Aminoguanidine reduces oxidative stress and structural lung changes in experimental diabetes mellitus*

Aminoguanidina reduz o estresse oxidativo e as alterações estruturais pulmonares em diabetes mellitus experimental

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Abstract

We evaluated the effect of aminoguanidine on pulmonary oxidative stress and lung structure in an experimental model of diabetes mellitus. Thiobarbituric acid reactive substances (TBARS), histology and arterial blood gases were evaluated in animals with diabetes mellitus (DM group), animals with diabetes mellitus treated with aminoguanidine (DM+AG group), and controls. The TBARS levels were significantly higher in the DM group than in the control and DM+AG groups (2.90 ± 1.12 vs. 1.62 ± 0.28 and 1.68 ± 0.04 nmol/mg protein, respectively), as was $PaCO_2$ when compared with that of the control group (49.2 ± 1.65 vs. 38.12 ± 4.85 mmHg), and PaO_2 was significantly higher in the control group (104.5 ± 6.3 vs. 16.30 ± 69.48 and 97.05±14.02 mmHg, respectively). In this experimental model of diabetes mellitus, aminoguanidine reduced oxidative stress, structural tissue alterations, and gas exchange.

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

Resumo

Avaliamos o efeito da aminoguanidina sobre o estresse oxidativo pulmonar e a estrutura pulmonar em um modelo experimental de diabetes mellitus. Foram determinados *thiobarbituric acid reactive substances* (TBARS, substâncias reativas ao ácido tiobarbitúrico), histologia e gasometria arterial em animais com diabetes mellitus (DM), animais com diabetes mellitus tratados com aminoguanidina (DM+AG) e controles. O nível de TBARS foi significativamente maior no grupo DM que nos grupos controle e DM+AG (2,90 ± 1,12 vs. 1,62 ± 0,28 e 1,68 ± 0,04 nmol/mg proteína, respectivamente), o mesmo ocorrendo com $PaCO_2$ em relação ao grupo controle (49,2 ± 1,65 vs. 38,12 ± 4,85 mmHg), e PaO_2 foi significativamente maior no grupo controle (104,5 ± 6,3 vs. 69,48 ±16,30 e 97,05 ± 14,02 mmHg, respectivamente). Neste modelo experimental de diabetes mellitus, a aminoguanidina reduziu o estresse oxidativo, alterações estruturais teciduais pulmonares e a troca gasosa no modelo experimental.

Descritores: Estresse oxidativo; Diabetes mellitus experimental; Pulmão.

Diabetes mellitus (DM) is a metabolic disorder that affects various organs. It is estimated that 171 million people are affected by the disease. This figure will probably have increased to 366 million by 2030, and the principal factors that promote the development of the disease are the advancing age of the population, the higher number of obese individuals, and greater urbanization. Worldwide, DM is currently the fifth leading cause of death.[1]

Several clinical and experimental studies have identified various pathophysiological mechanisms implicated in the development of DM-induced pulmonary changes, and it seems that acute inflammation is one of the principal triggers of this process.[2,3] Impaired surfactant production and difficulty in absorbing the edema are also present, as are oxidant/antioxidant imbalance, coagulation/fibrinolysis imbalance, and fibrosis/repair imbalance.

The principal factors associated with chronic pulmonary complications are oxidative stress and the generation of advanced glycation end products (AGEs).[4] In a study conducted by our group, oxidative stress was shown to be present in experimental DM, and animals with DM were

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shown to present structural changes in lung tissue, as well as altered blood gas values.[5]

The use of a treatment that blocks AGEs or reduces oxidative and nitrosative stress can be effective in lung disease.

Aminoguanidine prevents the formation of AGEs because its chemical structure contains hydrazine, which reacts with the compounds glyoxal, methylglyoxal, and 3-deoxyglucosone.[6] In addition to the effects of aminoguanidine on advanced glycation, lower doses of aminoguanidine can act specifically, inhibiting the activity of the iNOS enzyme and reducing nitrosative stress.[7] Aminoguanidine can also inhibit the metabolism of histamine, the catabolism of polyamines, and the activity of catalase, as well as potentiating the effects of angiotensin on the production of prostacyclin. However, no experimental studies have demonstrated the effects of aminoguanidine on DM-related pulmonary complications.[8]

The objective of the present study was to evaluate the effect of aminoguanidine on the reduction in oxidative stress and in the potential damage to the lung structure caused by DM. Blood gases were measured in order to evaluate changes in gas exchange. In addition, oxidative damage to lung tissue was evaluated using histological techniques to determine and quantify the changes in the lung structure.

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 three groups: control group; animals with DM (DM group); and animals with DM treated with aminoguanidine (DM+AG group). Each group comprised seven animals. The study period was 60 days, starting on the day the animals with DM presented glycemia greater than 250 mg/dL.

