EFFECTS OF EARLY DEXAMETHASONE TREATMENT ON SEVERAL MARKERS OF INFLAMMATION AND FIBROSIS IN AN ANIMAL MODEL OF LUNG SILICOSIS IN RATS – A PILOT STUDY

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Abstract

Lung silicosis is primarily caused by inhalation of particles of silicon oxide (silica). Despite a huge progress in understanding the interactions among the pathomechanisms of lung silicosis in the last years, there is a lack of effective therapy. With respect to a wide therapeutic action of corticosteroids, the purpose of this pilot study was to evaluate early effects of dexamethasone on several markers of inflammation and lung fibrosis in a rat model of silicosis. The silicosis model was induced by a single transoral intratracheal instillation of silica (50 mg/ml/animal), while the controls received an equivalent volume of sterile saline. The treatment with intraperitoneal dexamethasone initiated the next day after the silica instillation and was given 2-times a week at a dose of 1 mg/kg, while the controls received an equivalent volume of saline. The animals were euthanized 14 or 28 days after the treatment onset. Total and differential counts of leukocytes in the blood and bronchoalveolar lavage (BAL) fluid were determined. The presence of collagen in the bronchioles and lung vessels was detected by Sirius red staining and a smooth muscle mass was detected by smooth muscle actin. In comparison to saline, the instillation of silica increased the total count of circulating leukocytes after 14 and 28 days of the experiment (both $p<0.05$), which was associated with higher counts of lymphocytes ($p<0.05$ after 14 days, $p>0.05$ after 28 days) and slight but non-significant increases in neutrophils and eosinophils (both $p>0.05$). Although the total cell count in the BAL fluid did not change significantly, the percentages and absolute counts of neutrophils, eosinophils, and lymphocytes ($p<0.05$, $p<0.01$ or $p<0.001$) elevated after 14 and 28 days of the experiment. Silica induced an accumulation of collagen in the bronchioles ($p<0.001$ after both 14 and 28 days) and pulmonary vessels ($p<0.01$ after both 14 and 28 days) and elevated a formation of smooth muscle mass ($p<0.05$ after 14 days, $p<0.01$ or $p<0.001$ after 28 days). Treatment with dexamethasone decreased circulating leukocytes ($p<0.01$) and lymphocytes ($p<0.001$) and increased neutrophils ($p<0.05$), which was associated with a slightly decreased total cell count in BAL ($p>0.05$), decline in lymphocytes ($p<0.01$), and slight decreases in neutrophils and eosinophils after 28 days of the treatment. Moreover, dexamethasone reduced the accumulation of collagen ($p<0.01$ after 14 days and $p<0.001$ after 28 days) and the formation of smooth muscle mass ($p<0.01$ for bronchioles and $p>0.05$ for vessels after 24 days, $p<0.001$ for both bronchioles and vessels after 28 days). In conclusion, early dexamethasone treatment mitigated silica-induced granulocytic-lymphocytic inflammation and decreased a formation of collagen and smooth muscle mass in the bronchiolar and vascular walls, demonstrating a therapeutic potential of dexamethasone in the lung silicosis.

Key words: lung silicosis, animal model, inflammation, oxidative stress, dexamethasone

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INTRODUCTION

Lung silicosis, a serious fibrotizing disease, is primarily caused by massive or long-term inhalation of particles of silicon oxide (or silica) (1). Background of the pathological changes in silica-injured lungs has not been completely understood; however, they presumably result from direct cytotoxic effect of silica on macrophages, activation of macrophage surface receptors, lysosomal rupture, generation of reactive oxygen species (ROS), activation of inflammasome, overproduction of cytokines and chemokines, cell apoptosis/pyroptosis, and ongoing lung fibrosis (2-5). Nevertheless, despite a huge progress in understanding the complex interactions among the proinflammatory, prooxidant and profibrotic mechanisms in the pathogenesis of lung silicosis in the last years, there is still a lack of effective therapy. Various anti-inflammatory, antioxidant, and anti-fibrotic treatments including herbal compounds have been tested in experimental models of lung silicosis as well as in patients suffering from lung silicosis; however, effects of the given treatments were often contradictory (6-8).

