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ORIGINAL ARTICLE

Effects of the association of diabetes and pulmonary emphysema on cardiac structure and function in rats

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SUMMARY

Chronic obstructive pulmonary disease is often associated with chronic comorbid conditions of cardiovascular disease, diabetes mellitus and hypertension. This study aimed to investigate the effects of the association of diabetes and pulmonary emphysema on cardiac structure and function in rats. Wistar rats were divided into control non-diabetic instilled with saline (CS) or elastase (CE), diabetic instilled with saline (DS) or elastase (DE), DE treated with insulin (DEI) groups and echocardiographic measurements, morphometric analyses of the heart and lungs, and survival analysis conducted 50 days after instillation. Diabetes mellitus was induced [alloxan, 42 mg/kg, intravenously (iv)] 10 days before the induction of emphysema (elastase, 0.25 IU/100 g). Rats were treated with NPH insulin (4 IU before elastase plus 2 IU/day, 50 days). Both CE and DE exhibited similar increases in mean alveolar diameter, which are positively correlated with increases in right ventricular (RV) wall thickness ($P = 0.0022$), cavity area ($P = 0.0001$) and cardiomyocyte thickness ($P = 0.0001$). Diabetic saline group demonstrated a reduction in left ventricular (LV) wall, interventricular (IV) septum, cardiomyocyte thickness and an increase in cavity area, associated with a reduction in LV fractional shortening ($P < 0.05$), and an increase in LViv relaxation time ($P < 0.05$). Survival rate decreased from 80% in DS group to 40% in DE group. In conclusion, alloxan diabetes did not affect RV hypertrophy secondary to chronic emphysema, even in the presence of insulin. Diabetes *per se* induced left ventricular dysfunction, which was less evident in the presence of RV hypertrophy. Survival rate was substantially reduced as a consequence, at least in part, of the coexistence of RV hypertrophy and diabetic cardiomyopathy.

Keywords
diabetes mellitus, diabetic cardiomyopathy, mortality, pulmonary emphysema, right ventricular hypertrophy

It is increasingly recognized that chronic obstructive pulmonary disease (COPD) is often associated with chronic comorbid conditions of cardiovascular disease, diabetes mellitus and hypertension, which affect health-related quality of life and clinical outcomes (Fabbri *et al.* 2008; Mannino *et al.* 2008; Crisafulli *et al.* 2010).

Cardiovascular diseases, chronic respiratory diseases and diabetes are the most frequent chronic degenerative disorders, particularly in the elderly. Pulmonary vascular abnormalities are frequently present in patients with respiratory disorders, including COPD. Pulmonary hypertension may result in severe right ventricular (RV) dysfunction, known as cor pulmonale, which is generally associated with poor prognosis and increased mortality (Han *et al.* 2007). In a large population based on cohort study, it has been shown that per cent emphysema was inversely associated with RV end-diastolic volume, stroke volume and mass (Grau *et al.* 2013). Cardiac dysfunction is a well-known consequence of diabetes mellitus. Diabetes-related heart disease includes coronary artery disease, cardiac autonomic neuropathy and

diabetic cardiomyopathy, defined as a myocardial dysfunction occurring in patients with diabetes on the absence of coronary artery disease, hypertension or valvular heart disease (Rubler *et al.* 1972; Aneja *et al.* 2008).

In the attempt to investigate the relationship between COPD and diabetes, we first demonstrated that alloxan-induced diabetes in rats is associated with a greater emphysematous lesion, by impairing repair and remodelling of the alveolar walls (Di Petta *et al.* 2011). To our knowledge, no studies have investigated the effects of the association of COPD and diabetes on the development of heart disorders.

This study aimed to investigate the effects of the association of diabetes and pulmonary emphysema on cardiac structure and function in rats.

Materials and methods

Animals

Male Wistar rats weighing 200 ± 20 g (about 2 months of age) at the beginning of the experiments were used. The animals were maintained at 23 ± 2°C under a cycle of 12-h light/darkness and were allowed to food and water *ad libitum*.

