Experimental diabetes mellitus: oxidative stress and changes in lung structure*

Luiz Alberto Forgiarini Junior, Nélson Alexandre Kretzmann, Marilene Porawski, Alexandre Simões Dias, Norma Anair Possa Marroni

Abstract

Diabetes mellitus is an endocrine/metabolic disorder characterized by hyperglycemia. Its impact on the respiratory system is characterized by functional changes and alterations in gas exchange. The objective of this study was to evaluate the increase in oxidative stress and the potential damages to the lung structure in an experimental model of streptozotocin-induced diabetes. We conducted histological, biochemical and blood gas analyses in the lungs of diabetic rats. We concluded that the effects of experimental diabetes mellitus include oxidative stress, structural changes in the lung tissue and altered gas exchange.

Keywords: Lung; Diabetes mellitus; Oxidative stress; Diabetes mellitus, experimental; Free radicals.

Resumo

O diabetes mellitus é uma desordem endócrino-metabólica caracterizada pela hiperglicemia. O seu impacto no sistema respiratório é caracterizado por alterações funcionais e na troca gasosa. O objetivo deste estudo foi avaliar o aumento do estresse oxidativo e os possíveis danos na estrutura pulmonar no modelo de diabetes experimental induzido por estreptozotocina. Foram realizadas análises histológicas, bioquímicas e gasométricas no pulmão de ratos diabéticos. Concluiu-se que o estresse oxidativo está presente no diabetes mellitus experimental e que ocorrem alterações estruturais no tecido pulmonar, bem como alterações na troca gasosa.

Descritores: Pulmão; Diabetes mellitus; Estresse oxidativo; Diabetes mellitus experimental; Radicais livres.

The prevalence of diabetes mellitus (DM) has increased in recent years, principally due to the great number of patients with type 2 DM, which is related to the prevalence of obesity and sedentary lifestyle.[1]

Functional abnormalities in the respiratory system, such as reduced lung elastic recoil, lung volumes and diffusing capacity, are caused by DM.[2-4] Various cross-sectional studies[5-7] have shown the effect that type 1 and 2 DM have on pulmonary function tests in adults. It is known that DM is an independent risk factor for the development of sleep apnea,[8] and patients who present DM are more susceptible to contamination through airborne particulate matter.[9]

One of the factors responsible for pulmonary alterations can be oxidative stress. The mechanism responsible for this development is hyperglycemia, which activates the polyol pathway, increasing the production of sorbitol. This increase results in cellular stress that leads to a decrease in the intracellular antioxidant defenses. It can also result in the concentration of the products of advanced glycosylation, thus altering cell function. However, hyperglycemia can also activate nuclear transcription factors, triggering an increase in the expression of the inflammatory mediators. The combination of these mechanisms alters the production of oxidants, causing cellular stress and consequently the structural damage.[10]

With the objective of evaluating the increase of oxidative stress and possible damages to the lung structure caused by DM, the experimental

* Study conducted at the the *Hospital de Clínicas de Porto Alegre* – HCPA, Porto Alegre *Hospital de Clínicas* – Porto Alegre, Brazil.
Correspondence to: Norma Anair Possa Marroni. Rua José Kanan Aranha, 102, Jardim Isabel, CEP 91760-470, Porto Alegre, RS, Brasil.
Tel 55 51 3269-0663. E-mail: nmarroni@terra.com.br
Financial Support: This study received financial support from the *Fundo de Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre* (FIPE-HCPA, Research Incentive Fund of the Porto Alegre *Hospital de Clínicas*).
Submitted: 5 February 2009. Accepted, after review: 16 March 2009.

rat model of streptozotocin-induced DM was developed. Histological techniques were used in order to determine the alterations in the lung structure; biochemical measurements were taken in order to evaluate the oxidative injury, as were blood gas measurements, in order to evaluate gas exchange alterations.