With respect to a wide therapeutic action of a long-acting corticosteroid dexamethasone, the purpose of this pilot study was to evaluate early effects of dexamethasone on several markers of inflammation and lung fibrosis in a rat model of silicosis. We have presumed that early administration of dexamethasone may positively influence a development of the inflammation and thereby could mitigate the inflammation-associated lung fibrosis.

METHODS

This study was approved by the National Veterinary Board of Slovakia (Ro-1068/19-221/3) and the local Ethical Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava (EK 6/2019). Adult male Wistar rats of the mean body weight of 270-320 g were supplied by a certified animal breeding station (VELAZ, Czech Republic). Animals were kept in a certified faculty animal house where they underwent a 7-day quarantine and acclimation, with available food and water *ad libitum*.

Then, the animals (n=48 in total) were divided into three groups. In Sil group of animals, a model of lung silicosis was induced by a single transoral intratracheal instillation of silica (50 mg/ml/animal) in the inhalation anesthesia (4% isoflurane), while the control animals (Sal group) received an equivalent volume of sterile saline. The instillation of silica/saline was performed on an angled board by hooking the front teeth while positioning of the tongue laterally enables the instillation of silica suspension/saline by micropippetor into the trachea through opened vocal cords during inspiration (modified according to 9,10). In a half of the silica-instilled animals, the treatment with intraperitoneal dexamethasone (Dexamed, Medochemie, Cyprus) initiated the next day after the silica instillation and was given twice a week at a dose of 1 mg/kg (Dex group). The animals were euthanized 14 days (Sil14, Sal14, and Dex14 subgroups, each of n=8) or 28 days (Sil28, Sal28, and Dex28 subgroups, each of n=8) after the treatment onset by an overdosing of anesthetics (Zoletil, Virbac, France). The analysis after 14 days enables an observation of inflammatory changes, while the analysis after 28 days reveals an observation of early fibrotic changes (11,12).

Sample of blood was taken by a direct puncture of the heart. The total count of leukocytes was determined by a veterinary hematologic analyzer (Sysmex XT-2000iV, Japan). The differential count of leukocytes was estimated microscopically after staining by May-Grünwald/Giemsa-Romanowski and expressed in percents (%) and in absolute values (x 10^9/L).

The lung was excised. The left lung was lavaged with saline (0.9% NaCl, 2x10 ml/kg b.w.). The total count of cells was measured by a cell analyzer Countess (Thermo Fisher Scientific, USA). Then, the BAL fluid was centrifuged at 1500 rpm for 15 min. The differential

count of cells in the BAL fluid sediment was evaluated microscopically after staining by May-Grünwald/Giemsa-Romanowski and expressed in percents (%) and in absolute values (x 10^3/mL).

The right lung was washed by saline and stored in 4% formaldehyde. The presence of collagen (by Sirius red staining) and smooth muscle mass (detection of smooth muscle actin, SMA) in the walls of bronchioles and vessels of the right lung was verified immunohistochemically by a qualified histologist. The lung tissue was dehydrated through the series of graded ethanol baths, infiltrated with paraffin and cut into 4µm thick sections.

Detection of collagen by Sirius red: after deparaffinization the slides were firstly stained with Weigert's hematoxylin for visualization of the cell nuclei. After washing in running tap water (10 min), the slides were stained in picro-sirius red solution (Millipore Sigma, USA) for 1 hour. After washing twice in acidic distilled water, and dehydration in 100% ethanol, the slides were mounted in Entellan (Millipore Sigma, USA). The result of staining was red collagen fibers on pale yellow background. The slides were viewed with an Olympus BX43 microscope (Olympus, Japan). The image capture and airway wall thickness measurement was performed with the Quick Photo Micro software, version 3.2 (Olympus, Japan).

Detection of smooth muscle mass by SMA: after deparaffinization, revitalisation and rehydratation, the tissue slides were treated with 3% H_2O_2 solution for 10 min for blocking endogenous peroxidases. Washing with Tris buffer was used after each handling step. The sections were incubated with the primary rabbit polyclonal smooth muscle actin (SMA; 1:300, Cell Signaling Technology, USA) for 30 min at room temperature. The slides were then incubated by sequential 10 min incubation with LSAB2 System-HRP for use on rat specimens (Dako, Denmark), which detects primary mouse and rabbit antibodies. The sections were then counterstained with Mayer´s hematoxylin (Himedia Laboratories, India) and mounted with an Entellan (Merck, USA). The sections were viewed with an Olympus BX43 microscope (Olympus, Japan) equipped with a photo camera Canon E0S 2000D. The Quick Photo Micro program, version 3.2 (Olympus, Japan) was used to image capture the sections and measure the thickness of the smooth muscle layer in the bronchial wall and the *tunica media* in the blood vessel wall (expressed by dark brown cytoplasm of SMA-positive cells).

