those in control subjects (1.8 (1.0–2.9) pg/ml, p < 0.05). Serum IL-6 concentrations in patients with severe CHF (7.2 (2.0–42.3) pg/ml) at admission were significantly increased compared with those in the control subjects (p < 0.01) or in patients with mild CHF (p < 0.02). Among patients with higher IL-6 concentrations, the IL-6 concentrations in those with severe CHF decreased gradually in proportion to the severity of CHF during hospitalisation.

Serum IL-6 concentrations

Serum IL-6 concentrations in patients with mild CHF (2.2 (1.0–5.0) pg/ml) were significantly higher than those in control subjects (1.8 (1.0–2.9) pg/ml, p < 0.05). Serum IL-6 concentrations in patients with severe CHF (7.2 (2.0–42.3) pg/ml) at admission were significantly increased compared with those in the control subjects (p < 0.01) or in patients with mild CHF (p < 0.02). Among patients with higher IL-6 concentrations, the IL-6 concentrations in those with severe CHF decreased gradually in proportion to the severity of CHF during hospitalisation.

Correlations between urinary 15-F_{2t}-isoprostane concentrations and other markers

Urinary 15-F_{2t}-isoprostane concentrations were significantly correlated with plasma BNP (r = 0.87, p < 0.0001) (fig 2) and serum IL-6 (r = 0.64, p < 0.0001) (fig 3) concentrations. However, serum thrombomodulin concentrations were not correlated with other markers (data not shown).

DISCUSSION

Recent studies have focused on 15-F_{2t}-isoprostane, an index of lipid peroxidation, to investigate the involvement of oxidative stress in cardiovascular disease. In the present study, increased urinary 15-F_{2t}-isoprostane concentrations in patients with CHF with acute exacerbation gradually decreased in proportion to the severity of CHF during hospitalisation. Urinary 15-F_{2t}-isoprostane concentrations were significantly correlated with the concentrations of plasma BNP and serum IL-6, well established markers of CHF. These findings suggest that urinary 15-F_{2t}-isoprostane may be a marker of morbidity, as well as of oxidative stress, in patients with CHF.

Recent studies have focused on 15-F_{2t}-isoprostane, an index of lipid peroxidation, to investigate the involvement of oxidative stress in heart failure. Quantification of 15-F_{2t}-isoprostane has been suggested to be a reliable measure of oxidative injury in vivo. Cracowski and colleagues investigated urinary 15-F_{2t}-isoprostane formation in 25 patients suffering from severe heart failure and showed that concentrations of 15-F_{2t}-isoprostane in patients in NYHA class IV were significantly higher than those in NYHA class II and III. Mallat and colleagues reported that pericardial concentrations of 15-F_{2t}-isoprostane increased with the functional severity of heart failure (NYHA class I to III). Mehrabi and colleagues showed that an accumulation of 15-F_{2t}-isoprostane was increased in both pulmonary and aortic valves of patients with idiopathic dilated cardiomyopathy. In the present study, the urinary concentrations of 15-F_{2t}-isoprostane in patients with CHF increased with the functional severity of heart failure and urinary 15-F_{2t}-isoprostane concentrations in patients with severe CHF gradually decreased in proportion to the severity of CHF during hospitalisation. In addition, concentrations of serum thrombomodulin were not different between control subjects and patients with CHF. These observations suggest that increased concentrations of urinary 15-F_{2t}-isoprostane may result from leakage of the peptides from injured endothelial cells but are likely to be related to increased biosynthesis. Results also suggest that urinary 15-F_{2t}-isoprostane may be a useful marker of oxidative stress in patients with CHF and may have potential for development as a non-invasive quantitative tool for monitoring the response to treatment in patients with CHF.

Increased concentrations of various neurohumoral factors have been found in patients with CHF. Previous studies reported that BNP and IL-6 are established markers of morbidity and mortality in CHF. In the present study, concentrations of urinary 15-F_{2t}-isoprostane correlated significantly with concentrations of BNP and IL-6. These findings support the premise that urinary 15-F_{2t}-isoprostane may be a marker of morbidity in patients with CHF. However, further studies should be performed to clarify whether 15-F_{2t}-isoprostane is a prognostic marker in patients with CHF.

Several methods for measurement of F_{2t}-isoprostanes including gas chromatography–mass spectrometry are used. Results obtained using EIA differ from those obtained using gas chromatography–mass spectrometry assays. Therefore, EIA data should be regarded as semiquantitative indices of F_{2t}-isoprostanes.

Limitations of the study

Since urinary 15-F_{2t}-isoprostane is increased in a number of conditions, it is still difficult and presumptive to assign a unique role of this substance to oxidative stress alone. In addition, the number of patients in this study was quite small.
Conclusion
Increased urinary 15-F$_2$t-isoprostane concentrations in patients with CHF with acute exacerbation gradually decreased in proportion to the severity of CHF during hospitalisation. Urinary 15-F$_2$t-isoprostane concentrations significantly correlated with the plasma BNP and serum IL-6 concentrations. This may be caused by increased 15-F$_2$t-isoprostane production. These findings suggest that urinary 15-F$_2$t-isoprostane may be a marker of morbidity, as well as of oxidative stress, in patients with CHF.

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