Induction of diabetes mellitus

Diabetes mellitus was induced by an intravenous (penile vein) injection of 42 mg/kg alloxan monohydrate (Sigma Chemical Co., St. Louis, MO, USA) dissolved in physiological saline. Control rats were injected with physiological saline only. Ten days thereafter, the presence of diabetes was verified by blood glucose concentrations (>11.2 mmol/l) determined by a blood glucose monitor (Eli Lilly, São Paulo, SP, Brazil) in samples obtained from the cut tip of the rat tail. A group of diabetic rats received 4 IU of neutral protamine Hagedorn (NPH) insulin (Eli Lilly) subcutaneously, 2 h before porcine pancreatic elastase instillation, followed by 2 IU/day for the next 50 days.

Induction of pulmonary emphysema

Rats were anesthetized with an intraperitoneal injection of 300 mg/kg chloral hydrate, and a polyethylene tube (2 mm in diameter) was inserted into the trachea with the aid of a laryngoscope adapted for small animals. Under mechanical ventilation (Harvard Rodent Ventilator, Holliston, MA, USA), with a tidal volume of 10 ml/kg and a respiratory rate of 80 cycles per minute, a physiological saline solution (0.9% NaCl; 500 μl) containing 0.25 IU/100 g body weight of porcine pancreatic elastase (Sigma Chemical Co.) was slowly instilled into the lungs. The animals were randomly divided into five groups: control saline (CS) non-diabetic rats instilled with saline; diabetic saline (DS) diabetic rats instilled with saline; control elastase (CE) non-diabetic rats instilled with elastase; diabetic elastase (DE) diabetic rats instilled with elastase; diabetic elastase rats treated with insulin (DEI). Experiments were performed 50 days after instillation.

Echocardiographic measurements

Echocardiography was performed 50 days after the intratracheal instillation of elastase or saline. Rats were anesthetized with an intraperitoneal injection of 300 mg/kg chloral hydrate and allowed spontaneous breathing. Images were acquired with a 13-MHz linear transducer in a Sequoia 512 (Acuson, Mountain View, CA, USA) for the measurement of left ventricular (LV) dimension and function. Left ventricular mass was calculated by the following equation, assuming a spherical LV geometry and validated in rats. Left ventricular mass = $1047 \times [(\mathrm{LVd} + \mathrm{PWd} + \mathrm{IVSd})^3 - \mathrm{LVd}^3]$, where 1047 represents the specific density of cardiac tissue, LVd refers to the LV end-diastolic diameter, PWd is the posterior wall end-diastolic thickness, and IVSd is the interventricular septal end-diastolic thickness. Left ventricular fractional shortening was calculated as $(\mathrm{LVd} - \mathrm{LVs})/\mathrm{LVd} \times 100$, where LVs is the LV end-systolic diameter. Other parameters included LV ejection fraction and LV isovolumetric relaxation time, which were taken from aortic valve closure to the onset of mitral flow. All the parameters were analysed by an observer blinded to treatment group, according to the guidelines of the American Society of Echocardiography (Sahn *et al.* 1978; Bilate *et al.* 2003).

Heart morphometric analysis

The heart and lungs were fixed with 10% neutral-buffered formaldehyde solution at an inflation pressure of 20 cm H_2O for 48 h. After fixation, hearts were removed, and a transversal section of the ventricles was done at the midpoint of the distance between the heart apex and coronary sulk. Samples were photographed (Sony CyberShot, Sony Corporation, Tokyo, Japan) at a constant distance, and the digitized images were analysed using the software Image Tool (University of Texas Health Science Center, San Antonio, TX, USA). Measurements included the wall thickness (mm) of right and left ventricles, thickness of the interventricular septum and the area (mm^2) of right and left ventricular cavities. After the morphometric analysis, hearts were embedded in paraffin, and transversal sections (5 μm) were stained with haematoxylin and eosin. Aiming to analyse the fibre thickness of right and left ventricles, 10 randomly selected fields of each sample were examined at 400× magnification using the NIS-Elements Imaging Software (Nikon Instruments Inc., Tokyo, Japan). Results are presented as μm.

