International Journal of Cardiology 175 (2014) 268–273

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Plasma 15-F_{2t}-isoprostane in idiopathic pulmonary arterial hypertension

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ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form 21 April 2014
Accepted 11 May 2014
Available online 17 May 2014

Keywords:
5-F_{2t}-IsoP
Idiopathic pulmonary arterial hypertension
Prognosis
Biomarkers

ABSTRACT

Background: 15-F_{2t}-isoprostane (15-F_{2t}-IsoP), a prostaglandin F_2-like compound, is widely recognized as a biomarker of chronic heart failure. This study investigated the potential role and prognostic significance of plasma 15-F_{2t}-IsoP in patients with idiopathic pulmonary arterial hypertension (IPAH).
Methods: Plasma 15-F_{2t}-IsoP concentrations were determined in 80 consecutive IPAH patients at the time of their first right heart catheterization, and monitored for 30 ± 12 months. The expression of 15-F_{2t}-IsoP protein in autopsy lung samples was determined by immunohistochemical staining.
Results: Plasma 15-F_{2t}-IsoP concentrations were significantly increased in patients with IPAH compared with healthy controls (91 pg/ml vs. 30 pg/ml, respectively; $P < 0.001$). Patients with baseline 15-F_{2t}-IsoP concentrations ≥ 97 pg/ml had a significantly lower survival rate than those with lower baseline concentrations ($P < 0.001$). During follow-up, 15-F_{2t}-IsoP concentrations in survivors decreased, whereas concentrations in non-surviving patients increased further ($P < 0.05$). Elevated concentrations of 15-F_{2t}-IsoP were correlated with a severity of WHO functional class, lower 6-minute walking distance and mixed venous oxygen saturation, higher mean right atrial pressure and brain natriuretic peptide. Multivariate analysis revealed that the plasma 15-F_{2t}-IsoP concentration was an independent factor associated with mortality. Histological studies showed that the expression of 15-F_{2t}-IsoP was up-regulated in remodeled pulmonary vessels.
Conclusions: An elevated plasma 15-F_{2t}-IsoP concentration and a further increase during follow-up may be a risk factor for higher mortality in patients with IPAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a life-threatening condition characterized by progressive pulmonary vascular remodeling, which leads to right ventricular (RV) failure and death [1]. Although vasoconstriction and vascular wall remodeling are reported as common phenomena, the pathogenesis and the stimulus interplay are not completely understood. Enhanced oxidative stress is now recognized to be a prominent factor in many acute and chronic diseases, including cancer, cardiovascular disease and lung disease [2,3]. The lungs of patients with severe PAH have been shown to undergo oxidative stress, and increased free radicals may contribute to the pathogenesis and/or development of PAH [4,5]. The increased free radicals may lead to pulmonary vascular wall injury and initiate the process of vascular proliferation and structural remodeling [6].

15-F_{2t}-isoprostane (15-F_{2t}-IsoP, also named 8-*iso*-prostaglandin $F_{2\alpha}$) is a chemically stable lipid peroxidation product of arachidonic acid that is independent of the action of cyclooxygenase, and has been shown to have biological activity. It mediates vasoconstriction of pulmonary arteries and resistance microvessels, and mitogenesis in vascular smooth muscle cells, which can be reliably measured in urine and plasma in humans [7]. Its overproduction may contribute to increased platelet activation leading to increased thromboxane A_2 (TXA_2) production [7]. In addition, 15-F_{2t}-IsoP stimulates proliferation of endothelial cells and synthesis of endothelin-1 (ET-1) in pulmonary arteries [8]. In patients with pulmonary hypertension, the urinary concentration of 15-F_{2t}-IsoP has been found to be elevated and correlated with survival [9]. A study by Robbins et al. showed that chronic treatment with epoprostenol improved the outcome of patients with primary

Abbreviations: BMI, body mass index; BNP, brain natriuretic peptide; CI, cardiac index; 95% CI, 95% confidence interval; IPAH, idiopathic pulmonary arterial hypertension; 15-F_{2t}-IsoP, 15-F_{2t}-isoprostane; 6MWD, 6-minute walk distance; PAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; NO, nitric oxide; PDE5, phosphodiesterase type 5; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; ROC, receiver operation characteristic; SvO_2, mixed venous oxygen saturation.

