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Effect of inhaled N-acetylcysteine monotherapy on lung function and redox balance in idiopathic pulmonary fibrosis

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ABSTRACT

Background: An oxidant–antioxidant imbalance is considered to be involved in the pathogenesis of idiopathic pulmonary fibrosis (IPF). Therefore, administration of antioxidants, such as N-acetylcysteine (NAC), may represent a potential treatment option for IPF patients.
Methods: The aim of this study was to evaluate the effect of inhaled NAC monotherapy on lung function and redox balance in patients with IPF. A retrospective observational study was done, involving 22 patients with untreated early IPF (19 men; mean [±S.D.] age, 71.8 [±6.3] y). At baseline and at 6 and 12 months after initiating inhaled NAC monotherapy, we assessed forced vital capacity (FVC) and measured the levels of total glutathione, oxidized glutathione (GSSG), and the ratio of reduced to oxidized glutathione in whole blood (hereafter referred to as the ratio), and of 8-hydroxy-2′-deoxyguanosine in urine. To evaluate response to treatment, we defined disease progression as a decrease in FVC of $\geq$5% from baseline and stable disease as a decrease in FVC of $<$5%, over a period of 6 months.
Results: Change in FVC in the stable group at 6 and 12 months were 95±170 mL and −70±120 mL, while those in the progressive group at 6 and 12 months were −210±80 mL, −320±350 mL, respectively. The serial mean change in GSSG from baseline decreased as the ratio of reduced to oxidized glutathione increased in patients with stable disease, while it increased as this ratio decreased in patients with progressive disease. Receiver operating characteristic curve analysis revealed that a baseline GSSG level of $\geq$1.579 μM was optimal for identifying treatment responders.
Conclusion: Inhaled NAC monotherapy was associated with improved redox imbalance in patients with early IPF.

Abbreviations: 6MWD, 6-min walk distance; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; BAL, Bronchoalveolar lavage; FVC, vital capacity; GSH, Reduced levels of glutathione; GSSG, Oxidized glutathione; IPF, Idiopathic pulmonary fibrosis; NAC, N-acetylcysteine; ROS, Reactive oxygen species; tGSH, Total glutathione

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1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disease, and no optimal treatment has yet been established that can improve the prognosis of IPF [1]. One hypothesis regarding the pathogenesis of IPF is that an oxidant–antioxidant imbalance causes repeated epithelial cell injury, which is followed by pathological fibrotic repair. Reactive oxygen species (ROS) play a pivotal role in this process [2–4]. Moreover, patients with IPF have been found to have reduced levels of glutathione (GSH), a major antioxidant in lung, in epithelial lining fluid, bronchoalveolar lavage (BAL) fluid, and BAL cells [5–8].

N-acetylcysteine (NAC) is a tripeptide (γ-glutamylcysteinyl-glycine) that functions both as a precursor of glutathione synthesis and as a powerful direct antioxidant scavenger of ROS [9,10]. Furthermore, inhibition of transforming growth factor-β signaling, or its direct modification by NAC, may be beneficial in IPF, the pathogenesis of which is associated with excessive concentrations of this growth factor [11]. Administration of oral NAC increased intracellular and extracellular glutathione levels, such as BAL fluid, in patients with pulmonary fibrosis [6,8,12]. In 2005, the IFIGENIA study showed that IPF patients who received high-dose oral NAC therapy, combined with prednisone and azathioprine, had less reduction in vital capacity and carbon monoxide diffusing capacity (DLco) at 12 months than those receiving standard therapy involving prednisone and azathioprine [13]. However, in Japan, NAC is only available in liquid form for aerosol administration and has been used as a mucolytic agent, with few adverse effects. In 2012, a multicenter, prospective, randomized controlled trial showed that inhaled NAC monotherapy had beneficial effects for some patients with early IPF [14]. Recently, the PANTHER trial in the US could not demonstrate the effectiveness of orally administered NAC monotherapy [15]. Thus, the effectiveness of NAC inhalation is still disputed, and further study assessing different delivery methods of NAC is required. At present, it is unclear whether a subset of patients with IPF, with a higher burden of oxidative stress, may benefit from NAC monotherapy [16].

To date, no studies have investigated whether inhaled NAC monotherapy improved the systemic oxidant–antioxidant imbalance in patients with IPF. Thus, in this study, we assessed the correlation between the efficacy of inhaled NAC monotherapy on lung function and oxidant–antioxidant status of patients with IPF. Because of the possible increased risk of acute exacerbation of IPF after diagnostic BAL procedures [17], we sampled whole blood and urine to measure the status of redox balance in IPF patients.

2. Patients and methods

2.1. Study subjects

Between July 2007 and September 2010, we performed a retrospective observational study on 22 patients with untreated early IPF (19 men; mean [±S.D.] age, 71.8 [±6.3] y). In Japan, classification of the disease severity of IPF (stages I–IV) has been used to guide decisions on subsidization of medical care. These stages are defined as follows: stage I ($PaO_2 \geq 80$ Torr at rest), stage II (PaO_2 70–80 Torr at rest), stage III (PaO2 60–70 Torr at rest), and stage IV ($PaO_2 < 60$ Torr at rest). If patients with stage II or III experience desaturation during the 6-min walk test (6MWT), they are classified as stage III or IV, respectively [18].

Among 26 consecutive IPF patients with Japanese Respiratory Society stage I or II disease, 22 consenting patients were treated with inhaled NAC monotherapy. IPF was diagnosed according to the American Thoracic Society/European Respiratory Society Consensus Statement and the guidelines of the Fourth Edition of the Japanese Clinical Diagnostic Criteria for Idiopathic Interstitial Pneumonia [19,20]. The exclusion criteria were (i) improvement in symptoms during the preceding 3 months, (ii) use of prednisone, immunosuppressive therapy, or pirfenidone, and (iii) clinical evidence of collagen vascular disease or idiopathic interstitial pneumonia other than IPF.

2.2. Study protocol

Before treatment, all patients underwent pulmonary function tests, a 6-min walk distance (6MWD) test, analysis of serum markers of pneumocyte injury (Krebs von den Lungen-6 [KL-6] and surfactant protein D), and analysis of partial arterial oxygen concentration at rest. In addition, samples of peripheral whole blood and urine from all patients were examined to determine baseline levels of glutathione and 8-hydroxy-2′-deoxyguanosine (8-OHdG). All patients underwent treatment with inhaled NAC alone for 12 months via an ultrasonic nebulizer (NE-U07, Omron, Kyoto, Japan) during the study period. Ultrasonic nebulizers produce aerosol particles with a diameter of 1–8 μm. Patients inhaled 352.4 mg of NAC dissolved in saline, in a total volume of 6 mL, twice a day, based on the method used in the study of inhaled NAC monotherapy that had previously demonstrated beneficial effects in some patients with early stage IPF [14].

To evaluate the clinical response to inhaled NAC monotherapy, forced vital capacity (FVC) was measured in all patients at baseline, and at 6 and 12 months after treatment. Recently, Zappala et al. [21] reported that a marginal decline in FVC of 5–10% at 6 months was associated with poor prognosis in patients with IPF. We defined disease progression as a decrease in FVC of ≥5% from baseline to 6 months after the treatment, and stable disease as a decrease in FVC of <5%. Thus, patients were divided into two groups according

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