Allergology International 65 (2016) 425–431

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Changes in lung sounds during asthma progression in a guinea pig model

Chizu Habukawa [a, b, *], Katsumi Murakami [c], Kazuya Sugitani [b], Tomohiro Ohtani [b], Gabriel Pramudita Saputra [b], Katsuki Kashiyama [b], Yukio Nagasaka [d], Shigeo Wada [b]

[a] Department of Pediatrics, Minami Wakayama Medical Center, Wakayama, Japan
[b] Department of Mechanical Science & Bioengineering, Graduate School of Engineering Science, Osaka University, Osaka, Japan
[c] Department of Psychosomatic Medicine, Kinki University Sakai Hospital, Osaka, Japan
[d] Kyoto Pulmonary Center, Rakuwakai Otowa Hospital, Kyoto, Japan

ARTICLE INFO

Article history:
Received 16 January 2016
Received in revised form
17 March 2016
Accepted 22 March 2016
Available online 4 August 2016

Keywords:
Asthma
Bronchi
Guinea pigs
Lung
Respiratory sounds

Abbreviations:
f, respiratory frequency; TV, tidal volume; MV, minute volume; sRaw, specific airway resistance; sGaw, specific airway conductance; Raw, airway resistance; FRC, functional residual capacity; PIF, peak inspiratory flow; PEF, peak expiratory flow; Ti, inspiratory time; Te, expiratory time; dT, delta time; EF50, flow at the point at which 50% of TV is expired; Aair, total airway area; ASM, airway smooth muscle; Aw, area of airway wall; Ai, area of internal airway area; rAw, ratio of the airway wall thickness to Aair; rAi, ratio of the internal area to Aair; 21wsC, challenged for 12 weeks; 12wsC, challenged for 12 weeks; HE, Hematoxylin-Eosin

ABSTRACT

Background: Lung sound analysis is useful for objectively evaluating airways even in children with asymptomatic asthma. However, the relationship between lung sounds and morphological changes in the airways has not been elucidated. We examined the relationship between lung sounds and chronic morphological changes in the airways during the progression of asthma from onset in guinea pigs.
Methods: Eleven male guinea pigs were examined; of these, seven were used as asthma models and four as controls. The asthma models were sensitized and repeatedly challenged by inhaling albumin chicken egg. We measured lung sounds and lung function twice a week for 21 weeks. After the final antigen challenge, the lungs were excised for histological examination. We measured the ratio of airway wall thickness to the total airway area and the ratio of the internal area to the total airway area in the trachea, third bronchi, and terminal bronchioles.
Results: Among the lungs sounds, the difference between the two groups was greatest with respect to inspiratory sound intensity. The ratio of airway wall thickness to the total airway area of the terminal bronchioles was greater in the asthma models than in the controls, and it correlated best with the changes in inspiratory sound intensity in the 501−1000-Hz range ($r = 0.76$, $p < 0.003$).
Conclusions: Lung sound intensity in the middle frequency range from 501 to 1000 Hz correlated with peripheral airway wall thickness. Inspiratory sound intensity appeared to be an indicator of morphological changes in small airways in asthma.

Introduction

Chronic airway inflammation in bronchial asthma causes airflow limitation and leads to changes in the airway structure.[1,2] These morphological and functional changes are due to chronic allergic inflammation of the airways with increased airway wall thickness caused by thickening of smooth muscles, subepithelial fibrosis, accumulation of inflammatory cells, and airway edema.[3] These structural changes in the airways have been found in biopsy

* Corresponding author. Department of Pediatrics, Minami Wakayama Medical Center, 27-1 Takinai-machi, Tanabe-shi, Wakayama 646-0015, Japan.
E-mail address: gd6c-hbkw@asahi-net.or.jp (C. Habukawa).
Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2016.03.010

specimens of airways from adult patients with asthma.[4–7] In children and infants, these structural changes in the airways are observed from the onset of asthma.[8,9] Lung function tests are important tools for the assessment of these functional and possible morphological changes in asthma, but they are difficult to perform in small children.

Lung sound analysis is a noninvasive method that does not require cooperation of small children. Airway inflammation and airflow limitation affect breath sounds even in the absence of adventitious sounds.[10–12] We previously reported that lung sound intensity is affected by functional changes in the airways in children with asymptomatic asthma.[13,14]

It has been reported that acoustic transfer characteristics are affected by airway wall compliance, airway diameter, and energy dissipation caused by respiratory motion.[15–17] In this study, we investigated the changes in lung sounds during progression of asthma from onset and determined if lung sound analysis could detect histological changes.

Methods

Experimental animals

Eleven male Hartley strain guinea pigs (18 weeks old; weight, 812–922 g) were prepared for the examination (Japan SLC Inc., Shizuoka, Japan). All animal procedures were approved by our local animal care committee. The study protocol was approved by the ethics committee of our institution (approval number: 22-1-0). We divided the animals into two groups: seven asthma models and four controls. There was no significant difference in the weight of the asthma models and the controls.

