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Lung tissue mechanics in the early stages of induced paracoccidioidomycosis in rats

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Abstract

Pulmonary dysfunction represents the most important cause of death in patients with paracoccidioidomycosis (PBM). In order to investigate the functional changes of the lungs in the early stages of PBM, a model of benign disease was developed by intratracheal challenge of 12-week old isogenic Wistar rats with 1 x 10^6 yeast forms of *Paracoccidioides brasiliensis*. Animals were studied 30 and 60 days after infection, when fully developed granulomas were demonstrable in the lungs. Measurements of airway resistance, lung elastance and tissue hysteresis were made during sinusoidal deformations (100 breaths/min, tidal volume = 2 ml) with direct measurement of alveolar pressure using the alveolar capsule technique. Infection caused a significant increase in hysteresis (infected: 1.69, N = 13; control: 1.13, N = 12, P = 0.024, ANOVA), with no alterations in airway resistance or lung elastance. Histopathological analysis revealed the presence of fully developed granulomas located in the axial compartment of the lung interstitial space. These results suggest that alterations of tissue mechanics represent an early event in experimental PBM.

Key words
- Experimental paracoccidioidomycosis
- Pulmonary mechanics
- Tissue mechanics
- Alveolar pressure measurements
- Hysteresis

Introduction

Paracoccidioidomycosis is the most prevalent endemic mycosis in Latin America. *Paracoccidioides brasiliensis* is transmitted by the respiratory route through inhalation of conidia. Involvement of the phagocytic mononuclear system is the most common clinical finding in the acute form, mainly described in children or young adult patients. In contrast, the chronic form is prevalent in 30-50-year old adults with involvement of several organs, especially the lungs. Chronic granulomatous inflammation is a frequent histological finding, sometimes followed by intense fibrosis resulting in anatomical distortion of the oropharynx or upper respiratory tract (1,2) or severe sequelae represented by lymph node or pulmonary fibrosis (3). Protein-losing enteropathy, as well as cellular and humoral immunodeficiencies, have been described as consequences of lymph node

fibrosis (4). Besides this severe clinical evolution, another important cause of death is represented by *cor pulmonale* secondary to both obstructive and restrictive pulmonary insufficiency, which affects about 30% of chronic cases (5). Lung function has been studied only in the late stages of human paracoccidioidomycosis. The present study focuses on functional lung changes observed in the early phases of intratracheal paracoccidioidomycosis induced in isogenic rats.

Material and Methods

Fungi

Yeast forms of *Paracoccidioides brasiliensis* were obtained from 7-day old Fava-Netto culture medium (6). Cell viability was at least 80%, as assessed by Janus green staining (7). Total cell counts were performed with a hemocytometer chamber to estimate the inoculum size, which was 1 x 10^6 yeast forms of fungi/0.5 ml phosphate buffered saline.

Rats

A model of benign disease was developed by intratracheal challenge of twenty-five 12-week old isogenic Wistar-Furth rats. Animals were studied 30 and 60 days after infection. Corresponding groups injected with saline solution were used as controls.

Histopathological study

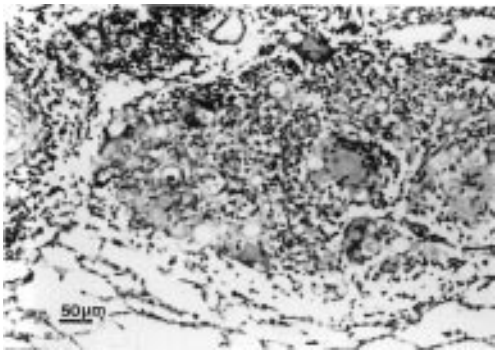
Histopathological studies were performed in three regions of the lungs: the perihilar area near the main bronchi, parenchyma including segmentary bronchi, and peripheral area usually with macroscopic alterations. Lymph nodes, liver, adrenals, kidney and brain were also studied. This analysis was done using paraffin-embedded slides stained with hematoxylin-eosin and by the Grocott-Gomori silver technique.

