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Tests Performed to Confirm the Presence of Pulmonary Arterial Hypertension

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Abstract

Additional, adenosine monophosphate-activated protein kinase (AMPK) is a highly conserved serine/threonine protein kinase that plays an important role in vascular homeostasis and is involved in the pathogenesis of Pulmonary arterial hypertension. Adenosine monophosphate-activated protein kinase exerts a pro-apoptotic effect in vascular smooth muscle cells and an anti-apoptotic effect in endothelial cells.

Keywords: Ventrilator surface; Lung parenchyma; Bronchiolar narrowing; Respiratory failure; Inflammatory stenosis; Emphysema

Introduction

AMP is a direct sensor activated by adenosine monophosphate-activated protein kinase through binding to the gamma subunit; this occurrence triggers the phosphorylation of the catalytic alpha subunit and may hence further exacerbate the pathogenesis of Pulmonary arterial hypertension. Teng demonstrated that AMPK activity and expression were decreased in pulmonary artery endothelial cells. Metformin, an AMPK activator, increases the bioavailability of NO and restores angiogenesis in pulmonary artery endothelial cells. Adenosine monophosphate-activated protein kinase activation also significantly reduces RVSP and RVH and inhibits the pulmonary artery remoulding in the MCT-induced rat Pulmonary arterial hypertension model. All these results imply that adenosine monophosphate-activated protein kinase may play a protective role in PAH, and the decreased AMP levels in the Pulmonary arterial hypertension group may adversely affect the adenosine monophosphate-activated protein kinase and consequently aggravate the phenotype of the disease [1]. Some of the other metabolic abnormalities detected in our analysis have been reported as potential biomarkers for early Pulmonary arterial hypertension diagnosis in previous studies. Betaine is a methyl donor in the formation of methionine, which is vital for protein synthesis in pulmonary arterial smooth muscle cell proliferation. In our study, the betaine level was significantly higher in the PAH group than in the control group [2]. Increased betaine may lead to abnormal mitochondrial structure and function and result in energy metabolism disorders. Acetylcarnitine is an acetic acid ester of carnitine that facilitates the movement of acetyl CoA into the mitochondria during fatty acid oxidation. Brittan et al. found that the circulating fatty acid long-chain acylcarnitines are elevated in patients with PAH and are associated with fatty acid accumulation in the myocardium caused by reduced fatty acid oxidation. High acylcarnitine levels were detected in our analysis and are consistent with previous study results. In future studies, a group of biomarkers reflecting different pathways dys-regulated in pulmonary vascular disease, including the NO pathway, mitochondrial bioenergetics, and fatty acid oxidation, can provide a comprehensive insight into the pathogenesis of Pulmonary arterial hypertension. In the present study, we adopted a feasible, accurate, and robust targeted metabolomic profiling platform that can simultaneously extract and quantify 126 metabolites covering the core network of lipid, energy, amino acid, and nucleotide metabolism from the same micro amount of biological sample [3]. Our results simultaneously highlighted the metabolic pathways dysregulated in Pulmonary arterial hypertension and provided new insight into the involvement of the urea cycle in the pathogenesis of PAH. However, the sample size in this study was relatively small [4].

Discussion

Further study utilizing a larger sample size and plasma or lung tissue samples from human Pulmonary arterial hypertension patients are needed to validate the present findings. We used a targeted metabolomic profiling platform to show a disrupted urea cycle pathway with increased urea, N-acetylornithine, and ornithine levels, 4-hydroxy-proline and decreased AMP metabolite levels in the plasma of a MCT-induced Pulmonary arterial hypertension model. Our results enabled the further understanding of the role of a disrupted urea cycle in the pathogenesis of Pulmonary arterial hypertension and also found five urea cycle related biomarkers and other two candidate biomarkers to facilitate early diagnosis of Pulmonary arterial hypertension in metabolomic profile [5]. Pulmonary arterial hypertension is a rare and devastating disease characterized by progressive pulmonary vascular remolding, which ultimately leads to right ventricle failure and death. Major advances have been achieved in the understanding of pathobiology and treatment of Pulmonary arterial hypertension; however, the disease remains to be an incurable condition associated with substantial morbidity and mortality. The 5- and 7-year survival rates for patients with Pulmonary arterial hypertension are 57 and 49%, respectively [6]. Pulmonary arterial hypertension is increasingly being recognized as a systemic disorder associated with substantial metabolic dysfunction. Recent studies have demonstrated the relationship of the metabolic syndrome with Pulmonary arterial hypertension and highlighted the features of insulin resistance, adiponectin deficiency, dyslipidemia, fatty acid oxidation, and the tricarboxylic acid cycle in the development of pulmonary vascular disease. The complex pathobiology of Pulmonary arterial hypertension involves various metabolic pathways related to inflammation, oxidative stress, plaque composition, and lipid metabolism, ultimately leads to endothelial damage, increased pulmonary vascular resistance, and right heart

