Correlation between serum H_2S and pulmonary function in children with bronchial asthma

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Abstract. Endogenous hydrogen sulfide (H_2S) has generated recent research interest because of its potential function as an inflammatory mediator. Despite its apparent functions in vascular smooth muscle, an important player in airway remodeling in asthma, little research has been done to assess the role of H_2S in the pathogenesis of asthma. To determine whether serum H_2S concentration is correlated with pulmonary function in children with asthma, we measured serum H_2S concentration and pulmonary function indices (FVC, FEV_1, PEF, FEF_{25-75}, MEF_{50} and MEF_{25}) in 64 children with asthma and 60 healthy children. Pearson's correlation was used to determine the relationship between serum H_2S concentration and lung function parameters. Compared to healthy children, both serum H_2S concentration and all lung function parameters were significantly decreased in children with asthma ($P<0.05$). Furthermore, serum H_2S concentration was positively correlated with lung function indices ($P<0.05$). Thus, decreasing levels of H_2S in the serum may be used to indicate decreasing lung function. Further investigation into the causality behind these findings is required.

Introduction

Recent studies have shown that endogenous hydrogen sulfide (H_2S) is produced in the human body. Together with nitric oxide (NO) and carbon monoxide (CO), H_2S is a gaseous signaling molecule with regulatory functions (1). Indeed, H_2S relaxes vascular smooth muscle, inhibits vascular smooth muscle cell proliferation and reduces oxidative stress to play an important role in the structure and function of pulmonary circulation (2,3). Notably, endogenous H_2S may be involved in the pathogenesis of airflow obstruction in chronic obstructive pulmonary disease (COPD) (4). In addition, epidemiological studies have shown that exogenous H_2S exposure increases the incidence of asthma (5). However, whether endogenous H_2S is involved in the pathogenesis of asthma and the precise effects of H_2S on pulmonary function remain unclear. While a previous study indicated that serum H_2S levels are lower in patients with asthma (6), serum H_2S has yet to be correlated with pulmonary function in these individuals. In the present study, we analyzed H_2S concentration in the serum in comparison to lung function parameters in 64 children with bronchial asthma and 60 healthy children to determine the effects of H_2S on pulmonary function in children with bronchial asthma.

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Materials and methods

Study population. Between June 2009 and June 2011, 64 children admitted to the Affiliated Nanjing Children's Hospital, Nanjing Medical University (China), with acute bronchial asthma were recruited to the study. The study population included 36 males and 28 females between 6 and 12 years of age (mean 9.03±1.84). Acute disease lasted between 6 and 25 days. Eighteen individuals (28.1%) experienced fever, with body temperatures between 37.5 and 38.4˚C. Asthma diagnosis followed the WHO criteria (7): all children had cough, asthma, obvious wheeze, rhonchus and moist rale in the lung, and other clinical manifestations; X-ray examination also showed increased lung markings or lung hyperinflation and infection. None of the children used asthma-related drugs prior to diagnosis. Children with combined respiratory and heart failure or other complications, or with congenital heart disease, tuberculosis infection and foreign body in the bronchus, were excluded. Sixty healthy children who had received physical examinations in our hospital during the same period were selected as controls. Control population included 31 males and 29 females between 6 and 12 years of age (mean 9.22±1.80). Mean age and gender distribution between the two groups of children were not statistically different. The study was approved by the Nanjing Health Bureau.

Detection of H_2S concentration in the serum. Venous blood (3 ml) was collected from children in the morning following a minimum 10-h fast. Samples were centrifuged

Table I. Mean measures of H_2S, FVC, FEV_1 and PEF in children with or without asthma ($\bar{\chi} \pm s$).

Study population	n	H_2S (μM)	FVC (%)	FEV_1 (%)	PEF (%)
Healthy control	60	52.60±5.56	104.51±8.20	99.02±7.81	100.59±7.66
Bronchial asthma	64	44.17±10.95	78.79±11.98	69.79±11.41	60.48±12.63
t		5.353	13.865	16.537	21.201
p-value		0.001	0.001	0.005	0.001

H_2S, hydrogen sulfide; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; PEF, peak expiratory flow.

Table II. Mean measures of FEF_{25-75}, MEF_{50} and MEF_{25} in children with or without asthma ($\bar{\chi} \pm s$).

Study group	n	FEF_{25-75} (%)	MEF_{50} (%)	MEF_{25} (%)
Healthy control	60	92.30±7.08	95.93±8.24	86.39±7.56
Bronchial asthma	64	54.78±12.19	57.84±12.22	50.67±11.70
t		20.779	20.212	20.049
p-value		0.001	0.002	0.001

FEF_{25-75}, forced midexpiratory flow rate; MEF_{50}, midexpiratory flow rate at 50% vital capacity; MEF_{25}, midexpiratory flow rate at 75% vital capacity.

at 3,000 rpm for 10 min to separate sera and were stored at -70˚C until testing for H_2S. H_2S was measured as described by Geng *et al* (8). A sensitive sulfur electrode [PXS-270 ion meter (Leici Company, Shanghai, China)] was used to determine H_2S content in the plasma. Briefly, standard sulfion and antioxidant solutions were prepared. The electrode was activated for at least 2 h in deionized water prior to use. The ion meter was adjusted to mV and rake ratio to 100. Sensitive sulfur electrode and reference electrode were immersed together in the sample until the reading stabilized. Standard curves were determined with standard sulfion solution before each test.