This was a controlled experimental study involving Wistar rats with a mean body weight of 300 g. All animals were treated in accordance with the World Health Organization Ethical Code for Animal Experimentation. The animals were divided into two groups, control and diabetic, each comprising 10 animals. The study period was 60 days, starting on the day the diabetic animals presented glycemia greater than 250 mg/dL. We induced DM using an only intraperitoneal injection of streptozotocin (70 mg/kg; Sigma Chemical, St. Louis, MO, USA).[11] A enzymatic colorimetric assay was used to determine the glycemia.

On day 60 of the experiment, the animals were sacrificed after having been i.p. anesthetized with ketamine (100 mg/kg) and xylazine (50 mg/kg). Subsequently, the thoraco-abdominal region was submitted to trichotomy, and a mid-ventral laparotomy was conducted. Blood from the abdominal aorta was collected in order to evaluate the arterial blood gases. An ABL 700 analyzer (Radiometer, Copenhagen, Denmark) was used to determine PaO_2, $PaCO_2$ and SaO_2. The lungs were removed and fixed in 4% paraformaldehyde for histological analysis and stored at −80°C in order to subsequently quantify the thiobarbituric acid reactive substances (TBARS) and evaluate the activity of the antioxidant enzyme superoxide dismutase (SOD).

In order to conduct the biochemical analysis, the lung tissue was homogenized,[12] after which protein levels were quantified in accordance with the Lowry et al. method.[13] Measurement of the TBARS was conducted as established by Buege & Aust.[14] Determination of the SOD activity was performed according to the technique described by Misra & Fridovich.[15]

The samples for the histological analysis of the lung tissue were collected and stored for 12 h in 10% formaldehyde solution, transferred to 70% alcohol and stained with H&E. The anatomopathological examination was performed in double-blind fashion by a pathologist in the Pathology Laboratory of the Porto Alegre *Hospital de Clínicas*.

Data were analyzed using the program Statistical Package for the Social Sciences, version 13 (SPSS Inc., Chicago, IL, USA). The Student-Newman-Keuls test was used. In all comparisons, the level of significance was set at 5%.

Blood glucose concentration was significantly higher in the diabetic group when compared with the control group, as in the evaluation of pulmonary lipid peroxidation, in which the TBARS concentration was significantly higher in the diabetic animals when compared with the controls. When evaluating the antioxidant enzyme SOD activity in the lung tissue, we observed a significant decrease in the diabetic group when compared with the controls.

In the blood gas analysis, we observed an increased $PaCO_2$ in the diabetic group when compared with the controls, and a decreased PaO_2. There was no difference regarding the SaO_2 between the groups (Table 1).

In histology, we evidenced the presence of intravascular macrophages in the diabetic group, which suggests the presence of inflammatory process. We also observed an increase in the extracellular matrix, expressed by the presence of fibrosis, as well as an increase in the thickness of the alveolocapillary membrane (Figure 1).

In our study, we observed an increase in lung oxidative stress in diabetic rats in relation to the controls, as well as a decrease in the antioxidant enzyme SOD activity. Those data are in accordance with the findings of other authors,[16] who demonstrated the increase of the oxidative stress

Table 1 - Comparison between the control group and the diabetic group in relation the glycemia, lipid peroxidation, superoxide dismutase and blood gas analysis.

Parameters	Control group	Diabetic group
TBARS, nmol/mg protein	0.889 ± 0.17	1.585 ± 0.55*
SOD, IU/mg protein	14.35 ± 3.98**	4.64 ± 2.3
$PaCO_2$, mmHg	46.2 ± 4.6	56.7 ± 9*
PaO_2, mmHg	105.9 ± 9.3**	90.2 ± 17.1
SaO_2, %	97.7 ± 0.4	95.7 ± 1.9

TBARS: thiobarbituric acid reactive substances; and SOD: superoxide dismutase enzyme. Values expressed as mean ± SD. *$p < 0.05$ *vs.* control group. **$p < 0.05$ *vs.* diabetic group.