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failure. Improved understanding of the specific metabolic pathobiology of Pulmonary arterial hypertension is critical in exploring the pathogenesis of Pulmonary arterial hypertension and uncovering the novel therapeutic targets for this devastating disease. Metabolomics targets the extensive characterization and quantitation of small molecular metabolites from exogenous and endogenous sources and has emerged as a novel avenue for advancing precision medicine. Recent evidence has shown the abnormalities of small molecular metabolites in patients with Pulmonary arterial hypertension and has led to the emergence of numerous metabolomic studies on Pulmonary arterial hypertension [7]. Yidan reported disrupted glycolysis, up regulated tricarboxylic acid cycle, and increased fatty acid metabolite production with altered oxidation pathways in patients with severe Pulmonary arterial hypertension. Lewis et al. also reported the plasma metabolite biomarkers of Pulmonary arterial hypertension, indoleamine 2,3 ioxy-genase, and the association with RV pulmonary vasculature dysfunction. These studies suggested that metabolomics is a powerful tool for the examining the pathology, prevention, diagnosis, and therapy of Pulmonary arterial hypertension. In the present work, we used integrated targeted metabolomics to detect lipids and polar metabolites from only 100 µl of a biosample. A monocrotaline-induced rat model was used to identify the metabolic profiles of PAH with the integrated targeted metabolomic strategy [8]. The potential biomarkers found in PAH rat plasma may facilitate earlier Pulmonary arterial hypertension detection and a thorough understanding of the Pulmonary arterial hypertension mechanism. The blood samples were collected from the euthanized rats by using EDTA as anticoagulant to obtain plasma by centrifugation and then maintained at −80 ℃. The plasma was thawed at 4℃ and re-homogenized through brief vortex mixing. Then, 100 µl of plasma was transferred into a 1.5 ml Eppendorf tube and combined with 20 µl of sphingolipid internal standards and 20 µl of polar metabolite internal standards. After the mixture was vortexed for 10 s, 400 µl of acetonitrile was added to the tube. The sample was vortexed for 5 min, allowed to stand for another 15 min, and then centrifuged at 13000 rpm for 10 min. Protein precipitation was removed, and the supernatant was transferred into another glass tube and evaporated under a nitrogen stream. The organic residue was then redissolved with 100 µl of acetonitrile/methanol for polar metabolite analysis followed by ultrasonication. The aliquots were consequently vortexed for 10 min and transferred into a 1.5 ml Eppendorf tube. After centrifugation for 10 min, the supernatant was transferred to a UPLC–MS/MS auto sampler vial. Rigorous method validation of polar metabolites was established before metabolomics analysis to ensure the accurate and reliable of the analytical method, such as linearity and lower limit of quantification, precision and accuracy, stability, replaceable matrix and carryover [9]. To ensure the accuracy of the analysis, pool sample and pool standard solution were used as quality control in the whole analytical batches. The metabolites with compound relative standard deviation less than 30% between pool sample and pool standard sample were further analysis. Plasma samples (100 µl) were analyzed using the targeted metabolomic profiling platform. In total, 126 polar metabolites were quantified from the MCT-treated and control rat plasma. Unpaired t test and Mann Whitney test were performed to determine the metabolite variations between the two groups. Thirteen plasma metabolites related to PAH were tentatively identified through the targeted metabolomic pattern analysis to be significantly altered between the MCT-treated and control groups ($p < 0.05$). The detailed information of the distinguished metabolites was summarized. The metabolites were ranked by significance on the basis of the p values. Our results demonstrated that many metabolites involved in different metabolic pathways were altered in rat plasma after MCT treatment. Thirteen differential metabolites were divided into five categories: organic acids (n = 7), nucleotides (n = 2), lipid (n = 1), organic compounds (n = 1) and others (n = 2), which comprised the materials that cannot clearly be classified into any of the other four categories. The organic acids accounted for the largest proportion of the metabolites. Among the 13 differential metabolites, only adenosine monophosphate (AMP) was significantly decreased in the PAH group than in the control group. The AMP concentration in the PAH group was only 0.03-fold of the control group. The rest of the differential metabolites (92.3%) in the PAH group were all elevated relative to those in the control group. In particular, phenyl-acetyl-glycine increased by 3.23 folds that in the control group. PLS-DA, a supervised method based on a partial least squares algorithm, shows a high sensitivity for biomarker detection [10]. In this study, PLS-DA was conducted to investigate the metabolite patterns of PAH model and control group. The score plot obtained though PLS-DA revealed that the PAH model aggregated to the right side, whereas the control group clustered to the left.There was a distinguished classification between the clustering of the PAH model and control groups in the plasma with R2Y and Q2 greater than 0.5, which suggested that the PLS-DA models showed good stability and predictability. Those results indicated that the differentially expressed metabolites can be used to separate the plasma samples into two distinct groups. Differential metabolites and their concentrations were imported to Meta-bo Analyst to exploit the most disturbed metabolic pathways via over representation analysis

Conclusion

In centri-lobular emphysema ventilatory disturbances were caused not only by the centriacinar dilated spaces delaying gas diffusion, but also by scattered bronchiolar stenosis situated at the termination of the conducting air passages. The stenosis seemed the more important cause. It was shown statistically that chronic arterial pulmonary hypertension and right ventricular hypertrophy were mainly the result of functional disturbances, especially hypoxia and abnormalities of VA/Q produced by the two structural changes situated at the end of the small airways.

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Conflict of Interest

None

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