Lung morphometric analysis

Lungs were fixed as described above, and midcoronal samples of both lungs were cut and embedded in paraffin, and 5-μm sections were then stained with haematoxylin and eosin. The morphometric analysis of lung parenchyma was

performed using an integrated eyepiece with a coherent system made of a 100-points and 50-lines grid of known length coupled with a light microscope (400×) (Weibel 1990). Results are presented as mean alveolar diameter (μm). Tissue slices also underwent specific staining methods to compute the amount of collagen and elastic fibres in the alveolar septa, as previously described (Di Petta *et al.* 2011). Briefly, for the analysis of collagen fibres, the tissue was stained with Sirius red and picric acid and observed under polarized light microscopy (Montes 1996). For elastic fibres, the Weigert's resorcin fuchsin method was used (Fullmer *et al.* 1974). Aiming to quantify collagen and elastic fibres, samples were examined at 400× magnification using the NIS-Elements Imaging Software (Nikon). Results are presented as the fraction of area (%) occupied by elastic and collagen fibres.

Statistical analysis

Data are presented as means ± SEM. ANOVA (one way) followed by Tukey–Kramer multiple comparisons test was used. Pearson's correlation was used to evaluate the relationship between the emphysematous lesion and the right ventricle. Survival was evaluated by the Kaplan–Meier test. The significance level was established as $P < 0.05$.

Ethical approval

The experimental protocol was approved by the Animal Subject Committee of the Heart Institute (InCor), University of São Paulo Medical School (approval number 3501/10/090).

Results

Relative to matching non-diabetic controls, animals rendered diabetic by the injection of alloxan exhibited approximately 25% in body weight gain, at 60-day interval, and sharply elevated blood glucose levels. Diabetic rats treated with insulin for 50 days presented 70% in body weight gain and an average 25% reduction in the levels of blood glucose, compared with non-treated diabetic rats. Results are summarized in Table 1.

Table 1 Characteristics of the animals at 60-day interval

Group	*n*	Blood glucose (mmol/l)	Body weight gain (g)
Matching control	13	5.78 ± 0.10	250 ± 11
Diabetic	16	30.05 ± 0.86*,†	61 ± 15*,†
Diabetic + insulin	7	22.59 ± 3.22*	173 ± 12$^{\delta}$

n, Number of animals in each group. Data are presented as means ± SEM and analysed by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test as follows: *$P < 0.001$ and $^{\delta}P < 0.01$ *vs.* corresponding values in control group; †$P < 0.001$ *vs.* values in diabetic + insulin group.

Morphologic alterations in characteristics of the emphysematous lesion were observed in lungs from elastase-instilled rats and were confirmed by the destruction of septa associated with the increase in alveolar spaces and the formation of structures at the ends of these ruptures known as 'drum sticks'. Photomicrographs of lung parenchyma and measurements of the mean alveolar diameter in saline-instilled control (CS) and diabetic (DS) rats and elastase-instilled rats, including control (CE), diabetic (DE) and insulin-treated diabetic (DEI) rats, are illustrated in Figure 1(a). Whereas maintenance of lobular and acinar architecture was observed in CS and DS rats, a distortion of acinar architecture due to hyperdistension of alveolar ducts, and rupture of alveolar septa are observed in CE, DE and DEI rats. Morphometric analyses of lung parenchyma showed that similar increases in the mean alveolar diameter were observed in the elastase-instilled groups (CE and DE) compared with the corresponding saline-instilled groups (CS and DS). Treatment of elastase-instilled diabetic rats with insulin (DEI) did not modify the magnitude of the emphysematous lesion compared to the CE group. In addition, an equivalent reduction in the proportion of elastic fibres and an equivalent increase in the proportion of collagen fibres were observed in the alveolar septa of CE, DE and DEI groups compared with the corresponding saline-instilled groups, CS and DS. Results are illustrated in Figure 1(b).