Detection of pulmonary function. MINAT (Japan) AS-407 pulmonary function instrument was used by specialized technicians to determine pulmonary function in all participants. Main indicators included forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), peak expiratory flow (PEF), forced midexpiratory flow rate (FEF_{25-75}), midexpiratory flow rate at 50% vital capacity (MEF_{50}) and midexpiratory flow rate at 75% vital capacity (MEF_{25}). All indicators are expressed as the percentage of the normal predicted value (based on the actual age, height and weight of children at sample collection).

Statistical analysis. SPSS13.0 statistical software was used for analyses. Data are expressed as the means ± standard deviation ($\bar{\chi} \pm SD$). Independent samples t-test was used to compare H_2S concentrations in the serum as well as differences in pulmonary function parameters between groups. Pearson's correlation was used to detect and analyze the correlation between H_2S concentration and each pulmonary function parameter in children with bronchial asthma. Analyses were performed with two-sided tests at α level 0.05. $p<0.05$ denoted statistically significant differences.

Results

H_2S serum concentration and pulmonary function differ in children with asthma. In children with bronchial asthma, H_2S concentration in the serum and indicators of pulmonary function (FVC, FEV_1, PEF, FEF_{25-75}, MEF_{50} and MEF_{25}) were all decreased in comparison to the healthy children. Each of these decreases were statistically significant between groups ($p<0.05$; Tables I and II).

Correlation between H_2S concentration in the serum and lung function in bronchial asthma. To establish a connection between the decreases in endogenous H_2S and the altered lung function in asthma patients, we assessed these results with Pearson's correlation. The H_2S concentration in the serum of children with bronchial asthma was positively correlated with each of the lung function parameters (FVC, FEV_1, PEF, FEF_{25-75}, MEF_{50} and MEF_{25}) ($p<0.05$; Table III); i.e., for increasing H_2S concentrations, lung function (FVC, FEV_1, PEF, FEF_{25-75}, MEF_{50} and MEF_{25}) also significantly increased (Fig. 1).

Discussion

H_2S exists in the human blood in two forms: gas and an ion, accounting for 18.5 and 81.5% of H_2S, respectively, constituting a dynamic balance (9). Metabolism of H_2S in the body is not fully understood. Two main metabolic pathways have been proposed (10): i) H_2S is rapidly oxidized into trisulfide in mitochondria and is further converted to sulfite and sulfate, as occurs in the colon; ii) H_2S is methylated in the cytoplasm by sulfo-S-methyltransferase and combined with metahemoglobin to form sulfhemoglobin. Despite such uncertainties, the functions of H_2S as a signaling molecule within the body

Table III. Correlation between H_2S concentration and pulmonary function parameters in children with asthma.

Statistics	FVC	FEV_1	PEF	FEF_{25-75}	MEF_{50}	MEF_{25}
r	0.550	0.554	0.555	0.543	0.540	0.567
p-value	0.001	0.001	0.001	0.001	0.001	0.001

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; PEF, peak expiratory flow; FEF_{25-75}, forced midexpiratory flow rate; MEF_{50}, midexpiratory flow rate at 50% vital capacity; MEF_{25}, midexpiratory flow rate at 75% vital capacity.