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http://dx.doi.org/10.1016/j.ijcard.2014.05.014
0167-5273/

pulmonary hypertension (PPH) and reduced urinary excretion of 15-F_{2t}-IsoP but not that of TXA_2 [10]. However, the potential role of plasma 15-F_{2t}-IsoP in patients with PAH is currently unknown.

The aim of the present study was to access whether: (1)15-F_{2t}-IsoP may provide prognostic information and long-term outcome in patients with idiopathic pulmonary arterial hypertension (IPAH); (2) 15-F_{2t}-IsoP concentrations correlate with hemodynamic parameters and baseline characteristics.

2. Methods

2.1. Study subjects

Eighty consecutively diagnosed adult patients with IPAH (≥18 to 60 years of age at diagnosis) were enrolled at one center in China between April 1, 2007 and July 30, 2010. IPAH was diagnosed according to standard criteria: a mean pulmonary artery pressure (mPAP) > 25 mmHg and pulmonary vascular resistance (PVR) > 3 Woods units at rest in the presence of a normal pulmonary capillary wedge pressure (PCWP < 15 mmHg) [11]. Patients with PAH associated with a definite cause such as connective tissue disease and congenital heart disease, and those with portopulmonary hypertension, chronic pulmonary thromboembolism, and pulmonary hypertension due to left heart disease and lung diseases and/or hypoxemia were excluded. Eighty control subjects were selected from a cohort of healthy volunteers. The median age of control subjects was 30 (19–57) years, and the ratio of men to women was 1:4. Other exclusion criteria for the study included potential confounding factors associated with increased 15-F_{2t}-IsoP production: cigarette smoking and excessive alcohol consumption [12], hypercholesterolemia [13], and diabetes mellitus [14]. The study subjects were prohibited from consuming alcoholic drinks 24 h before study [12]. We also excluded participants who had received cyclooxygenase (COX) inhibitors, including nonsteroidal anti-inflammatory medications, aspirin or COX-2 inhibitors within 14 days of blood sample collection [10].

All patients received PAH-specific drug treatment according to established guidelines [15,16]. The PAH-targeted treatments included the endothelin receptor antagonist (ERA) bosentan, the phosphodiesterase type 5 (PDE5) inhibitors sildenafil or vardenafil, and the inhaled prostacyclin analog iloprost, either as monotherapy or as part of combination therapy. Conventional therapy included digitalis, diuretics, supplemental oxygen and anticoagulants, as appropriate.

The study was conducted according to the principles of the Declaration of Helsinki, and was approved by the Shanghai Pulmonary Hospital Ethics Committee. Written informed consent was obtained from all participants.

2.2. Blood sampling and laboratory analysis

Blood samples were collected in ethylene diamine tetra-acetic acid (EDTA) tubes at the time of the first right heart catheterization using the central venous line, while patients were in a stable hemodynamic state and not receiving vasodilator drugs. The blood samples were immediately centrifuged at 4 °C. Plasma was isolated and stored in cryotubes at −80 °C until assayed. The 15-F_{2t}-IsoP concentration in plasma was measured by a specific enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). This assay has been validated by gas chromatography/mass spectrometry [17], and there is a high correlation (0.95) between known amounts of 15-F_{2t}-IsoP and the concentration measured by EIA.

2.3. Follow-up and endpoints

The primary study endpoint was cardiopulmonary death. The cut-off date for survival analysis was August 30, 2011. After baseline blood sampling, patients were monitored at outpatient clinic visits and/or hospitalizations for 30 ± 12 months. Each blood sampling was repeated twice at least. No patients received lung or heart–lung transplantation during the follow-up period. Patients lost to follow-up were considered as censored data at the time of their last visit.