Chronic asthma model: sensitization and exposure protocol

We sensitized the animals according to the sensitization methods previously described.[18,19] In brief, asthma models were sensitized by intraperitoneal injections of average 7 mg of ovalbumin (OA: albumin chicken egg, Grade V; Sigma Chemical, St. Louis, MO, USA) in 2 ml of Freund's complete adjuvant (Daitron, Tokyo, Japan) twice on days 0 and 14.

Chronic allergen challenge was started 1 week after the second intraperitoneal injection of the antigen. The allergen challenge was repeated once or twice a week for 18 weeks or 9 weeks.[18,19] We chose one of the sensitization methods according to the condition of the animals.

For the allergen challenge, the animals were placed in a nebulization unit box (8.5 × 9.3 × 22 cm) without being anesthetized. The animals were challenged with an aerosolized inhalational solution comprising 3 mg of OA in 3 ml of 0.9% saline delivered by an ultrasonic nebulizer (NE-U12; Omron, Tokyo, Japan) for 10 min.

The animals inhaled 3–30 mg of OA to avoid OA tolerance at a given concentration.[20] The nebulization box allowed up to four guinea pigs to simultaneously breathe OA under identical conditions. Diphenhydramine (20 mg/kg; Sigma Chemical) was intraperitoneally administered 1 h prior to each challenge to prevent severe anaphylaxis.

When an animal showed a severe change in breathing pattern or cyanosis within 10 min of exposure, the inhalation challenge was immediately discontinued for ethical reasons.

Measurement of respiratory resistance and lung function

Respiratory resistance was measured using a double-chamber whole-body plethysmograph (Buxco, Sharon, CT, USA) according to the method of Pennock *et al.*[21] We measured respiratory frequency (f), tidal volume (TV), minute volume (MV), specific airway resistance (sRaw), specific airway conductance (sGaw), airway resistance (Raw), functional residual capacity (FRC), peak inspiratory flow (PIF), peak expiratory flow (PEF), inspiratory time (Ti), expiratory time (Te), and delta time (dT). Delta time is the delay in time observed between nasal and thoracoabdominal flows, the flow at the point at which 50% of TV is expired (EF50).

Sound recording and signal processing

Before recording lung sounds, we shaved the hairs of the bilateral chest. Lung sounds were recorded on the right and left side of the anterior chest for ≥120 s while the animals breathed freely before measurement of airway resistance and the following antigen challenge.

Lung sounds were recorded using an air-coupled microphone. We inserted a Primo EM147 electret condenser microphone in plastic couplers with a cylindrical air chamber and recorded the lung sounds of the guinea pigs.

Audio signals of the lung sounds were amplified and high-pass filtered at 50 Hz (AT-MA2, AUDIO-TECHNICA). The optimal amplifier gain was adjusted empirically before the study and fixed throughout the experiments. The audio signal was digitized at 48 kHz and 16 bits per sample using an audio interface (Fireface UC; RME, Germany) and analyzed using audio editor software (Adobe Auditoin CS6; Adobe, USA). Fast Fourier transformation was performed on 4096 points of signal data using a Hanning window with 50% overlap into adjacent segments.

The upper section of Figure 1 shows an example of lung sound analysis in a guinea pig. The audio signal of the inspiratory breath sounds had greater amplitude than that of the expiratory breath sounds.

Noise handling

Prior to sound analysis, we carefully listened to all the recordings and reviewed the spectrogram to exclude noise, such as friction and environmental sound. It was apparent that the intensity of the inspiratory breath sounds was larger than that of the background noise; the average intensity of the inspiratory breath sounds was −74.4 ± 12.0 dB, whereas the average intensity of the background noise was −90.6 ± 13.1 dB.

Lung sound index

The spectral shape of the lung sounds shows that the intensity of the inspiratory breath sounds was larger than that of the expiratory breath sounds before the antigen challenge (The upper section of Fig. 1).

We calculated the lung sound index to compare lung sound intensities before and after the antigen challenge. The lung sound index is the difference between the averaged power of 3 inspiratory breath sounds before and after the antigen challenge.

We calculated the lung sound index in 4 frequency bands: 0–500 Hz, 501–1000 Hz, 1001–1500 Hz, and 1501–2000 Hz.

Histological examination and airway dimensions

We examined the lung tissues of 5 asthma models and 3 controls. The animals were sacrificed by administering an overdose of pentobarbital (500 mg/kg i.h). A cannula was inserted into the proximal portion of the trachea, and the lungs were inflated with ethanol at a constant pressure of 25 cmH_2O.

Tissue preparation was performed as previously described.[22,23] The lung tissue specimens were obtained from the trachea, third bronchi, and terminal bronchioles of the right and left lungs and then embedded in paraffin. Then, 4-μm-thick sections were cut and