Physiological measurements

Rats were anesthetized with intraperitoneal sodium pentobarbital (35 mg/kg) and a polyethylene cannula was inserted into the trachea by direct visualization. The rats were ventilated with a rodent ventilator (Harvard 683, Harvard Apparatus Co., South Natik, MA) at a constant tidal volume (2.5 ml) with the respiratory frequency set at 100 breaths/min. Airflow ($\dot{V}$) was measured with a pneumotachograph connected to the tracheal cannula through a Valydine DP 45-16-2114 differential pressure transducer. Volume (V) was obtained by electronic integration of the $\dot{V}$ signal. Tracheal pressure (Ptr) was measured with a Valydine DP 45-28-2114 differential pressure transducer. Positive end-expiratory pressure of 5 cmH_2O was used to maintain a functional residual capacity close to a normal value. Alveolar pressure (Palv) was measured according to the alveolar capsule technique described by Saldiva et al. (8). The capsules were made from 3-ml plastic syringes with the distal end cut off and connected to a Valydine DP 45-28-2114 differential pressure transducer through a 15-cm long polyethylene catheter (1.6 mm ID). The pleural surface was punctured with an 18-gauge needle, and the capsule was glued to this surface with cyanoacrylate. The depth of the holes on the pleural surface was less than 0.5 mm to avoid sampling of bronchial pressures. A single capsule was used for each rat but placed at different sites in each animal.

Flow, Ptr and Palv signals were sampled at 200 Hz with a 12-bit analog-to-digital converter (DT-2801A, Data Translation, Marlboro, MA) and stored in a microcomputer. In each measurement, one data point was obtained by the average of 14-16 breaths.

Airway pressure (Paw) was obtained by subtracting Palv from Ptr. Airway resistance (Raw) was obtained by dividing Paw by $\dot{V}$ during lung inflation. Pulmonary dynamic



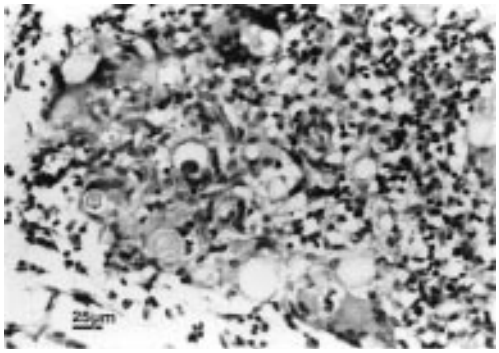


Figure 1 - Compact granulomas in the lungs of Wistar rats 60 days after inoculation with *P. brasiliensis* (hematoxylin and eosin, *left* = 200X, *right* = 400X).

elastance (lung elastic modulus), tissue resistance and area of hysteresis of the pressure volume loop during tidal ventilation were obtained by the method of Fredberg and Stamenovic (9). Seven to eight breaths were averaged to complete the mechanical parameter for each animal.

Statistical analysis

The significance of the results was assessed by analysis of variance considering two factors, i.e., infection and time of infection. Variance of the parameters of interest was stabilized by applying logarithmic transformation of elastance, resistance and hysteresis.

Results and Discussion

The infection was limited to the lungs. Epithelioid granulomas rich in fungi (Figure 1, left) were observed in the lungs 15 to 150 days after the infection. Giant cells, macrophages and some polymorphonuclear cells, eosinophils and lymphocytes (Figure 1, right) were also found in the granulomas. Occasional necrosis was observed in a small number of granulomas during the period of follow-up.

Tables 1, 2 and 3 show the mean and corresponding standard deviation (SD) of functional variables for hysteresis, elastance and resistance, respectively, measured for each period of time, as well as the corresponding output of ANOVA analysis for each variable.

Table 1 - Effect of paracoccidioidomycosis infection on pulmonary hysteresis.

P = 0.024, significance of paracoccidioidomycosis infection (ANOVA). P = 0.491 and P = 0.174, time of infection and interaction infection *vs* time (not significant, ANOVA).

	Hysteresis (cmH_2O/ml)		
	30 days	60 days	Total
Control			
Mean	0.76	1.39	1.13
Median	0.75	1.16	0.88
Standard deviation	0.26	0.99	0.81
Minimum	0.50	0.51	0.50
Maximum	1.09	3.34	3.34
No. of cases	5	7	12
Infected			
Mean	1.79	1.61	1.69
Median	1.50	1.66	1.54
Standard deviation	0.79	0.88	0.80
Minimum	0.87	0.68	0.68
Maximum	2.82	3.23	3.23
No. of cases	6	7	13
Total			
Mean	1.32	1.50	1.42
Median	1.09	1.40	1.16
Standard deviation	0.78	0.90	0.84
Minimum	0.50	0.51	0.50
Maximum	2.82	3.34	3.34
No. of cases	11	14	25

Increased hysteresis (P = 0.024) was observed in infected animals at 30 and 60 days after inoculation compared to control animals. No difference in elastance or resistance was observed between groups during either period of infection.