Morphometric analyses of the right ventricle showed a 40% increase in the wall thickness in elastase-instilled groups, CE and DE, compared with the respective saline-instilled groups, CS and DS. Results are illustrated in Figure 2. This alteration was accompanied by an equivalent increase (35%) in RV cavity area in both CE and DE compared with the respective saline-instilled groups. No differences were observed regarding the DEI group when compared with DS or DE. A significant positive correlation was demonstrated between the RV wall thickness and the mean alveolar diameter (Figure 2a) and between the cavity area and the mean alveolar diameter (Figure 2b).

As illustrated in Figure 3(a), histological analyses of cardiomyocytes demonstrated similar increases in fibre thickness of the right ventricle in elastase-instilled groups, CE and DE, compared with the respective saline-instilled groups, CS and DS. Values observed in elastase-instilled diabetic rats treated with insulin (DEI) did not differ from the values attained in DE group. A significant positive correlation was demonstrated between the RV fibre thickness and the mean alveolar diameter (Figure 3a). Representative photomicrographs of RV muscle in saline- and elastase-instilled rats are shown in Figure 3(b).

Results, summarized in Table 2, show that the wall thickness of the left ventricle and IV septum decreased 45% and 40%, respectively, in DS rats compared with the other groups. This was accompanied by a 12% reduction in LV fibre thickness. The cavity area increased in DS compared with DEI and the control groups, CS and CE. There were no differences in the cavity area between DE and DEI ($P = 0.2087$).

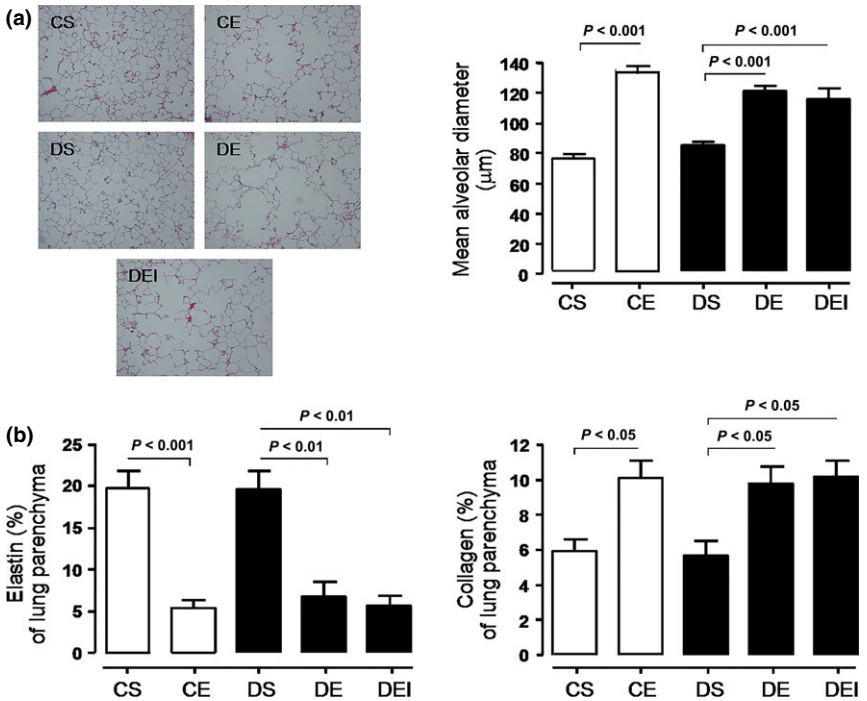

Figure 1 Morphometric analysis of lung parenchyma. (a) Photomicrographs of lung parenchyma histology (HE, 400×) and the mean alveolar diameter (μm) in rats instilled with saline or elastase. CS, control saline (n = 6); CE, control elastase (n = 7); DS, diabetic saline (n = 8); DE, diabetic elastase (n = 7); DEI, diabetic elastase treated with insulin (n = 7). Data are presented as means ± SEM. The analysis was performed in 10 fields/rat. (b) Fraction of area (%) occupied by elastin and collagen in the lung parenchyma of the animals. Data are presented as means ± SEM of 10 fields/rat, 03 rats/group, and analysed by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test.

