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Changes in gene expression profiles in patients with pulmonary arterial hypertension associated with scleroderma treated with tadalafil☆

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ABSTRACT

Objective: Pulmonary arterial hypertension (PAH) is one of the most devastating complications in scleroderma (SSc) patients and has a poorer outcome than other PAH subgroups. Tadalafil (Adcirca®) is a phosphodiesterase-5 inhibitor (PDE5-I) approved by the FDA for treatment of PAH; however, its effectiveness specifically in SSc-PAH patients is unclear. We investigated whether there were differences in gene expression associated with 16 weeks of treatment with tadalafil and, if so, whether these changes differed with respect to treatment outcome.

Methods: We enrolled 10 SSc-PAH subjects who were naïve to PDE5-I treatment, profiled gene expression in whole blood prior to and following treatment with tadalafil, measured changes in genomic profiles before and after treatment with tadalafil, and correlated them with changes in clinical outcomes, such as cardiopulmonary hemodynamics, six-min walk distance (6MWD), Borg Dyspnea Index (BDI), NYHA/WHO functional class (FC), B-type natriuretic peptide (BNP), and cardiac magnetic resonance imaging (cMRI).

Results: Genes associated with IL-12 signaling and extracellular matrix maintenance were coordinately up- or down-regulated with treatment, respectively, across all subjects. Interestingly, we found that genes encoding voltage-gated potassium channels and genes related to innate immunity were coordinately up-regulated in subjects who improved with tadalafil treatment compared to those patients who did not. In contrast, up-regulation of Golgi-related gene sets was associated with clinical worsening during the treatment period.

Conclusion: The results of this pilot study suggest that outcomes of SSc-PAH patients treated with tadalafil are associated with specific changes in gene expression and biological pathways.

Introduction

Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by vasoconstriction and vascular remodeling of pre-capillary pulmonary arterioles, leading to restriction of blood flow, increases in pulmonary arterial pressure and pulmonary vascular resistance, and eventually right ventricular failure and death. Untreated, the median survival is 2.8 years; however, even with treatment, the prognosis remains poor (mortality rate of 10–15% yearly). The following three pathways have been associated with the pathobiology of PAH: the prostacyclin pathway, the endothelin pathway, and the nitric oxide (NO) pathway [1]. Currently approved therapies have been based on these three pathways—(1) prostacyclin analogues; (2) endothelin receptor antagonists (ERAs); and (3) phosphodiesterase-5 inhibitors (PDE5-I) or soluble guanylate cyclase (sGC) stimulators [2,3].

Patients with systemic sclerosis (SSc) and PAH (SSc-PAH) have a poorer response to treatment and a poorer prognosis than other major subgroups of PAH. Despite more favorable hemodynamics, these patients have a poorer functional capacity, greater elevations in PAH biomarkers and a greater mortality than patients with idiopathic PAH (IPAH). Since SSc-PAH patients represent the second largest group of PAH patients, more optimal management of these individuals is necessary, but clinical trials or directed studies of these patients are currently lacking. In fact, almost all data are derived from subgroup analyses of available clinical trials, since only one prospective study has been performed in SSc-PAH patients alone [4].

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To advance the treatment and improve outcomes of SSc-PAH patients, a better understanding and characterization of the underlying pathogenesis of SSc-PAH is vital. One potential way to achieve this would be to investigate differences in gene expression and systems biology in SSc-PAH patients before and after treatment with PAH-specific medications. This pilot study was undertaken to determine whether there are significant changes in gene expression during tadalafil treatment and, if so, whether these changes differ with respect to the clinical response to treatment.

Methods and materials

Study design

This proof-of-concept study (named PIONEER) was approved by the Institutional Review Board of Boston University Medical Campus (BUMC). PIONEER was a single center, open-label, prospective study to investigate changes in gene expression profiles in SSc-PAH subjects treated with tadalafil (Adcirca®) at the FDA-approved dose of 40 mg once daily. Hemodynamic parameters and multiple clinical parameters were assessed and blood samples were collected prior to initiation of tadalafil and again after 16 weeks of treatment (see below).

Study population

A total of 10 subjects with a diagnosis of SSc-PAH were recruited from the Pulmonary Hypertension Center at Boston Medical Center (BMC). Inclusion criteria included age 18–75 years, a diagnosis of SSc by American College of Rheumatology (ACR) criteria, confirmation of PAH by right heart catheterization (RHC) within 6 months of the screening visit, and no previous treatment with, nor contraindication to, PDE5-I therapy. All subjects received tadalafil (Adcirca®) as their first PAH-specific treatment during the study (PAH-specific monotherapy).