We induced DM using a single i.p. injection of streptozotocin (70 mg/kg; Sigma Chemical, St. Louis, MO, USA).[9] The rats that were treated with aminoguanidine (50 mg/kg, i.p., aminoguanidine hemisulfate salt; Sigma-Aldrich, St. Louis, MO, USA) for the last 30 days of the experiment.[10]

An enzymatic colorimetric assay was used to determine the glycemia, and the animals were sacrificed on day 60 of the experiment. All of the animals were anesthetized through i.p. injection of ketamine (100 mg/kg) and xylazine (50 mg/kg). Subsequently, the thoracoabdominal region was shaved, and a mid-ventral laparotomy was performed. 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 subsequently removed and fixed in 4% paraformaldehyde for histological analysis, portions being stored at –80°C in order to subsequently quantify the thiobarbituric acid reactive substances (TBARS). Measurement of the TBARS was conducted as established by Buege & Aust.[11]

In order to perform lipid peroxidation, the lung tissue was homogenized,[12] after which protein levels were quantified in accordance with the method proposed by Lowry et al.[13]

Picrosirius red staining was used for the histological analysis of the lungs. 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 by the program Statistical Package for the Social Sciences, version 13 (SPSS Inc., Chicago, IL, USA). We used ANOVA to compare the groups, and post hoc comparisons were made using the Student-Newman-Keuls test. The level of significance was set at 5% ($p < 0.05$).

Blood glucose concentrations were significantly higher in the DM group than in the control group. After treatment with aminoguanidine, no reduction in hyperglycemia was observed. Pulmonary lipid peroxidation was greater in the DM group than in the control group, and the use of aminoguanidine significantly reduced the levels of tissue lipid peroxidation, which nearly returned to baseline values. The blood gas analysis revealed an increase in $PaCO_2$, as well as a decrease in PaO_2 and SaO_2, in the DM group when compared with the control group. However, the analysis of the blood gases in animals from the DM+AG group revealed a significant increase in PaO_2 and SaO_2 (Table 1).

Histological analysis revealed the presence of inflammatory cells in the DM and DM+AG groups when compared with the control group. In the DM group, we also observed an increase

Table 1 - Groups compared in terms of glycemia, thiobarbituric acid reactive substances, and blood gases.

Parameters	Group		
	Control	DM	DM+AG
	(n = 7)	(n = 7)	(n = 7)
Glycemia, mg/dL	217.6 ± 11.84	561 ± 35.60*	549.62 ± 42.30**
TBARS, nmol/mg protein	1.62 ± 0.28	2.90 ± 1.12*	1.68 ± 0.40***
PaO_2, mmHg	104.58 ± 6.33	69.48 ± 16.30*	97.05 ± 14.02***
$PaCO_2$, mmHg	38.12 ± 4.85	49.2 ± 1.65*	45.28 ± 10.84
SaO_2, %	97.54 ± 0.55	84.92 ± 7.80*	94.6 ± 3.24***

DM: animals with diabetes mellitus; DM+AG: animals with diabetes mellitus treated with aminoguanidine; and TBARS: thiobarbituric acid reactive substances. Values expressed as mean ± SD. *p < 0.05 (DM group vs. control group). **p < 0.05 (DM+AG group vs. control group). ***p < 0.05 (DM+AG group vs. DM group).

in the extracellular matrix, which determines the presence of tissue fibrosis. In comparison with the control group, the DM group presented significantly greater fibrosis and the DM+AG group presented significantly less fibrosis (Figure 1).

In the present study, increased lipid peroxidation in lung tissue was observed in animals with DM, as reported in a previous study.[5] In the present study, aminoguanidine reduced lipid peroxidation. Although it is an indirect measurement for the evaluation of oxidative stress, lipid peroxidation analysis can indicate cell damage mediated by oxidative and nitrosative agents. Previous studies have demonstrated the ability of aminoguanidine to reduce the apoptosis induced by reactive oxygen species and by lipid peroxidation in cells from the retinas and kidneys of animals with DM.[14,15]

Blood gas analysis revealed changes in gas exchange in the DM group, since PaO_2 and SO_2 were lower and $PaCO_2$ was higher. A similar result was found in patients with DM, since these individuals presented with significantly lower diffusing capacity than that observed for the healthy individuals. One of the potential

