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Effect of lung resection and sham surgery on the frequency of infection in alloxan-diabetic rats

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Abstract

The present study was carried out in order to determine the effect of lung resection on the frequency of infections in alloxan-diabetic rats. Adult female Wistar rats were injected with alloxan (40 mg/kg, *iv*) to induce diabetes mellitus (group D; N = 45) or with vehicle (1.0 ml/kg, *iv*) to be used as controls (group C; N = 45). Thirty-six days after receiving alloxan both groups were randomly divided into three subgroups: no operation (NO; N = 15), sham operation (SO; N = 15), and left pneumonectomy (PE; N = 15). The rats were sacrificed 36 days after surgery and their lungs were examined microscopically and macroscopically. The occurrence of thoracic wall infection, thoracic wall abscess, lung abscess and pleural empyema was similar in groups D and C. In contrast, the overall infection rate was higher ($P<0.05$) in the diabetic rats (SO-D and PE-D subgroups, but not in the NO-D subgroup). Considering that the overall infection rate was similar in the SO-D and PE-D subgroups, we suggest that surgery but not pneumonectomy was related to the higher prevalence of infection in diabetic rats.

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Diabetic patients are more prone to infections than nondiabetic patients (1), showing higher rates of unusual infections such as rhinocerebral and lung mucormycosis, emphysematous cholecystitis and pyelonephritis, necrotizing cellulitis or fasciitis, and malignant external otitis (2-5). In agreement, abnormalities of all aspects of polymorphonuclear leukocyte function have been described in diabetic patients, particularly decreased phagocytosis, intracellular killing, adherence and cell movement (6,7).

Since chest surgery is very common today, the possibility of a higher incidence of infections in diabetic patients after this type of surgery should be considered. However, there are few studies showing the occurrence of infections after heart (8-10) or lung surgery (11) in diabetic patients. Because diabetes causes higher predisposition to lung diseases (12,13) and considering that lung surgery is often contaminated or infected, the possibility of an infection after lung surgery should be considered. Thus, we investigated the prevalence of infection in alloxan-diabetic rats after lung resection.

Adult female Wistar rats weighing 220 ± 20 g were used. The rats were housed at controlled temperature (23ºC) on a 12-12-h light-dark cycle. Water and food (Nuvital®) were given *ad libitum* to all rats.

The Ethics Committee on Research of

UNIFESP-EPM approved the experimental procedures.

In order to induce diabetes, group D rats (N = 45) were fasted for 24 h and received a single intravenous dose (45 mg/kg) of alloxan. The control rats (group C, N = 45) were 24-h fasted rats which received a single intravenous dose of saline (1.0 ml/kg, the same volume injected into the group D). Immediately after the injection of alloxan or saline all animals were maintained with free access to food during the investigation.

Six days after the injection of alloxan or saline, glycemia (14) was measured (always at 4 pm), and the animals with glycemia ≥200 mg/dl were maintained in the D group. In addition, 36 days after the injection of alloxan, glycemia was measured again. Similarly, body weight was measured on days 6 and 36.

Thirty-six days after the injection of alloxan or saline both groups were divided randomly into three subgroups: no operation (NO; N = 15), sham operation (SO; N = 15), and left pneumonectomy (PE; N = 15). On day 36 the animals were anesthetized [2-(2,6-xylidine)-5,6, chlorhydrate, dehydro-4H-1,3-thiazine and ketamine chlorhydrate, intramuscularly] and placed in a plastic box (41 x 33 x 17 cm) with oxygen overflow (1 liter/min) for 10 min.

The surgery was performed as described (15), i.e., under natural ventilation, without orotracheal intubation and with oxygen flow (1 liter/min) through a bell glass covering the head of the animal.

The rat was positioned in right lateral decubitus. The left hemithorax was disinfected with polyvinylpyrrolidone iodine in an aseptic environment. A left thoracotomy was carried out in the fourth intercostal space by an incision in the skin, subcutaneous tissues, serratus muscle, intercostal muscles and parietal pleura. The trunk of the left pulmonary lobe was sutured with 000 cotton thread and left pneumonectomy was executed in subgroups PE-C and PE-D. In order to keep the subatmospheric intrathoracic pressure, the pleural cavity was aspirated with a catheter and a 3-ml syringe at the junction of the costal arches with sutures of 000 cotton thread. In addition, thoracotomy without lung resection, i.e., sham operation was performed in subgroups SO-C and SO-D.

The duration of surgery was about 3-4 min which represents the maximal period of apnea tolerated by the rat (15).

After surgery, the animals remained in the oxygenated plastic box until they recovered from anesthesia (about 30 min).

On the 36th postoperative day, the animals were anesthetized again and submitted to laparotomy by a transverse incision in the subcostal abdominal region. The diaphragm muscle was released from the thoracic wall, the costal arches were sectioned and the anterior wall of the thorax was retracted for inspection of the thoracic wall. Finally, the heart was removed, the trachea and the bronchus were dissected and the material was prepared for macroscopic and microscopic analysis.