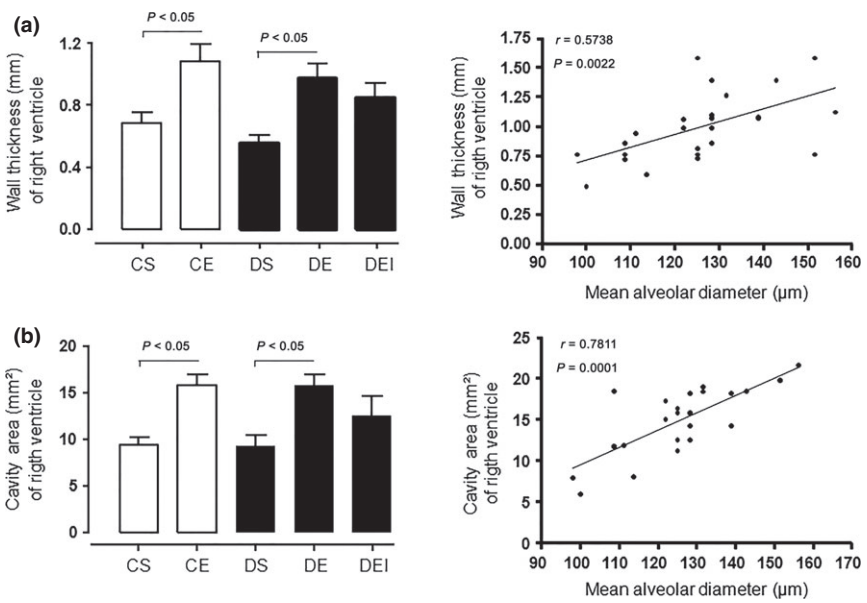

Figure 2 Morphometric analysis of the right ventricle. (a) Wall thickness (mm) and its correlation with mean alveolar diameter (μm). (b) Cavity area (mm^2) and its correlation with mean alveolar diameter (μm). CS, control saline (n = 6); CE, control elastase (n = 7); DS, diabetic saline (n = 8); DE, diabetic elastase (n = 7); DEI, diabetic elastase treated with insulin (n = 7). Morphometric data are presented as means ± SEM and analysed by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test.

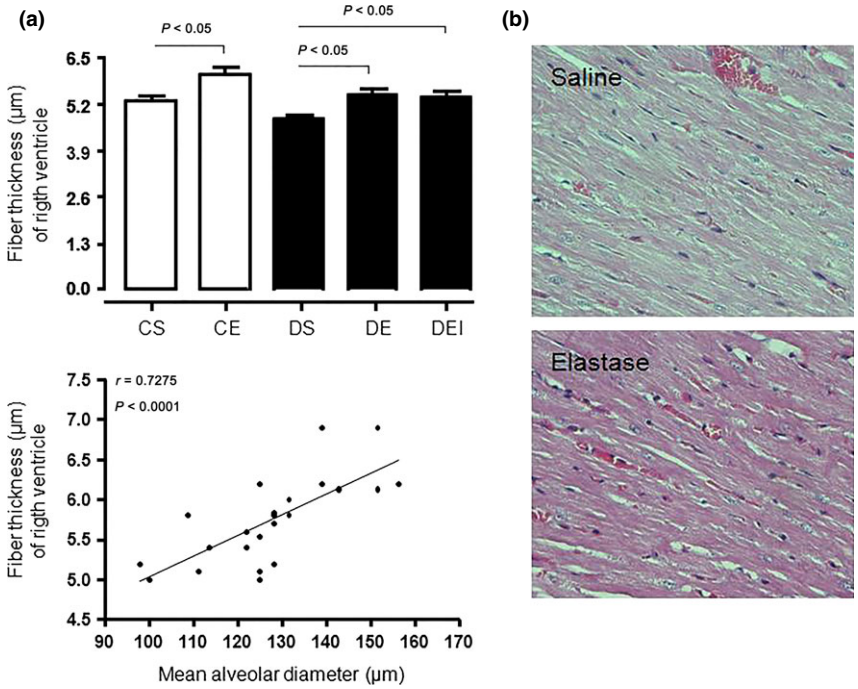

Figure 3 Histomorphometric analysis of the right ventricle. (a) Fibre thickness (µm) and its correlation with mean alveolar diameter (µm). CS, control saline (n = 6); CE, control elastase (n = 7); DS, diabetic saline (n = 8); DE, diabetic elastase (n = 7); DEI, diabetic elastase treated with insulin (n = 7). Histomorphometric data are presented as means ± SEM of 10 fields/rat and analysed by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test. (b) Photomicrographs of right ventricular myocardium histology (HE, 400×) of a saline-instilled rat and an elastase-instilled rat.