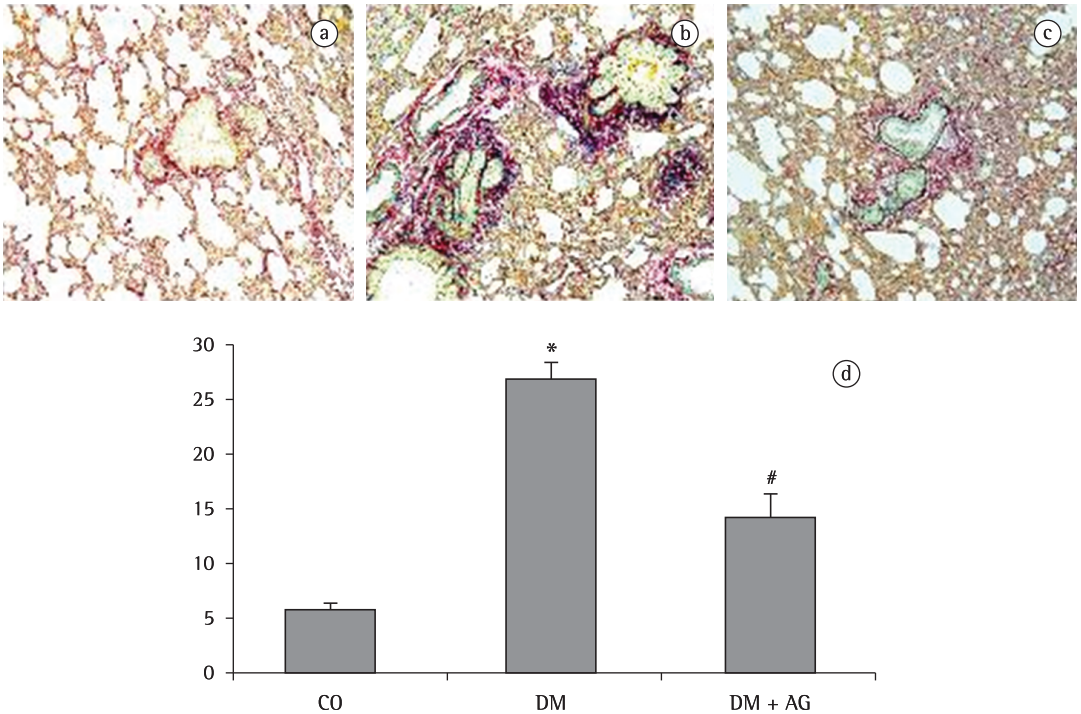

Figure 1 - Photomicrographs of the samples of lung tissue from animals in the different groups: in a), control (CO); in b), diabetes mellitus (DM); and in c), diabetes mellitus treated with aminoguanidine (DM+AG; picrosirius red; magnification, ×200). In d), graph of the quantification of fibrosis (percentage of pixels). *p < 0.001 vs. CO and DM+AG groups; #p < 0.001 vs. DM group.

predictive factors for this clinical change was the microalbuminuria presented by patients, since there was an inverse correlation between microalbuminuria and the diffusing capacity of the lung.[16]

One of the factors that might have been responsible for this alteration in the respiratory system was the increase in the basement membrane thickness, which might be caused by the inter- and intra-molecular bindings with collagen, which are the result of the effect of AGEs. This mechanism can also increase rigidity and resistance to proteolytic digestion, as well as affecting extracellular matrix proteins (fibronectin, type III collagen, type IV collagen, type VI collagen, and laminin) and upregulating cytokine production.[17]

Aminoguanidine proved effective in an experimental model of diabetic nephropathy, in which it reduced the thickening of the glomerular basement membrane.[15] In the present study, aminoguanidine improved the diffusing capacity for blood gases, as evidenced by an increase in PaO_2 and SaO_2. However, there was no reduction in $PaCO_2$ after the treatment. This might be due to the potential changes in the respiratory mechanics and in the effect of the central and peripheral chemoreceptors that are also affected by DM.[5,18]

The histological analysis of the lung tissue revealed an increase in the alveolar-capillary membrane resistance in the animals with DM. These changes were also reported by other authors,[18,19] who demonstrated that DM is the principal factor responsible for changes in lung structure. A study investigating nuclear factor kappa B (NF-κB) inhibition in an experimental model of DM demonstrated that the lungs of animals with DM presented changes in tissue structure, which were caused by increased oxidative stress, and that there was a reduction in DM-induced lung injury when NF-κB was inhibited.[20]

It is possible that aminoguanidine acts as an AGE inhibitor, because it reduces the levels of pulmonary oxidative stress and the changes in lung structure caused by increased collagen synthesis and deposition.

In summary, structural changes in the lung tissue of animals with DM lead to changes in gas exchange, as well as to increased oxidative stress. In the present study, the use of aminoguanidine reduced lipid peroxidation and minimized changes in lung structure (manifested as fibrosis), as well as normalizing the levels of PaO_2 and SaO_2.

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