For analysis of the data, the statistical package SYSTAT for Windows (Systat Software Inc., USA) was used. Differences among the groups were analyzed by one-way ANOVA with posthoc Fisher's LSD test. A value of $p<0.05$ was considered statistically significant. The data are expressed as means ± SD.

RESULTS

Total count of leukocytes in the blood

Determination by a cell counter revealed that the total count of leukocytes did not change in the saline-instilled animals (3.68±1.06 x 10^9/L in Sal28 vs 3.65±1.24 x 10^9/L in Sal14, $p>0.05$). Contrary, significant increases were found for the silica-instilled vs the saline-instilled animals after 14 days (5.37±1.19 x 10^9/L in Sil14 vs 3.65±1.24 x 10^9/L in Sal14, $p<0.05$) as well as after 28 days of the experiment (4.96±1.18 x 10^9/L in Sil28 vs 3.68±1.06 x 10^9/L in Sal28 $p<0.05$). The administration of dexamethasone treatment caused a decrease in circulating leukocytes after 14 days (3.22±1.31 x 10^9/L in Dex14 vs 5.37±1.19 x 10^9/L in Sil14, $p<0.01$) as well as after 28 days of the experiment in comparison to the non-treated silica-instilled animals (2.98±1.31 x 10^9/L in Dex28 vs 4.96±1.18 x 10^9/L in Sil28, $p<0.01$).

Differential count of leukocytes in the blood

Analysis of the differential count of leukocytes in the blood did show any significant differences between the Sal28 vs Sal14 groups (all $p>0.05$) neither in percentual nor in absolute expressions (Fig. 1, Table 1). Contrary, the instillation of silica resulted in a significant

decrease in a percentage (p<0.05) but non-significant decrease expressed in absolute numbers (p>0.05) of circulating monocytes in Sil14 vs Sal14 group (Fig. 1, Table 1). In addition, silica instillation after 14 days caused increases in absolute numbers of blood lymphocytes (p<0.05), neutrophils (p=0.056), and eosinophils (p>0.05) compared to the saline-instilled animals (Table 1). After 28 days, relative (p<0.05, Fig. 1) and absolute (p>0.05, Table 1) values of monocytes in the blood elevated in Sil28 vs Sil14 group. Similar but non-significant trend was also observed for percentages of eosinophils and lymphocytes while neutrophils had a slight tendency to decrease (all p>0.05, Fig. 1). These changes were consistent with changes in the absolute values of eosinophils and neutrophils, but the absolute counts of lymphocytes slightly decreased (all p>0.05, Table 1).

The administration of dexamethasone after 14 days resulted in increased percentages of circulating neutrophils and monocytes and decreased lymphocytes in comparison to the non-treated animals (Dex14 vs Sil14, all p<0.05, Fig. 1). This trend is consistent with increases in absolute numbers of neutrophils, monocytes, and eosinophils (all p>0.05), while the number of circulating lymphocytes declined (p<0.001, Table 1). After 28 days of the treatment delivery, the elevated percentages of circulating neutrophils and decreased lymphocytes (both p<0.05) were found in dexamethasone-treated compared to thenon-treated animals (Dex28 vs Sil28, Fig. 1). The analysis of absolute numbers confirmed a decrease in lymphocytes (p<0.001) and an increase in neutrophils (p<0.05) as well as slightly decreased counts of monocytes and eosinophils (both p>0.05) in Dex28 vs Sil28 groups (Table 1).