2.4. Lung tissue sampling and immunohistochemical staining

Lung tissue samples were obtained from 7 patients with IPAH at autopsy (median age 29 [IQR 24 to 42] years; 5 females, 2 males) as well as from 3 brain-dead organ donors (median age 31 [IQR 25 to 40] years; 2 females, 1 male). Immunohistochemical staining was done with primary antibody against 15-F_{2t}-IsoP (Oxford Biomedical Research, MI) and secondary antibody according to the manufacturers' recommendations. Briefly, the lung was cut sagittally into slices, followed by fixation in 10% buffered formalin. Then the paraffin-embedded lung tissues were deparaffinized, rehydrated and treated with 0.3% hydrogen peroxide for 20 min to block endogenous peroxidase. Sections were incubated with 10% normal rabbit serum in phosphate buffer for 10 min and incubated with 15-F_{2t}-IsoP antibody (dilution 1: 100 of 6 mg/ml protein concentration) overnight at 4 °C. A biotinylated goat secondary antibody (ProteinTech Group, Chicago, USA; dilution 1: 400) was subsequently applied for 1 h at room temperature followed by incubation with horseradish peroxidase (HRP)-conjugated streptavidin. Diaminobenzidine was used as a substrate for HRP. The slides prepared were mounted for light microscopic examination (Leica DM 2500). Arteries of 15–200 μm were evaluated analyzed by Intel® Integrated Performance Primitives version 5.0 software (Santa Clara, CA, USA).

3. Statistical methods

Data was recorded as numbers, percentages, means with corresponding standard deviations, or medians with corresponding 25th and 75th percentiles [interquartile range (IQR)]. The two-sided Mann–Whitney *U*-test was used to compare baseline characteristics, hemodynamic parameters, and biomarker levels. The proportions were compared with the χ^2 test. For comparison of the prognostic values of 15-F_{2t}-IsoP, brain natriuretic peptide (BNP), and selected hemodynamic parameters, receiver operating characteristic curves (ROC) were generated and the areas under the curves (AUCs) were calculated. Spearman's ρ was investigated for correlations with a Bonferroni correction for each variable.

Survival analyses were performed using the Kaplan–Meier method and were compared by means of the log-rank test. Factors associated with survival were examined by univariable Cox regression analysis, such as demographic, medical history and hemodynamic variables measured at initial right heart catheterization. Continuous variables were assessed for linearity of their relationship with the outcome variable. If these variables were not found to be linearly related to the outcome, they were grouped into quartiles and modeled to avoid violating model assumptions. Univariate hazard ratios were estimated for each variable of interest in turn. Variables were all incorporated into a forward stepwise multivariable Cox proportional hazards model. A 2-tailed P value < 0.05 was used to indicate statistically significant differences. All statistical analyses were performed using the SPSS 14.0 statistical software package (SPSS Inc; Chicago, IL, USA).

4. Results

4.1. 15-F_{2t}-IsoP concentrations and characteristics of the study population

The study cohort consisted of 80 newly diagnosed patients with IPAH (median age 33 years, range 19–59 years; 84% females). In comparison with control subjects, patients with IPAH had significantly higher plasma 15-F_{2t}-IsoP concentration (30 pg/ml; 95% confidence interval [CI], 29 to 34 pg/ml vs. 91 pg/ml; 95% CI, 84 to 98 pg/ml, respectively; P < 0.001) (Fig. 1). The clinical and biochemical characteristics of the patients are summarized in Table 1.

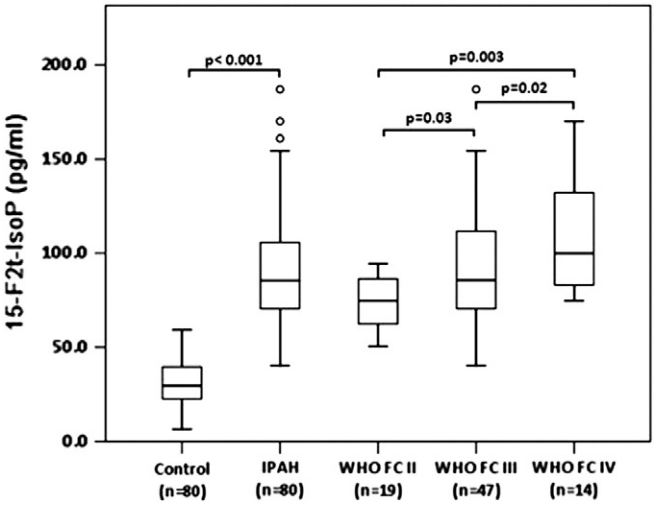

Fig. 1. Plasma concentrations of 15-F2t-IsoP in patients with IPAH and control subjects a) and b) association of baseline plasma 15-F2t-IsoP concentrations with WHO functional class in patients with IPAH. The line through the middle of the boxes represents the median.