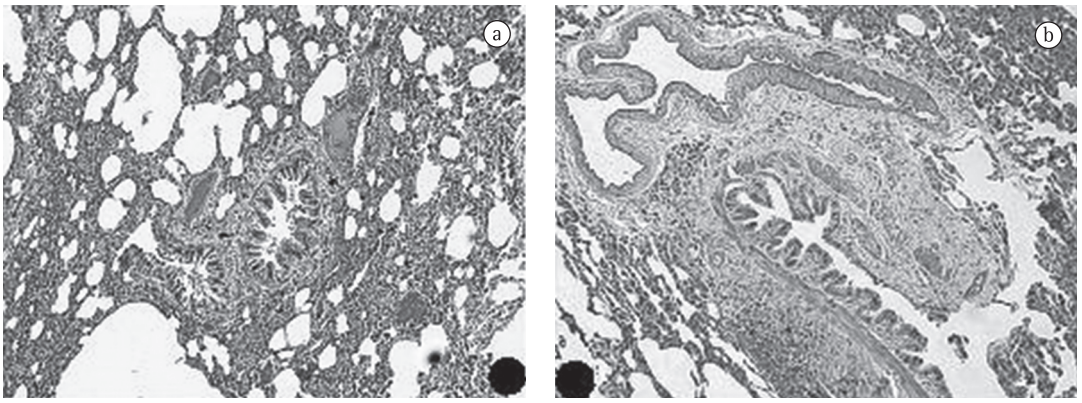

Figure 1 - Photomicrographs of lung tissue samples: a) control; b) diabetic (H&E; magnification, ×200).

and the decrease of the antioxidant enzyme SOD in the lungs of diabetic rats. Those authors also demonstrated that there is an increase in the expression of inducible nitric oxide synthase in the lung tissue of the diabetic animals. The same finding was exposed by another group of authors.[17] However, they used, as an experimental model, alloxan-induced DM in rabbits.

When the blood gas values were analyzed, we observed an alteration in the gas exchange in the diabetic animals, expressed through the decreased PaO_2 and the increased $PaCO_2$. This finding is described as an alteration frequently found in diabetic patients, who present a decreased diffusing capacity. This decrease can be correlated with the glycemic control and the duration of the disease. One of the factors responsible for this alteration can be the increase in the basement membrane thickness. In a study of the pulmonary biopsies of 171 patients,[18] it was concluded that individuals with DM present an increase in basement membrane thickness similar to that seen in asthma patients. This increase was greater in individuals with cancer and pulmonary fibrosis, as well as in patients presenting TB or sarcoidosis.

When we analyzed the histology of the lung tissue, we found an increase in the alveolo-capillary barrier in the diabetic animals. These alterations were also observed by another group of authors,[19] who studied the lungs of diabetic hamsters and concluded that these structural modifications sustain the functional disorders observed in the patients, and that the lung is one of the organs affected by DM. These alterations can be explained by variations in the synthesis of collagen and elastin, as well as by the fact that the phospholipid and phosphatidylcholine content is decreased in proportion to the alveolar surface. We also observe, in the lung tissue of diabetic animals, the occurrence of alterations in the morphology of the type II pneumocytes.[20]

We concluded that oxidative stress is present in experimental DM, and that structural alterations in the pulmonary tissue are observed, as are alterations in blood gases.