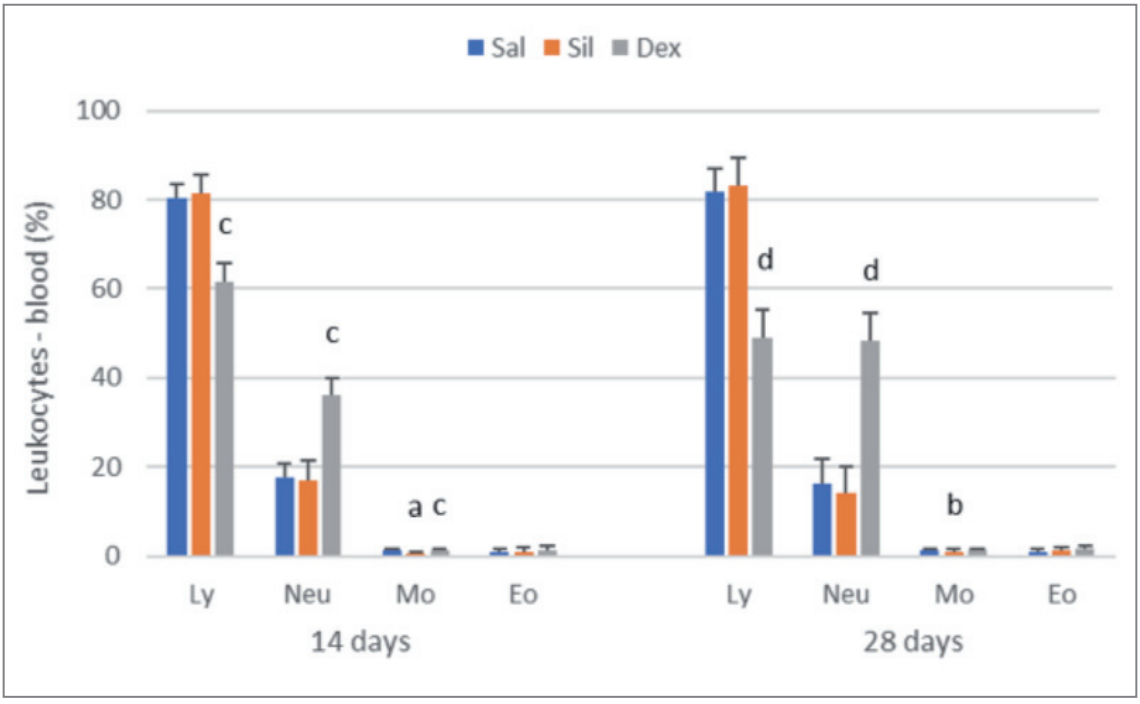


Fig. 1 Differential count of leukocytes in the blood (%) in the control groups with saline instead of silica (Sal), in the silica-injected and non-treated animals (Sil), and in the silica-injected and dexamethasone-treated animals (Dex) after 14 or 28 days of the treatment delivery.

Abbreviations: Ly: lymphocytes, Neu: neutrophils, Mo: monocytes, Eo: eosinophils. Statistical differences among the groups: [a] p<0.05 for Sil14 vs Sal14; [b] p<0.05 for Sil28 vs Sil14; [c] p<0.05 for Dex14 vs Sil14; [d] p<0.05 for Dex28 vs Sil28.

Table 1 Absolute counts of leukocytes in the blood and in the BAL fluid in the control groups with saline instead of silica (Sal), in the silica-injected and non-treated animals (Sil), and in the silica-injected and dexamethasone-treated animals (Dex) after 14 or 28 days of the treatment delivery.

	14 days			28 days		
Leukocytes in the blood (x 10^9/L)						
	Sal14	**Sil14**	**Dex14**	**Sal28**	**Sil28**	**Dex28**
Ly	2.95±1.07	4.33±0.84 [a]	1.99±0.87 [g]	3.21±0.83	4.18±1.23	1.42±0.53 [j]
Neu	0.63±0.19	0.97±0.40	1.14±0.40	0.61±0.22	0.67±0.22	1.49±0.79 [h]
Mo	0.04±0.02	0.03±0.01	0.04±0.02	0.05±0.03	0.05±0.02	0.03±0.01
Eo	0.03±0.03	0.04±0.04	0.05±0.05	0.03±0.04	0.06±0.04	0.04±0.02
Leukocytes in the BAL fluid (x 10^3/mL)						
	Sal14	**Sil14**	**Dex14**	**Sal28**	**Sil28**	**Dex28**
Ma	97.7±41.5	95.4±37.7	146±38.8 [f]	107±45.9	102±43.8	84.3±42.4
Neu	2.29±1.66	14.3±3.83 [c]	35.1±49.9	3.51±1.35	26.8±24.1 [d]	8.68±5.23
Eo	0.25±0.32	0.81±0.23 [b]	1.47±1.57	0.23±0.23	2.39±2.17 [d]	0.62±0.35 [h]
Ly	0.99±0.64	2.35±1.70	4.06±2.10	0.71±0.27	4.92±2.89 [e]	1.40±0.87 i

