Morphometric analysis of the bronchiolar arterioles through the normal aging process

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Changes in dimensions of bronchiolar arterioles have been proposed as a mechanism in pulmonary diseases of the elderly, such as chronic obstructive pulmonary disease. The aim of this study was the morphometric assessment of the bronchiolar arterioles in the lung of mouse through the normal aging process. Lung specimens from CD1 mice at the age of 2, 6, 12, 18 or 24 months were fixed in 10% neutral-buffered formalin and paraffin-embedded. After staining of 5-μm sections with Masson trichrome technique, bronchiolar arterioles were analyzed by morphometry. The results of the ANOVA analysis indicated that there was no a significant difference in total perimeter (F = 1.33, p = 0.265), total area (F = 0.66, p = 0.621), adventitia layer area (F = 0.25, p = 0.907), muscular layer area (F = 0.27, p = 0.893), and lumen area (F = 1.04, p = 0.393) of the bronchiolar arterioles among the analyzed ages. Our findings indicate that there are no significant changes in dimensions of bronchiolar arterioles in the normal aging process. More research is necessary to assess the possible role of small blood vessels in chronic pulmonary diseases.

Keywords: morphometry; lung; aging; bronchiolar arterioles; mouse

1. Introduction

The respiratory system consists of the nasal cavity, paranasal sinuses, pharynx, larynx, trachea, and lungs. The upper respiratory tract includes the nose and nasal passages, paranasal sinuses, the pharynx, and the portion of the larynx above the vocal cords. The lower respiratory tract includes the trachea and within the lungs, the bronchi, bronchioles, and alveoli. This system performs or participates in several functions: air conduction, gas exchange, olfaction (reception of odor), and phonation (production of sound) [1, 2].

During the first decade of life, the lungs undergo a stage of growth and maturation. Around 10-12 years old the maximum number of alveoli is reached, and thereafter the maturation of the respiratory system accelerates until the maximum function is achieved, approximately at the age of 20 years old for women and 25 years old for men [3]. Afterwards, aging is also associated with structural changes in the lung. The best known change that occurs in the lung as it ages is the alveolar enlargement [4-6]. Structural changes lead to physiological changes in the aged lung. These changes make adults more likely to suffer chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD) and emphysema. As life expectancy increases, these diseases represent an increasingly serious public health problem [7, 8].

Structure and function of the vascular system are also altered in the aging process [9, 10]. Changes in dimensions of bronchiolar arterioles have been hypothesized as a mechanism of pulmonary diseases of the elderly [11]. The aim of this study was the morphometric assessment of the bronchiolar arterioles in the lung of mouse through the normal aging process.

2. Material and Methods

Animals and most of experimental procedures used were described in a previous paper [12]. Male CD1 mice were examined; they were kept in standard conditions: stainless-steel cages, receiving chow and water ad libitum, 18-21 °C, 55-60% relative humidity, and 12:12 h day-night cycle. Three animals were sacrificed at the age of 2, 6, 12, 18 or 24 months by cervical dislocation. To more consistently control the selection of sample sections, only the right lungs were processed and analyzed. Lungs were fixed in 10% neutral-buffered formalin and paraffin-embedded. Serial 5-μm sections were cut, deparaffinized in xylene and hydrated in a graded series of alcohol. Sections were stained with Masson trichrome technique [13]. Analyses were conducted using three tissue sections per animal, taken from the middle of the lung, so that a portion of each lung lobe was included in each section. All mice received care in compliance with the “Guide for the care and use of laboratory animals” [14].

All available bronchiolar arterioles for each mouse were analyzed. On average, two bronchiolar arterioles per slide were analyzed (range 0–6). The arterioles analyzed in this study were those sectioned transversely and that were next to
a bronchiole [12, 15]. Arterioles were excluded from analysis if the entire arteriole could not be included in the photograph, or if the adventitia or muscular layers were not well defined or were disrupted.

Sections were examined using a Primo Star light microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany), and high-resolution color images (400x) were captured using a Axio-Cam ICc1 camera (Carl Zeiss Microscopy GmbH) linked to image analysis software Zen lite 2011 (Carl Zeiss Microscopy GmbH) to measure areas and lengths. All analyses were performed on coded slides by a single observer blinded to the age groups.

