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Serum levels of tumor necrosis factor-related apoptosis-inducing ligand correlate with the severity of pulmonary hypertension

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ABSTRACT

Pulmonary hypertension (PH) is a rapidly progressive disease that eventually leads to right heart failure and death. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptors (TRAIL-Rs) play an important role in the survival, migration, and proliferation of vascular smooth muscle cells. However, the association between serum TRAIL levels and PH is unknown. In this study, we assayed the serum soluble TRAIL (sTRAIL) levels in 78 patients with PH and 80 controls. The sTRAIL concentrations were elevated in the PH patients compared with the controls (138.76 ± 6.60 pg/mL vs. 80.14 ± 3.38 pg/mL, $p < 0.0001$). The presence of sTRAIL levels of >103 pg/mL could discriminate PH patients from healthy individuals, with a sensitivity of 75.6% and specificity of 81.2%. Moreover, elevated sTRAIL concentrations were associated with eventual pathological complications; this is consistent with the finding that sTRAIL levels decreased in patients who responded to treatment. In a hypoxia-induced PH mouse model, sTRAIL levels were significantly higher compared with those in normoxia mice, and clearly decreased when the mice were treated with treprostinil. The sTRAIL levels were positively correlated with right ventricular systolic pressure and the index of right ventricular hypertrophy. In conclusion, serum sTRAIL could be a biomarker for diagnosis and effective therapy for PH patients.

1. Introduction

Pulmonary hypertension (PH) is a malignant disease that is associated with progressive deterioration and poor prognosis [1]. Without treatment, the median survival of patients with idiopathic pulmonary arterial hypertension (IPAH) is ≤ 3 years from diagnosis [2]. Despite the improved therapeutic options available for PH in recent years, the prognosis of these patients remains poor. In fact, the current medications only slow down the progression of the disease in the majority of patients, who eventually develop right-side heart failure. A majority of these patients develop right-side heart failure because, at the time of diagnosis, most patients are in the middle-to-late stage of the disease, when it is generally not reversible. The best option to halt the progression of PH is early diagnosis. At present, echocardiography is recommended as an initial noninvasive approach for the screening and evaluation of PH [3]. Although echocardiography has definitely improved the prediction of PH, it is reserved for patients with severe PH. Thus, a simple and direct method for screening for PH at an early stage is urgently needed.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) family of cytokines, which play vital roles in regulating cell death and inflammation [4]. TRAIL exists as either a type II membrane protein or a soluble protein (sTRAIL) following the enzymatic cleavage of the transmembrane carboxyl-terminal domain [5]. Moreover, TRAIL interacts with four high-affinity membrane receptors. TRAIL-R1 and TRAIL-R2 transduce apoptotic signals after binding with TRAIL, whereas TRAIL-R3 and TRAIL-R4 lack the intracellular death

Abbreviations: 6MWD, 6-min walk distance; CAD, coronary artery disease; CHD, congenital heart disease; CTD, connective tissue disease; CTEPH, chronic thromboembolic pulmonary hypertension; hs-CRP, high-sensitivity C-reactive protein; IPAH, idiopathic pulmonary arterial hypertension; LHD, left-side heart disease; LVEF, left ventricular ejection fraction; PAH, pulmonary arterial hypertension; PASMC, pulmonary artery smooth muscle cell; PH, pulmonary hypertension; RV/(LV + S), right ventricle/(left ventricular + ventricular septal); RVH, right ventricular hypertrophy; RVSP, right ventricular systolic blood pressure; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

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domain and apoptosis-inducing capability. Thus, TRAIL-R3 and TRAIL-R4 have been proposed to function as decoy receptors, and protect normal cells, including endothelial cells, from apoptosis [6].

TRAIL has also been shown to play an important role in the modulation of immune response [7] and inflammation resolution [8]. Moreover, TRAIL is expressed in the medial smooth cell layer of the aorta and pulmonary artery, and mediates the survival, migration, and proliferation of vascular smooth muscle cells [9], thus facilitating the neointima formation response to vascular injury [10]. TRAIL particularly plays an important role in the development of plaque rupture that leads to myocardial infarction, which is one of the major causes of HF. Clinical studies reported that serum TRAIL levels were significantly lower in patients with acute coronary syndrome [11] and acute myocardial infarction (AMI) [12] than in healthy subjects. Another study reported an inverse association between serum TRAIL levels and the severity of coronary artery disease (CAD), with a significant deterioration in TRAIL levels in patients with severe three-vessel disease as compared to subjects without CAD [13]. Decreased serum TRAIL levels may have a negative prognostic significance in AMI patients [14], whereas lower concentrations of circulating TRAIL may be related to the clinical evolution of cardiovascular disease in older adults [15]. The levels of TRAIL have been found to be elevated in HF patients [16] and are inversely associated with events in advanced HF patients, and thus appear to have a protective role [17]. This suggests that TRAIL may be useful as a biomarker of CAD and HF. Recently, investigators have demonstrated that TRAIL is upregulated in pulmonary artery smooth muscle cells isolated from PH patients, and that TRAIL promotes the development of pulmonary artery hypertension (PAH) in different rodent models [18]. However, the serum sTRAIL levels in PH patients and its relationship with the severity of PH have not yet been determined.

In the present study, we aimed to assess the serum TRAIL concentrations in PH patients and hypoxia-induced PH mice and to investigate its possible association with various disease parameters and severity.