Table 2 Morphometric variables of the left ventricle

Parameters	CS (6)	CE (7)	DS (8)	DE (8)	DEI (7)	*P* ANOVA
LV wall thickness (mm)	3.31 ± 0.19	3.60 ± 0.07	1.82 ± 0.12*	3.18 ± 0.19	3.02 ± 0.12	<0.0001
LV cavity area (mm^2)	1.99 ± 0.26	2.00 ± 0.35	7.25 ± 1.10‡,§	4.43 ± 0.92	1.84 ± 0.48	<0.0001
IV septum thickness (mm)	3.28 ± 0.09	3.58 ± 0.13	2.01 ± 0.25‡,†	3.22 ± 0.20	3.61 ± 0.28	<0.0001
LV fibre thickness (µm)	6.07 ± 0.15	6.46 ± 0.17	5.46 ± 0.05$^{\#}$	6.22 ± 0.16	6.18 ± 0.14	<0.0012

Number of animals is indicated in parenthesis. LV, left ventricular; IV, interventricular; CS, control saline; CE, control elastase; DS, diabetic saline; DE, diabetic elastase; DEI, diabetic elastase + insulin. Data are presented as means ± SEM and analysed by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test as follows: *P < 0.0001 *vs.* values in other groups; $^{\ddagger}P$ < 0.0001 *vs.* DEI; $^{\S}P$ < 0.001 *vs.* CS, CE; $^{\dagger}P$ < 0.01 *vs.* CS, and CE; $^{\#}P$ < 0.05 *vs.* values in other groups.

Table 3 Echocardiographic measurements

Parameters	CS (6)	CE (7)	DS (8)	DE (8)	DEI (7)	*P* ANOVA
LV mass (g)	0.90 ± 0.02	0.94 ± 0.04	0.83 ± 0.02	0.87 ± 0.03	0.86 ± 0.02	0.0589
LVs diameter (mm)	3.25 ± 0.31	2.86 ± 0.19	3.18 ± 0.19	3.90 ± 0.30	3.47 ± 0.32	0.1114
LVd diameter (mm)	6.53 ± 0.13	6.26 ± 0.30	6.10 ± 0.27	6.65 ± 0.39	6.24 ± 0.44	0.7692
IVSd thickness (mm)	1.06 ± 0.08	1.00 ± 0.05	1.07 ± 0.09	1.17 ± 0.13	0.88 ± 0.05	0.2587
PWd thickness (mm)	1.25 ± 0.14	1.16 ± 0.08	1.21 ± 0.12	1.17 ± 0.11	1.00 ± 0.03	0.5308
LV fractional shortening (%)	53.60 ± 3.12	54.62 ± 2.10	43.16 ± 1.37*	44.50 ± 1.89†	44.75 ± 2.28†	0.0009
LViv relaxation time (ms)	23.67 ± 1.68	26.25 ± 2.13	31.29 ± 1.44*	23.57 ± 1.8	27.29 ± 1.60	0.0304
LV ejection fraction (%)	85.50 ± 3.53	89.00 ± 1.60	81.71 ± 2.49	81.42 ± 1.19	80.57 ± 2.69	0.0844

Number of animals is indicated in parenthesis. LV, left ventricular; s, end systolic; d, end diastolic; IVS, interventricular septal; PW, posterior wall; iv, isovolumetric; CS, control saline; CE, control elastase; DS, diabetic saline; DE, diabetic elastase; DEI, diabetic elastase + insulin. Data are presented as means ± SEM and analysed by one-way ANOVA followed by the Tukey–Kramer multiple comparisons test as follows: *P < 0.05 *vs.* values in CS; $^{\dagger}P$ < 0.05 *vs.* values in CE.

