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Nicorandil ameliorates pulmonary inflammation and fibrosis in a rat model of silicosis

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ABSTRACT

Nicorandil, an antianginal and potassium channel opener agent, has different useful impacts on cardiovascular and respiratory systems. Its effect against silicosis has not been discussed yet, therefore, this is an attempt to decide whether nicorandil can reduce silica-induced lung injury in rats. Silica model was induced by intranasal instillation of silica dust once. Rats were given nicorandil for 56 days after exposure to silica. Results showed that nicorandil significantly alleviated silica-induced inflammation as it decreased the elevated levels of total and differential cell counts, pulmonary edema (revealed by decreased lung/body weight ratio and W/D weight ratio), LDH and total protein levels in BALF. Notably, nicorandil decreased collagen deposition as evidenced by reduction in levels of hydroxyproline and collagen in lung tissues as well as obvious alleviation in silica-induced fibrosis in histopathological findings. Nicorandil effectively reduced the increased expression of NF-κB and iNOS and decreased MPO levels in lung tissues. Moreover, nicorandil abolished oxidative and nitrosative stress via reducing levels of pulmonary MDA and NOx concomitant with elevating levels of pulmonary GSH and SOD. Meanwhile, nicorandil decreased the levels of TNF-α and TGF-β, up regulated Nrf-2 and HO-1 levels in BALF suggesting antioxidant, anti-inflammatory and antifibrotic properties. In summary, nicorandil can confer protection against silica-induced lung inflammation and fibrosis. This impact might be due to its ability to down regulate the production of inflammatory and fibrotic cytokines in addition to restoring oxidant/antioxidant balance.

1. Introduction

Silicosis is a widespread chronic occupational disease; that arises due to exposure to crystalline silica (silicon dioxide), a component of sand or rock. The most susceptible persons to silicosis are those working in mines and construction industries. Lung silicosis is described by severe inflammation, loss of alveolar architecture and marked fibrosis of lungs [1]. There are many pathological similarities between fibrotic reactions in human and rodent lungs. Therefore, rat model is a useful tool to scrutinize pathologic alterations in vivo.

However, the exact mechanism of silicosis is still unclear, it has been accepted that progression of lung fibrosis is accompanied with infiltration of inflammatory cells including eosinophils, neutrophils and macrophages. After exposure to silica particles, macrophages phagocyte these particles and become activated to liberate numerous mediators comprising histamine as well as serotonin which are retained in lung throughout inflammation and mediate cascade of events leading to fibrotic response [2]. Prolonged inflammation develops chronic fibrosis, in which extracellular matrix is replaced by abnormal collagen producing fibroblasts [3]. Previous reports proposed that nitric oxide (NOx) plays an important role in silica-induced injury in lungs and that the expression of inducible nitric oxide synthase (iNOS) is elevated in bronchoalveolar lavage fluid (BALF) of rats after instillation of silica. Also, it has been stated that the inflammatory cytokines may induce production of NOx [4,5]. Earlier studies reported that oxidative stress is involved in the development of pulmonary fibrosis. During inflammation, inflammatory cytokines produce huge quantities of reactive oxygen species (ROS) that might contribute to tissue injury and lead to pulmonary fibrosis. Disturbance in the oxidant/antioxidant hemostasis is observed in lungs of pulmonary fibrosis patients [3].

Nicorandil is a hybrid between nitrates and potassium channel openers, used as antianginal drug. It has proved efficiency in preventing pulmonary arterial pressure induced by monocrotaline [6]; in addition to its prophylactic effect against lung injury induced by cyclophosphamide and ova albumin [7,8]. Nicorandil administration has been documented to decrease oxidative stress via stimulating the opening of KATP channel [9]. Consequently, the usage of nicorandil might be a proper tactic to diminish silica-induced toxicity.

The current study aimed to perceive the possible beneficial effect of nicorandil against silica-induced lung toxicity and to investigate the

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Table 1
Effect of nicorandil (100 mg/kg) on lung/body weight ratio and W/D ratio in silicotic rats.

Groups	Lung/body weight ratio	Lung W/D ratio
Control	3.54 ± 0.11	2.34 ± 0.06
Silica	5.7 ± 0.26*	4.34 ± 0.34*
Silica + nico	3.22 ± 0.04$	2.64 ± 0.11$

Values are expressed as means ± standard error of mean (n = 5). Comparisons were performed using one-way ANOVA followed by Tuckey–Kramer's multiple comparisons post hoc test.
* $p < 0.05$ vs. control.
$ $p < 0.05$ vs. silica group.

probable mechanisms.

2. Materials and methods

2.1. Chemicals

Crystalline silica was provided by U.S. Silica Company (Berkeley Springs, West Virginia, USA) as a gift. Nicorandil was obtained as commercial tablets (Adancor®, Merck, Egypt) and suspended in CMC (0.5%). All chemicals used were of highest grade.