Abbreviations: Ly: lymphocytes, Neu: neutrophils, Mo: monocytes, Eo: eosinophils, Ma: macrophages. Statistical differences among the groups: [a] $p<0.05$, [b] $p<0.01$ and [c] $p<0.001$ for Sil14 vs Sal14; [d] $p<0.05$ and [e] $p<0.01$ for Sil28 vs Sal28; [f] $p<0.05$ and [g] $p<0.001$ for Dex14 vs Sil14; [h] $p<0.05$, [i] $p<0.01$ and [j] $p<0.001$ for Dex28 vs Sil28.

Total count of cells in the BAL fluid

No significant differences in total counts of cells in the BAL fluid in the saline-instilled animals after 28 days vs 14 days were found (111.7±47.1 x 10^3/mL in Sal28 vs 101.3±43.6 x 10^3/mL in Sal14, $p>0.05$). Similarly, no significant differences were detected for comparison of the silica-instilled vs the saline-instilled animals after 14 days (112.9±35.5 x 10^3/mL in Sil14 vs 101.3±43.6 x 10^3/mL in Sal14, $p>0.05$) or after 28 days of the experiment (135.7±68.5 x 10^3/mL in Sil28 vs 111.7±47.1 x 10^3/mL in Sal28 $p>0.05$). Dexamethasone treatment showed a trend to increase count of BAL cells after 14 days (186.3±43.6 x 10^3/mL in Dex14 vs 112.9±35.5 x 10^3/mL in Sil14, $p<0.05$) but non-significantly decreased the total cell count after 28 days of the experiment in comparison to the non-treated silica-instilled animals (95.0±43.8 x 10^3/mL in Dex14 vs 135.7±68.5 x 10^3/mL in Sil28, $p>0.05$).

Differential count of leukocytes in the BAL fluid

Differential count of leukocytes in the BAL fluid did not change significantly between the Sal14 vs Sal28 groups (all $p>0.05$, Fig. 2 and Table 1). Contrary, in response to the instillation of silica an increase in lymphocyte percentage ($p<0.05$) as well as a slight elevation in an absolute count ($p=0.065$) of lymphocytes was observed in Sil28 vs Sil14 group (Fig. 2, Table 1).

More obvious changes were determined for the silica-instilled animals vs the saline-instilled controls after 14 days of the treatment delivery (Sil14 vs Sal14) as expressed by a decrease in

percentage of macrophages ($p<0.001$), while the absolute numbers of macrophages remained unchanged ($p>0.05$). In addition, silica instillation caused increases in percentages and absolute numbers of neutrophils (both $p<0.001$), increases in percentages ($p<0.001$) and absolute numbers ($p<0.01$) of eosinophils, and elevation in percentage ($p<0.05$) and absolute counts ($p=0.054$) of lymphocytes in Sil14 vs Sal14 groups (Fig. 2, Table 1).

Similar differences were also observed after 28 days of the treatment delivery where the percentage of macrophages was lower ($p<0.001$), while the absolute numbers of macrophages remained unchanged ($p>0.05$). However, increases in relative and absolute counts of other cells persisted till the end of the experiment as demonstrated on increases in the percentage ($p<0.001$) and absolute numbers ($p<0.05$) of neutrophils, percentage and absolute numbers of eosinophils (both $p<0.05$), and percentage ($p<0.001$) and absolute numbers ($p<0.01$) of lymphocytes in Sil28 vs Sal28 group (Fig. 2, Table 1).