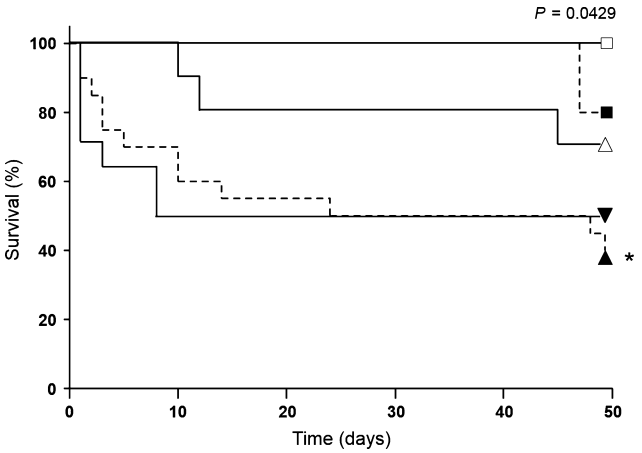

Figure 4 Kaplan–Meier survival analysis of animals. Control saline (□, $n = 6$); control elastase (△, $n = 10$); diabetic saline (■, $n = 10$); diabetic elastase (▲, $n = 19$); diabetic elastase treated with insulin (▼, $n = 14$). *$P < 0.05$ comparing diabetic-elastase rats with diabetic-saline rats.

As summarized in Table 3, the echocardiographic analysis shows that diabetic rats (DS, DE and DEI) exhibited a 20% reduction in LV fractional shortening compared with the respective control group (CS, CE). LViv relaxation time increased 30% in DS rats compared with CS. No differences were observed in LV mass, LVs and LVd diameters, IVSd thickness, PWd thickness and LV ejection fraction among groups.

The survival analysis of the animals showed that there was a significant difference between elastase-treated diabetic rats which exhibited 40% survival compared with saline-treated diabetic rats which presented 80% survival. Data are illustrated in Figure 4.

Discussion

Data presented suggest that RV hypertrophy secondary to emphysema is not affected by alloxan diabetes. The suggestion is supported by the following observations. First, similar increases in RV wall thickness and cavity area, accompanied by an increase in RV cardiomyocyte thickness, were observed in elastase-instilled control non-diabetic and diabetic rats. Second, these alterations are positively correlated with increases in mean alveolar diameter, which were equivalent in both groups of rats. Third, RV hypertrophy was not modified by treatment of diabetic rats with insulin.

It has been previously demonstrated that insulin modulates the inflammatory and repair responses in elastase-induced emphysema in diabetic rats (Di Petta *et al.* 2011). However, as demonstrated herein, the magnitude of the emphysematous lesion and the associated RV hypertrophy was not modified by the presence of diabetes or by treatment of diabetic rats with insulin. It should be considered that in the former study (Di Petta *et al.* 2011), the induced lesions are of mild intensity, whereas in the present study, rats exhibited a moderate-to-severe emphysema to ensure the development of RV hypertrophy. In this condition, the fine adjustment of insulin on the early response to elastase limiting the intensity of the lesion was not observed, as demonstrated by the values attained in mean alveolar diameter as well as in elastic and collagen fibres density, which were equivalent in control non-diabetic and diabetic rats.

The dose and timing of insulin treatment were chosen based on its ability to restore the initial inflammatory response to elastase instillation into the lung of diabetic rats, as previously demonstrated (Di Petta *et al.* 2011). Despite an average 25% reduction in the levels of blood glucose after treatment of diabetic rats with insulin, animals still were hyperglycaemic when compared with non-diabetic control rats. However, diabetic rats treated with insulin presented 70% in body weight gain, whereas non-treated diabetic rats exhibited only 25%, at 60-day interval.