Primary and secondary outcomes

The primary outcome was the change in gene expression profiles before and after 16 weeks of treatment with tadalafil. Secondary outcomes included changes in (1) cardiopulmonary hemodynamics [mean right atrial pressure (mRAP), mean pulmonary artery pressure (mPAP), and pulmonary capillary wedge pressure (PCWP), cardiac output/cardiac index (CO/CI), pulmonary vascular resistance (PVR)], (2) six-minute walk distance (6MWD), (3) Borg Dyspnea Index (BDI); (4) NYHA/WHO functional class (FC), and (5) B-type natriuretic peptide (BNP) level. An exploratory outcome for this study was a change in cardiac magnetic resonance imaging (cMRI) parameters, such as right ventricular (RV) end-diastolic volume index (RVEDVI), and RV mass index and ejection fraction (RVEF%). For this study, we arbitrarily defined response to tadalafil, a priori, as $\geq$20% decrease in PVR and/or a $\geq$30 m improvement in 6MWD from baseline to end of treatment at week 16.

Study visits and data collection

The study consisted of a screening period of 28 days prior to the baseline visit (day 0) and study visits every 4 weeks thereafter until week 16. The 6MWD assessments and BDI were performed per standard protocol outlined by the American Thoracic Society (ATS). FC was assessed according to standard definition. Blood samples for gene expression profiling, BNP and creatinine (prior to cMRI) were collected according to standard protocols. RHC was performed per standard guidelines and protocols; all RHCs were performed by a single operator (HWF). Subjects were followed for 1 year after completion of the 16-week study period.

Sample collection and processing

Whole blood samples were collected prior to the initiation of tadalafil and at week 16 of treatment during right heart catheterization using PAXgene tubes and kept at room temperature for at least 4 h prior to storage at −20°C. Samples were then transferred to storage at −80°C prior to sample analysis. Total RNA was extracted using the QIAGEN RNeasy kit (QIAGEN, Valencia, CA). Sample integrity was verified using RNA 6000 Pico Assay RNA chips and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

Peripheral blood samples were used for RNA extraction because they are easily assessable, could be measured longitudinally [5], and have been used previously by us in studies of the pulmonary complications of scleroderma (CORT) [6,7].

Microarray analysis

Biotin labeling was performed using the Ambion WT Expression Kit (Life Technologies, Grand Island, NY) according to the manufacturer's protocol, followed by the GeneChip WT Terminal Labeling and Controls Kit (Affymetrix, Santa Clara, CA). Labeled, fragmented DNA was hybridized to the Affymetrix Human Gene 1.0 ST Array for 18 h in a GeneChip Hybridization oven 640 at 45°C with rotation (60 rpm). The hybridized samples were washed and stained using an Affymetrix 450 fluidics station. After staining, microarrays were immediately scanned using an Affymetrix GeneArray Scanner 3000 7G Plus. Raw Affymetrix CEL files were normalized to produce gene-level expression values using the implementation of the Robust Multiarray Average (RMA) [8,9] in the "affy" R package (version 1.36.1) [6] included within the Bioconductor software suite (version 2.12) [10] and an Entrez Gene-specific probeset mapping from Brainarray (version 14.0.0) (http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF). These data have been deposited in the Gene Expression Omnibus (GEO) as Series GSE75173. Differential gene expression was assessed by Student's paired *t*-test as performed on coefficients of linear models created with the "limma" R package (version 3.14.4). Linear mixed-effects modeling and the associated analysis of variance were carried out using the "nlme" R package (version 3.1-108). Correction for multiple hypothesis testing was performed using the Benjamini-Hochberg false discovery rate (FDR) [11]. All microarray analyses were performed using the R environment for statistical computing (version 2.15.1).

Gene set enrichment analysis (GSEA)

GSEA [12,13] was used to identify biological terms, pathways and processes that were coordinately up-regulated or down-regulated after 16 weeks of treatment with tadalafil or which were associated with clinical response to tadalafil treatment. The Entrez Gene identifiers of the genes on the array were ranked according to either (1) the paired *t* statistic computed between 0 and 16 weeks of treatment or (2) sign ($Beta_{int}$) * sqrt(LR_{int}), where LR_{int} is the likelihood ratio of the interaction term (response:timepoint) of a linear mixed-effects model that included response, timepoint, and response:timepoint as fixed effects and subject as a random effect, and $Beta_{int}$ is the coefficient of the interaction term in that model. This signed sqrt(LR) metric approximately follows a standard normal distribution. Each list was then used to perform a pre-ranked GSEA analysis (default parameters with random seed 1234) using the Entrez Gene versions of the Biocarta, KEGG, Reactome, and Gene Ontology