The treatment with dexamethasone had no effect on percentages of BAL cells after 14 days (for Dex14 vs Sil14 all $p>0.05$), but revealed slight increases in absolute counts of macrophages ($p<0.05$), neutrophils, eosinophils, and lymphocytes (all $p>0.05$). However, the situation dramatically changed after 28 days of the treatment delivery when a percentage of macrophages increased ($p<0.05$) but the absolute number of macrophages slightly decreased ($p>0.05$). In addition, in the comparison of Dex28 vs Sil28 groups we found a decrease in percentage and absolute counts of lymphocytes (both $p<0.01$), a slight but non-significant decreases in percentages of neutrophils ($p=0.061$) and eosinophils ($p=0.057$), which was associated with decreases in their absolute values ($p<0.05$ for eosinophils and $p=0.056$ for neutrophils) (Fig. 2, Table 1).

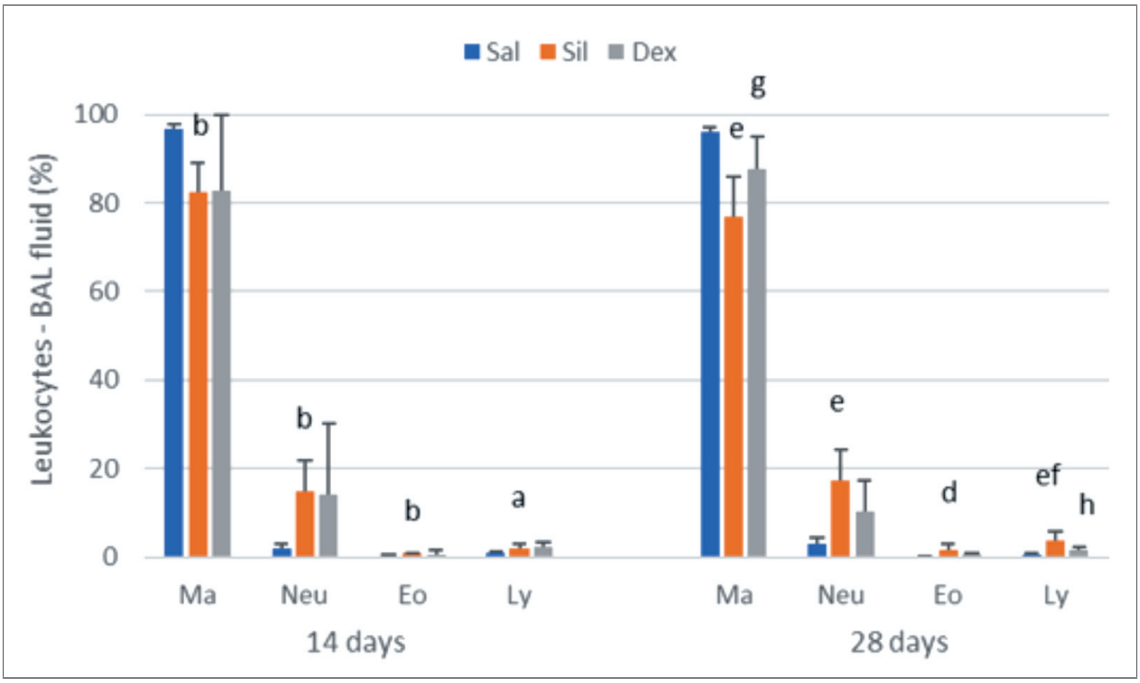


Fig. 2. Differential count of leukocytes in the BAL fluid (%) in the control groups with saline instead of silica (Sal), in the silica-injected and non-treated animals (Sil), and in the silica-injected and dexamethasone-treated animals (Dex) after 14 or 28 days of the the treatment delivery.

Abbreviations: Ma: macrophages, Neu: neutrophils, Eo: eosinophils, Ly: lymphocytes. Statistical differences among the groups: [a] $p<0.05$ and [b] $p<0.001$ for Sil14 vs Sal14; [d] $p<0.05$ and [e] $p<0.001$ for Sil28 vs Sal28; [f] $p<0.05$ for Sil28 vs Sil14; [g] $p<0.05$ and [h] $p<0.01$ for Dex28 vs Sil28.