It is well recognized that cardiovascular complications of COPD are frequently observed in the advanced stage of the disease, with pulmonary hypertension being the best described consequence of lung disease. Mild-to-moderate pulmonary hypertension is a common complication, may first appear during exercise and is associated with increased risk of exacerbation (Kessler *et al.* 1999). Pulmonary hypertension is the major determinant of cor pulmonale (Burrows *et al.* 1972; Incalzi *et al.* 1999), characterized by RV hypertrophy, dilatation or both and has been described in COPD patients with severe hypoxaemia (Han *et al.* 2007). In fact, concentric RV hypertrophy is already present in COPD patients with normoxia or mild hypoxia as an early sign of adaptation of the RV to intermittent pressure overload (Vonk-Noordegraaf *et al.* 2005). In addition, as previously demonstrated in the elastase-treated hamster model (Avelar *et al.* 2005), data presented herein suggest that the elastase-treated rat model has some features in common with RV hypertrophy secondary to emphysema, including increases in mean alveolar diameter, RV wall thickness and cavity area.

In the presence of RV hypertrophy, the thickness of LV wall, IV septum and LV cardiomyocyte was not modified in the elastase-instilled groups, including diabetic and control rats, as depicted by the morphometric analysis. In addition, LV ejection fraction did not differ among groups, as demonstrated by echocardiography. Although LV ejection fraction is frequently decreased in patients with emphysema, analyses of magnetic resonance images of the heart in patients with COPD and healthy subjects are in support of the hypothesis that flattening of the IV septum during systole might explain the relatively normal LV ejection fraction in patients with marked RV hypertrophy (Vonk Noordegraaf *et al.* 1997).

Notwithstanding this, morphological and functional changes in the left ventricle of diabetic rats without emphysema suggest the presence of a cardiomyopathy associated with type 1 diabetes. As demonstrated herein, a reduction in LV wall and IV septum thickness, cardiomyocyte thickness, an increase in cavity area, increased LV isovolumetric relaxation time and a decrease in LV fractional shortening were observed in diabetic rats without emphysema, characterizing

the diabetic cardiomyopathy. Because diabetic rats with emphysema also demonstrated a decrease in LV fractional shortening but normal LV isovolumetric relaxation time, compared with non-diabetic control rats, the suggestion is that RV hypertrophy might prevail over the diabetic cardiomyopathy. Indeed, the presence of diabetic cardiomyopathy, assessed by echocardiography, magnetic resonance imaging and histology, has been consistently demonstrated in the streptozotocin-induced diabetes model in rats (Hoit *et al.* 1999; Mihm *et al.* 2001; Akula *et al.* 2003; Borges *et al.* 2006; Weytjens *et al.* 2008, 2010; van den Brom *et al.* 2009, 2010).

Heart failure is commonly associated with diabetes mellitus. Patients with diabetes develop a cardiomyopathy that is independent of coronary artery disease or hypertension and contributes to the increased morbidity and mortality (Zarich *et al.* 1988; Riggs & Transue 1990; Boyer *et al.* 2004).

Evidence reviewed by Haire-Joshu *et al.* (1999) indicates that there are consistent results from both cross-sectional and prospective studies showing enhanced risk for micro- and macrovascular disease and premature mortality from the combination of smoking and diabetes. As demonstrated herein, survival rate decreased from 80% in diabetic rats to 40% in rats which presented both chronic diseases – diabetes and pulmonary emphysema.

In conclusion, alloxan diabetes did not affect RV hypertrophy secondary to emphysema, even in the presence of insulin. Diabetes *per se* induced left ventricular dysfunction, which was less evident in the presence of RV hypertrophy. Survival rate was substantially reduced as a consequence, at least in part, of the coexistence of RV hypertrophy and diabetic cardiomyopathy.

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Author contribution

ADP involved in study conception and design, analysis and interpretation of the data, and drafting of the manuscript and served as principal author. RS, VLC, VMCS and CLF participated in data collection and review of the manuscript. LFPM and PS involved in study conception and design, data analysis and interpretation and drafting and reviewing of the manuscript for important intellectual content. All authors participated in the approval of the manuscript.

Conflict of interest

There is no conflict of interest from any of the authors. The sponsor had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.

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