2.2. Animals

Adult male Sprague Dawley rats (220 ± 40 g) were obtained from "Vacsera", Helwan, Egypt. Animals were kept in adjusted conditions of temperature, humidity and systematic 12 h light/dark cycle. Whole experiments were performed in agreement with the protocol accepted by "Research Ethics Committee, Faculty of Pharmacy, Mansoura University" which is in agreement with the guidelines of Laboratory Animal Care (NIH publication no. 85–23, revised 1985).

2.3. Experimental protocol

Silicosis was induced according to the method described by ref. [10]. Concisely, silica particles were suspended in normal saline (50 mg in 0.1 ml saline per rat) and the mixture was shaken well. Also 20,000 IU penicillin was added to the previous mixture. The animals were slightly anesthetized then silica was administered by intranasal instillation [11].

Rats were randomized into 3 groups (n = 10); control, silica and silica + nico. In silica + nico group, rats received the drug once daily (100 mg/kg, orally) [7] for 56 days. Both silica and silica + nico groups were subjected to intranasal instillation of silica once at day 1. On the end day, all animals were weighed then sacrificed after anesthesia. Only five rats of each group were used for collection of bronchoalveolar fluid (BALF), in which the chest was opened and trachea become exposed then cannula was inserted in it. Six milliliters of sterile saline (0.9% w/v) were used to lavage lungs and obtain BALF. Afterwards, BALF was centrifuged at 4 °C and the supernatant was kept at −80 °C for biochemical analysis. Cell pellets residue after centrifugation were suspended in 100 µl saline for quantification of inflammatory cell counts.

Table 2
Effect of nicorandil (100 mg/kg) on total and differential cell counts in BALF.

Groups	Total cell count ($\times 10^4$)	Neutrophils ($\times 10^4$)	Lymphocytes ($\times 10^4$)	Macrophages ($\times 10^4$)	Eosinophils ($\times 10^4$)	Basophils ($\times 10^4$)
Control	92.5 ± 3.7	58.3 ± 2.6	30.7 ± 1.2	1.6 ± 0.6	1.8 ± 0.07	0 ± 0
Silica	835 ± 49*	638 ± 36.1*	167 ± 9.8*	5 ± 0.4*	16.7 ± 0.98*	8.4 ± 0.5*
Silica + nico	250 ± 22.3*,$	165.5 ± 18.5*,$	72 ± 2.6*,$	3.3 ± 2$	5 ± 0.44*,$	4.2 ± 0.42*,$

Values are represented as means ± SEM (n = 5). Analyses were performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test.
* $p < 0.05$ vs. control.
$ $p < 0.05$ vs. silica.

Table 3
Effect of nicorandil (100 mg/kg) on LDH and total protein levels in BALF.

Groups	LDH (U/L)	Total protein (mg/dl)
Control	166.5 ± 13.2	1.4 ± 0.12
Silica	437.2 ± 18.9*	4.2 ± 0.06*
Silica + nico	177.2 ± 22.4$	2.5 ± 0.5$

Values are represented as means ± SEM (n = 5). Analyses were performed using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test.
* $p < 0.05$ vs. control.
$ $p < 0.05$ vs. silica group.

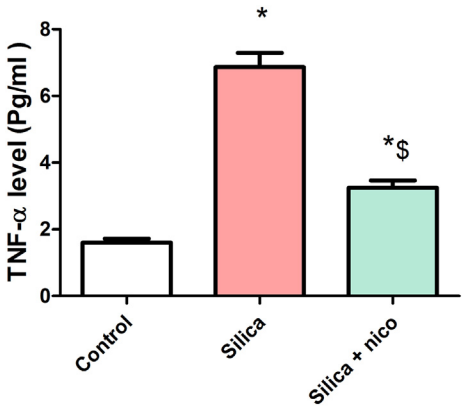

Fig. 1. Effect of nicorandil (100 mg/kg) on TNF-α levels in BALF. Bars are expressed as means ± standard error of mean (n = 5). Comparisons were performed using one-way ANOVA followed by Tukey–Kramer's multiple comparisons post hoc test. *$p < 0.05$ vs. control; $$p < 0.05$ vs. silica group; ANOVA: analysis of variance.

Lungs from the remaining five rats of each group were rapidly excised, rinsed then weighed to obtain lung/body weight ratio. Left lung were homogenized in ice-cold 1.15% KCl (pH = 7.4) to obtain 10% w/v homogenates. After centrifugation of these lung homogenates, the supernatant was stored in −80 °C to be used in further assay of oxidative stress biomarkers. Right lungs were fixed in buffered formalin for histopathological and immunohistochemical analysis.

2.4. Estimation of pulmonary edema

To evaluate pulmonary edema, these parameters were estimated.

2.4.1. Lung/body weight ratio

It was deduced by the following formula (right lung weight + left lung weight divided by body weight).

2.4.2. Lung wet/dry (W/D) weight ratio

Part of left lung was isolated then weighed to estimate (wet) weight, then kept in oven with temperature 80 °C overnight to estimate (dry) weight.