2. Methods

2.1. Study population

Study participants were prospectively enrolled from the Cardiology Department of Ruijin Hospital (between July 2009 and June 2012) at Shanghai Jiao Tong University School of Medicine. Patients with suspected or confirmed long-term or intercurrent inflammatory diseases that tended to be associated with a short-term phase response (i.e., patients with infections, malignancies, liver diseases, or renal diseases) were excluded. The diagnosis of PH was made on the basis of pulmonary artery systolic pressure (PASP) $\geq$35 mmHg (Pulmonary Hypertension Diagnosis and Treatment Guidelines as recommended by the European Society of Cardiology), as estimated by echocardiography and confirmed by right cardiac catheterization, and the presence of typical symptoms such as fatigue and dyspnea after exertion. The current study included 158 participants, stratified into 2 groups. The PH group consisted of 78 patients (mean age, 59.40 ± 1.98 years) and the control group included 80 participants without PH (mean age, 57.69 ± 1.19 years) who were matched with the PH group for age, gender, diabetes, and hypertension. As indicated by the report of the 4th World Symposium on PH in Dana Point, CA, in 2008, the PH group included patients with PAH (n = 28), chronic thromboembolic PH (CTEPH, n = 9), and PH with left heart disease (LHD-PH, n = 41). The PAH group contained IPAH (n = 13), congenital heart disease-related PAH (CHD-related PAH, n = 13), and connective tissue disease-related PAH (CTD-related PAH, n = 2). During hospitalization, all participants underwent thorough clinical and laboratory assessments with special attention to CAD, hypertension, and diabetes. Moreover, 151 individuals underwent coronary angiography (including all the controls) or right heart catheterization, and 7 PH patients were unable to tolerate the procedures. Body mass index (BMI) was calculated for all the participants. Diabetes was defined as repeated fasting plasma glucose levels $\geq$7.0 mmol/L or if the participant was prescribed oral hypoglycemic drugs or insulin. Hypertension was diagnosed as a repeated systolic blood pressure >140 mmHg, a repeated diastolic blood pressure >90 mmHg, or current use of antihypertensive medications. CAD was defined as >50% luminal diameter stenosis of a major epicardial coronary vessel. Right heart insufficiency was diagnosed, in accordance with previously proposed criteria [19], if jugular vein distension, hepatomegaly, and peripheral edema were present. All patients received appropriate pharmacologic therapy if there were any signs of illness. All individuals were also classified based on the World Health Organization (WHO) functional classification. The demographic, clinical, and biochemical profiles are presented in Table 1. Informed consent was obtained from all participants. The study was approved by the Ethic Review Board in Ruijin Hospital, at Shanghai Jiao Tong University.

2.2. Right-sided catheter procedure

Twenty-seven patients underwent a right-sided catheter examination and their hemodynamic data were obtained. A 7-F Swan-Ganz catheter (Nihon Kohden, Tokyo, Japan) was introduced from the femoral jugular vein. The hemodynamic

Table 1
Characteristics of the study population.

	PH(n = 78)	Control(n = 80)	P-Value
Age, years	59.40 ± 1.98	57.69 ± 1.19	0.6287
Gender, M/F	32/46	36/44	0.6362
BMI, kg/m^2	24.17 ± 0.40	25.50 ± 0.37	0.0123
hs-CRP, mg/dL	6.22 ± 1.17	3.12 ± 0.45	0.0121
Diabetes mellitus, n (%)	8 (10.2)	15 (18.8)	0.1858
Hypertension, n (%)	38 (48.7)	45 (56.2)	0.3528
Left-heart disease, n (%)	41 (52.6)	–	–
PH classification, n (%)		–	–
PAH group	28 (35.9)		–
IPAH	13 (16.7)		–
CHD-related PAH	13 (16.7)		–
CTD-related PAH	2 (2.6)		–
LHD-related PH	41 (52.6)		–
CTEPH	9 (11.5)		–
WHO		–	–
I	8 (10.3)		–
II	17 (21.8)		–
III	50 (64.1)		–
IV	3 (3.8)		–
6MWD, m	347.8 ± 24.36	–	–
PASP, mm Hg	57.92 ± 2.80	<30	–
LVEF, %	62.74 ± 1.03	66.46 ± 0.61	0.0037
Pulmonary hypertension-associated symptoms			
Right heart insufficiency, n (%)			
Jugular vein distention	4 (5.1)	0 (0)	0.0571
Hepatomegaly	0 (0)	0 (0)	–
Peripheral edema	34 (43.6)*	0	<0.0001
Other symptoms, n (%)			
Dyspnea on exertion	47 (60.3)*	14 (17.5)	<0.0001
Fatigue	35 (44.9)*	5 (6.2)	<0.0001
Chest distress	60 (76.9)*	67 (62.0)	0.2481
Chest pain	32 (41.0)	39 (48.8)	0.2709
Palpitations	20 (25.6)	25 (31.2)	0.7354

*P < 0.05 versus controls.
BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; PH, pulmonary hypertension; PAH, pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; CHD, congenital heart disease; CTD, connective tissue disease; LHD, left-heart disease; CTEPH, chronic thromboembolic pulmonary hypertension; WHO, World Health Organization; 6MWD, 6-min walking distance; PASP, pulmonary artery systolic pressure; LVEF, left ventricular ejection fraction.