Lung fragments were selected as follows: NO-C, SO-C, NO-D and SO-D, left lung, cranial, medium, accessory and caudal lobes of the right lung; PE-C and PE-D, cranial, medium, accessory and caudal lobes of the right lung. These fragments were immersed in 10% formaldehyde for histological processing by a standard technique and stained with hematoxylin/eosin for the evaluation of inflammatory reaction by leukocyte exudates (neutrophils).

Microscopic parameters were scored as present or absent and considering the involvement of the anatomical area of the pleura and/or lung: lobar pneumonia, bronchopneumonia, interstitial pneumonia, abscess, and pleurisy.

Glycemia and body weight were analyzed by the paired Student *t*-test. The presence of infection was evaluated statistically by the chi-square test. In addition, the presence of systemic infections was evaluated statistically by Fisher's test on the basis of

the occurrence of lobar pneumonia, bronchopneumonia and interstitial pneumonia. A 95% level of confidence ($P<0.05$) was accepted for all comparisons. The results are reported as means ± SD.

A single dose of alloxan (45 mg/kg) was effective in inducing a maintained diabetic state in all rats. Glycemia of the NO-D, SO-D and PE-D subgroups on day 6 after alloxan injection was 348.3 ± 44.5, 379.1 ± 66.6, 376.1 ± 74.6 mg/dl, respectively. In addition, glycemia of the NO-D, SO-D and PE-D subgroups on day 36 after alloxan injection was 338.3 ± 59.7, 361.5 ± 62.9 and 338.3 ± 52.9 mg/dl, respectively.

The body weight of the NO-D, SO-D and PE-D subgroups on day 6 after alloxan injection was 218.8 ± 20.4, 203.1 ± 17.1 and 211.1 ± 19.7 g, respectively. In addition, the body weight of the NO-D, SO-D and PE-D subgroups on day 36 after alloxan injection was 225.5 ± 27.7, 208.5 ± 37.7 and 219.1 ± 32.6 g, respectively. Thus, all diabetic groups showed unchanged glycemia and body weight from the 1st to the 36th day.

In contrast with the results of many studies showing higher susceptibility to infections (1-11,16-20), the presence of thoracic wall infection, thoracic wall abscess, lung abscess and pleural empyema (Table 1), and lobar pneumonia, bronchopneumonia and interstitial pneumonia (Table 2) did not differ between diabetic and nondiabetic rats.

Finally, group D rats (SO-D and PE-D subgroups, but not the NO-D subgroup) showed a higher ($P<0.05$) prevalence of infection (Table 2). In addition, considering that the results of overall infection rate reported in Table 2 were obtained from the other data reported in Table 2 (using Fisher's test), it should be emphasized that we did not observe D rats with two or more infections.

Since the overall infection rate was similar in the SO-D and PE-D subgroups we suggest that surgery but not pneumonectomy was related to the higher prevalence of infection in diabetic rats.

Table 1. Thoracic wall infection, thoracic wall abscess, lung abscess and pleural empyema in diabetic (group D) and nondiabetic (group C) rats.

	Infected rats/uninfected rats (% infection)		Significance (C *vs* D)
	Group C	Group D	
Thoracic wall infection			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	0/15 (0.0)	
PE	1/14 (6.67)	0/15 (0.0)	P = 0.5000
Total	1/44 (2.22)	0/45 (0.0)	
Thoracic wall abscess			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	2/13 (13.33)	P = 0.2414
PE	0/15 (0.0)	1/14 (6.67)	P = 0.5000
Total	0/45 (0.0)	3/42 (6.67)	
Lung abscess			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	0/15 (0.0)	
PE	0/15 (0.0)	1/14 (6.67)	P = 0.5000
Total	0/45 (0.0)	1/44 (2.22)	
Pleural empyema			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	0/15 (0.0)	
PE	0/15 (0.0)	1/14 (6.67)	
Total	0/45 (0.0)	1/44 (2.22)	

NO, no operation; SO, sham operation; PE, left pneumonectomy. Data were evaluated by the chi-square test.

Table 2. Presence or absence of lobar pneumonia, bronchopneumonia, interstitial pneumonia and overall infection rate in diabetic (group D) and nondiabetic (group C) rats.

	Infected rats/uninfected rats (% infection)		Significance (C *vs* D)
	Group C	Group D	
Lobar pneumonia			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	2/13 (13.33)	P = 0.2414
PE	0/15 (0.0)	0/15 (0.0)	
Total	0/45 (0.0)	2/43 (4.44)	
Bronchopneumonia			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	1/14 (6.67)	P = 0.5000
PE	1/14 (6.67)	2/13 (13.3)	P = 0.5000
Total	1/44 (2.22)	3/42 (6.67)	
Interstitial pneumonia			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	1/14 (6.67)	P = 0.5000
PE	0/15 (0.0)	2/13 (13.33)	P = 0.2414
Total	0/45 (0.0)	3/42 (6.67)	
Overal infection rate			
NO	0/15 (0.0)	0/15 (0.0)	
SO	0/15 (0.0)	6/9 (40.0)	P = 0.008429
PE	2/13 (13.33)	7/8 (46.67)	P = 0.005432
Total	2/43 (4.44)	13/32 (28.88)	

NO, no operation; SO, sham operation; PE, left pneumonectomy. Data were evaluated by the chi-square test, except for overall infection data that were analyzed by Fisher's test.

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