In human paracoccidioidomycosis, initial involvement of the lungs may occur at

Table 2 - Paracoccidioidomycosis (PBM) infection has no effect on pulmonary elastance.

Neither PBM infection (P = 0.283), time of infection (P = 0.496) nor interaction infection *vs* time (P = 0.561) were statistically significant (ANOVA).

	Elastance (cmH_2O/ml)		
	30 days	60 days	Total
Control			
Mean	2.37	2.91	2.68
Median	2.19	3.35	2.72
Standard deviation	0.81	0.92	0.88
Minimum	1.67	1.13	1.13
Maximum	3.73	3.79	3.79
No. of cases	5	7	12
Infected			
Mean	2.99	3.09	3.04
Median	2.83	3.18	3.05
Standard deviation	0.82	1.02	0.90
Minimum	2.10	1.80	1.80
Maximum	4.00	4.38	4.38
No. of cases	6	7	13
Total			
Mean	2.71	3.00	2.87
Median	2.38	3.27	3.05
Standard deviation	0.84	0.94	0.89
Minimum	1.67	1.13	1.13
Maximum	4.00	4.38	4.38
No. of cases	11	14	25

Table 3 - Paracoccidioidomycosis (PBM) infection has no effect on pulmonary resistance.

Neither PBM infection (P = 0.098), time of infection (P = 0.609) nor interaction infection *vs* time (P = 0.993) were statistically significant (ANOVA).

	Resistance ($cmH_2O\ ml^{-1}\ s^{-1}$)		
	30 days	60 days	Total
Control			
Mean	0.047	0.058	0.053
Median	0.033	0.037	0.035
Standard deviation	0.057	0.052	0.052
Minimum	0.002	0.013	0.002
Maximum	0.143	0.160	0.160
No. of cases	5	7	12
Infected			
Mean	0.085	0.097	0.091
Median	0.083	0.073	0.080
Standard deviation	0.043	0.065	0.054
Minimum	0.019	0.000	0.000
Maximum	0.150	0.190	0.190
No. of cases	6	7	13
Total			
Mean	0.068	0.077	0.073
Median	0.067	0.064	0.067
Standard deviation	0.051	0.060	0.055
Minimum	0.002	0.000	0.000
Maximum	0.150	0.190	0.190
No. of cases	11	14	25

the alveolar or interstitial level, as a granulomatous or an exudative pattern, which usually does not lead to abnormalities in spirographic tests. As a consequence of lymphatic spreading of the lesions along the peribronchial tissue, centrifugal histological changes are commonly observed. These findings, sometimes with simultaneous fibrosis and alveolar destruction, may be responsible for the functional disturbances observed in patients with chronic pulmonary paracoccidioidomycosis (10). Obstruction and restriction have been the most frequent spirographic patterns described in these patients.

Similarly to the human disease, hamsters infected by the intratracheal route presented with hilar bronchial involvement with centrifugal dissemination after regional lymph node involvement by confluent granulomas (11). These changes might represent lesions which could later evolve to fibrosis. An experimental model more similar to human pulmonary fibrosis seems to be represented by mice intranasally inoculated with conidia, as described by Restrepo et al. (12). However, an intriguing difference between experimental and human pulmonary disease is the long period of time between the initial infection and the development of chronic disease in human paracoccidioidomycosis.

We suggest that benign mycosis in isogenic Wistar-Furth rats provides a useful model to study changes in lung function during the early phases of paracoccidioidomycosis, mainly due to the presence of predominantly cellular granulomas in response

to the infectious agent and the absence of significant scarring. As a consequence of the granulomatous infection, a significant increase in hysteresis (P = 0.024) was observed at 30 days, which remained elevated up to the end of the experiment (60 days). The present study represents the first description of increased tissue resistance independent of bronchoconstriction. Histopathological examination performed in three different areas of the lung at day 30 and 60 after the infection showed fully developed granulomas only in infected rats. These results indicate that changes in tissue mechanics due to granuloma formation along the axial compartment of lung connective tissue are an early event in experimental paracoccidioidomycosis. In addition, it is probable that the granuloma may act as a viscous or plastic element in the pulmonary interstitium causing an increase of tissue pressure losses at the tissue level, which probably precedes the restrictive and/or obstructive functional patterns observed in chronic human disease.

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