References

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001;414(6865):782-7.
2. Davis TM, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: the Fremantle Diabetes Study. Diabetes Res Clin Pract. 2000;50(2):153-9.
3. Goldman MD. Lung dysfunction in diabetes. Diabetes Care. 2003;26(6):1915-8.
4. Walter RE, Beiser A, Givelber RJ, O'Connor GT, Gottlieb DJ. Association between glycemic state and lung function: the Framingham Heart Study. Am J Respir Crit Care Med. 2003;167(6):911-6.
5. Davis TM, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: the Fremantle Diabetes Study. Diabetes Res Clin Pract. 2000;50(2):153-9.
6. Lawlor DA, Ebrahim S, Smith GD. Associations of measures of lung function with insulin resistance and Type 2 diabetes: findings from the British Women's Heart and Health Study. Diabetologia. 2004;47(2):195-203.
7. Lange P, Parner J, Schnohr P, Jensen G. Copenhagen City Heart Study: longitudinal analysis of ventilatory capacity in diabetic and nondiabetic adults. Eur Respir J. 2002;20(6):1406-12.
8. Reichmuth KJ, Austin D, Skatrud JB, Young T. Association of sleep apnea and type II diabetes: a population-based study. Am J Respir Crit Care Med. 2005;172(12):1590-5.

9. Zanobetti A, Schwartz J. Are diabetics more susceptible to the health effects of airborne particles? Am J Respir Crit Care Med. 2001;164(5):831-3.
10. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: a clinical perspective. Endocr Rev. 2001;22(1):36-52.
11. Dias AS, Porawski M, Alonso M, Marroni N, Collado PS, González-Gallego J. Quercetin decreases oxidative stress, NF-kappaB activation, and iNOS overexpression in liver of streptozotocin-induced diabetic rats. J Nutr. 2005;135(10):2299-304.
12. Llesuy SF, Milei J, Molina H, Boveris A, Milei S. Comparison of lipid peroxidation and myocardial damage induced by adriamycin and 4'-epiadriamycin in mice. Tumori. 1985;71(3):241-9.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
14. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-10.
15. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170-5.
16. Hürdağ C, Uyaner I, Gürel E, Utkusavas A, Atukeren P, Demirci C. The effect of alpha-lipoic acid on NOS dispersion in the lung of streptozotocin-induced diabetic rats. J Diabetes Complications. 2008;22(1):56-61.
17. Gumieniczek A, Hopkała H, Wójtowicz Z, Wysocka M. Changes in antioxidant status of lung tissue in experimental diabetes in rabbits. Clin Biochem. 2002;35(2):147-9.
18. Watanabe K, Senju S, Toyoshima H, Yoshida M. Thickness of the basement membrane of bronchial epithelial cells in lung diseases as determined by transbronchial biopsy. Respir Med. 1997;91(7):406-10.
19. Popov D, Simionescu M. Alterations of lung structure in experimental diabetes, and diabetes associated with hyperlipidaemia in hamsters. Eur Respir J. 1997;10(8):1850-8.
20. Hsia CC, Raskin P. The diabetic lung: relevance of alveolar microangiopathy for the use of inhaled insulin. Am J Med. 2005;118(3):205-11.

About the authors

Luiz Alberto Forgiarini Junior
Doctoral Student in Pulmonology. *Universidade Federal do Rio Grande do Sul* – UFRGS, Federal University of Rio Grande do Sul – Porto Alegre, Brazil.

Nélson Alexandre Kretzmann
Doctoral Student. Postgraduate Program in Hepatology, *Universidade Federal de Ciências da Saúde de Porto Alegre* – UFCSPA, Federal University of Health Sciences of Porto Alegre – Porto Alegre, Brazil.

Marilene Porawski
Associate Researcher. Laboratory of Experimental Hepatology and Physiology of the *Hospital de Clinicas de Porto Alegre* – HCPA, Porto Alegre *Hospital de Clínicas* –of the *Universidade Federal do Rio Grande do Sul* – UFRGS, Federal University of Rio Grande do Sul – Porto Alegre, Brazil.

Alexandre Simões Dias
Professor. Professional Masters Program in Rehabilitation and Inclusion, Methodist University Center of the *Instituto Porto Alegre* – IPA, Porto Alegre Institute – Porto Alegre, Brazil.

Norma Anair Possa Marroni
Coordinator. Laboratory of Experimental Hepatology and Physiology, *Universidade Federal do Rio Grande do Sul* – UFRGS, Federal University of Rio Grande do Sul – Porto Alegre, Brazil.