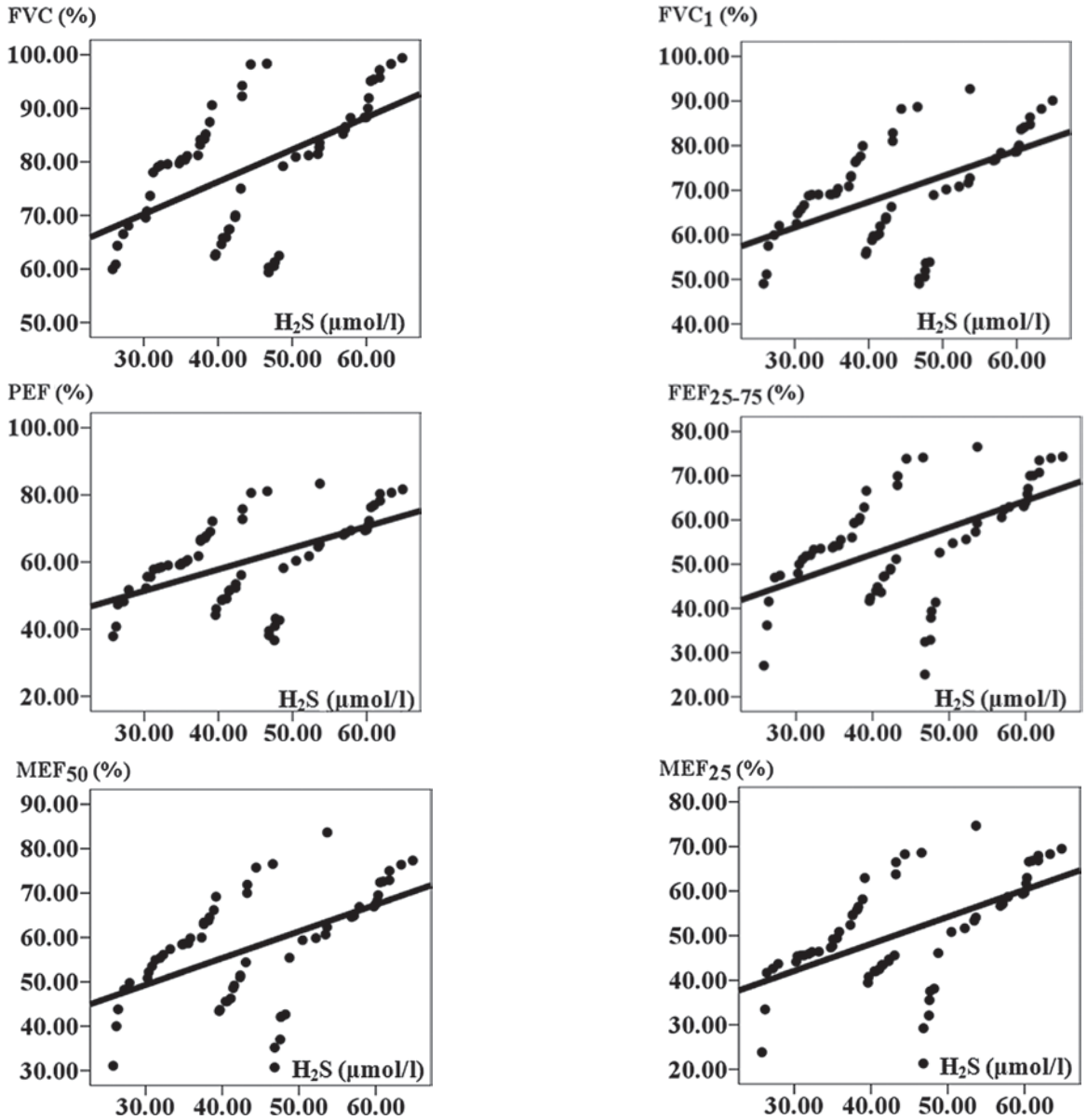

Figure 1. Relationship between serum H_2S concentration and pulmonary function parameters in children with asthma. FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; PEF, peak expiratory flow; FEF_{25-75}, forced midexpiratory flow rate; MEF_{50}, midexpiratory flow rate at 50% vital capacity; MEF_{25}, midexpiratory flow rate at 75% vital capacity.

are becoming increasingly evident. For example, H_2S relaxes vascular smooth muscle, which, as reported by Zhao *et al* (11), occurs via direct regulation on vascular smooth muscle tone. H_2S also inhibits proliferation of vascular smooth muscle cells (12,13). Additionally, conflicting evidence suggests both anti- and pro-inflammatory properties of this compound (14,15), making it of increasing interest for its potential involvement in promoting or preventing disease.

One disease in which H_2S may play a preventative role is bronchial asthma. A chronic inflammatory disease of the airway, bronchial asthma involves changes in various cell types, eosinophils, mastocytes, T lymphocytes, neutrophils

and airway epithelial cells, and cellular components (15). Of note, vascular smooth muscle changes are involved in the airway remodeling noted in asthma and may suggest a role of H_2S in this disease (16,17). In the past 20 years, the incidence of asthma has significantly increased and it has become one of the most common chronic diseases worldwide (18). The pathogenesis of asthma is very complex and has not yet been fully elucidated. Uncovering a role of H_2S in asthma pathogenesis may lead to new therapeutic or preventative approaches. Here, we confirmed that H_2S concentration in the serum is lower in children with bronchial asthma than in healthy children, which indicates that endogenous H_2S may play an anti-inflammatory role in the lung. Endogenous H_2S may therefore be useful as a non-invasive indicator for monitoring bronchial asthma.

Pulmonary function is impaired in individuals with bronchial asthma (15,16). Lung function testing is commonly employed in the diagnosis and prognosis of this disease. Our findings of reduced lung function in children with bronchial asthma are not surprising. However, this is the first report of a correlation between indicators of lung function and concentration of endogenous H_2S in the serum of patients with bronchial asthma. Increasing serum H_2S concentrations corresponded to improved lung function. This result indicates that H_2S concentration may predict disease severity. It is possible that endogenous H_2S causes bronchoconstriction via effects on smooth muscle of bronchi to influence pulmonary function of both large and small airways. Indeed, a study in a rat model of asthma indicated that endogenous H_2S reduced airway remodeling (19). Taken together, these findings suggest that further investigation of endogenous H_2S in the context of asthma is warranted.

In conclusion, few studies have focused on the biological effects of endogenous H_2S on airway and function in respiratory diseases. Our findings of a correlation between endogenous H_2S and lung function indicate that further investigation of this compound in the context of airway disease is required, possibly leading to improvements in the diagnosis and treatment of such diseases.

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