The following parameters were analyzed in the bronchiolar arterioles: total perimeter, total area, adventitia layer area, muscular layer area, and lumen area. The perimeter was calculated using the approximate formula for the perimeter of an ellipse, \( P = 2\pi \sqrt{(a'^2 + b'^2)/2} \) where \( a' \) and \( b' \) are one-half of the short and long axes of the arteriole (Fig. 1).

The results are presented as means ±1 standard error (SE). The data were statistically analyzed using a one-way ANOVA test. A \( p \) value less than 0.05 was considered significant. The information was analyzed using the SPSS for Windows software (SPSS, Inc., Chicago, IL, USA), release 21.0.

3. Results and Discussion

Figure 2 shows a representative example of a micrograph of a bronchiolar arteriole with analyzed parameters in the morphometric analysis. Means ± SE are reported in Table 1. The results of ANOVA analysis indicated that there was no significant difference in total perimeter (\( F = 1.33, p = 0.265 \)), total area (\( F = 0.66, p = 0.621 \)), adventitia layer area (\( F = 0.25, p = 0.907 \)), muscular layer area (\( F = 0.27, p = 0.893 \)), and lumen area (\( F = 1.04, p = 0.393 \)) of the bronchiolar arterioles among the analyzed ages (Fig. 3).

Fig. 1  Schematic representation of bronchiolar arteriole showing morphometric measurements. The total perimeter was calculated using the formula \( P = 2\pi \sqrt{(a'^2 + b'^2)/2} \) where \( a' \) and \( b' \) are one-half of the short and long axes of the arteriole.

Fig. 2  Photomicrograph of a bronchiolar arteriole stained with Masson trichrome technique showing measured parameters in the morphometric analysis. L, lumen area; M, muscular layer area; A, adventitia layer area; TA, total area; LA, long axis of the arteriole; SA, short axis of the arteriole; B, bronchiole. The total perimeter was calculated using the formula \( P = 2\pi \sqrt{(a'^2 + b'^2)/2} \) where \( a' \) and \( b' \) are one-half of the SA and LA of the arteriole.
Table 1  Morphometric parameters of the bronchiolar arterioles in the lung of mouse through the normal aging process.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age (months)</th>
</tr>
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<tbody>
<tr>
<td>Total perimeter (µm)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>207.8 ± 14.3</td>
</tr>
<tr>
<td>Total area (µm²)</td>
<td>3732.0 ± 508.5</td>
</tr>
<tr>
<td>Adventitia layer area (µm²)</td>
<td>787.7 ± 95.6</td>
</tr>
<tr>
<td>Muscular layer area (µm²)</td>
<td>875.4 ± 119.2</td>
</tr>
<tr>
<td>Lumen area (µm²)</td>
<td>2068.9 ± 303.6</td>
</tr>
</tbody>
</table>

Data are reported as mean ± standard error (SE). Data were analyzed by the one-way ANOVA test. Results were considered statistically significant at \( p<0.05 \). There were no significant differences in the studied parameters among the analyzed ages.

Fig. 3  Analysis of morphometric measurements in bronchiolar arterioles from healthy 2-, 6-, 12-, 18-, and 24-month-old mice. a) total perimeter, b) total area, c) adventitia layer area, d) muscular layer area, e) lumen area. Data are reported as mean ± standard error (SE). The data were statistically analyzed using a one-way ANOVA test. Results were considered statistically significant at \( p<0.05 \). There were no significant differences in the studied parameters among the analyzed ages.
Aging is associated with structural and functional changes in the lung. The aim of this study was the morphometric assessment of the bronchiolar arterioles in the lung of mouse through the normal aging process.

We did not find significant changes in dimensions of bronchiolar arterioles in the normal aging process. Development of respiratory diseases of the elderly might be attributed to other causes such as smoking, exposure to airborne pollutants, occupational exposure to certain particles, alpha-1-antitrypsin deficiency, and et cetera [16]. More research is necessary to assess the possible role of small blood vessels in chronic pulmonary diseases.

References