Collagen accumulation in the bronchioles and pulmonary vessels

Immunohistochemical analysis by Sirius red staining showed a slightly increased accumulation of collagen in bronchioles and pulmonary vessels in Sal28 vs Sal14 groups (both $p<0.05$). Similar trend of higher collagen accumulation was detected in Sil28 vs Sil14 groups; however, the differences were not statistically significant (both $p>0.05$) (Fig. 3). The production of collagen obviously raised in the silica-instilled animals in comparison to the controls (Sil vs Sal) where the increase was statistically significant in bronchioles ($p<0.001$) and vessels ($p<0.01$) after both 14 days and 28 days of the treatment delivery. The treatment with dexamethasone reduced formation of collagen compared to the non-treated animals already after 14 days of the experiment ($p<0.01$ for both bronchioles and vessels, Dex14 vs Sil14) and this trend was even more obvious after 28 days of the treatment delivery ($p<0.001$ for both bronchioles and vessels, Dex28 vs Sil28) (Fig. 3).

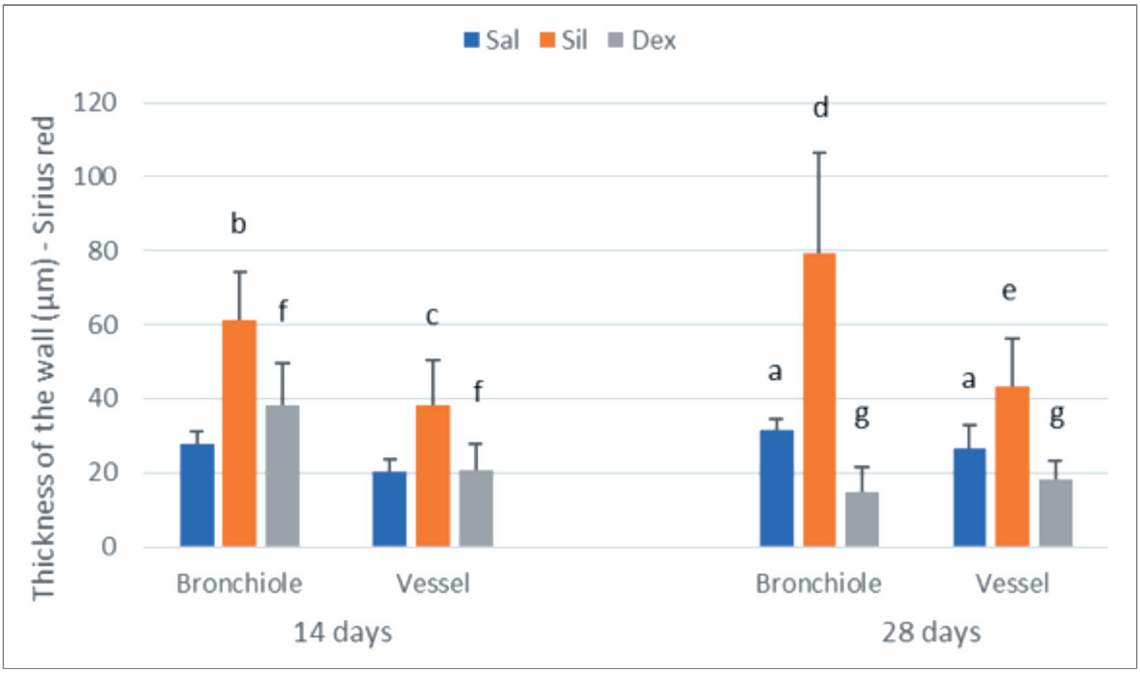


Fig. 3. Thickness of the wall of bronchioles and pulmonary vessels (in µm) – staining for presence of collagen by Sirius red in the control groups with saline instead of silica (Sal), in the silica-injected and non-treated animals (Sil), and in the silica-injected and dexamethasone-treated animals (Dex) after 14 or 28 days of the treatment delivery.

Statistical differences among the groups: [a] $p<0.05$ for Sal28 vs Sal14, [b] $p<0.001$ for Sil14 vs Sal14, [c] $p<0.01$ for Sil14 vs Sal14, [d] $p<0.001$ for Sil28 vs Sal28, [e] $p<0.01$ for Sil28 vs Sal28, [f] $p<0.01$ for Dex14 vs Sil14, [g] $p<0.001$ for Dex28 vs Sil28.

Smooth muscle mass accumulation in the bronchioles and pulmonary vessels

Immunohistochemical analysis by means of antibody against SMA showed no significant differences for Sal28 vs Sal14 or Sil28 vs Sil14 (all $p>0.05$). Silica instillation caused an increase in the smooth muscle mass in the wall of bronchioles and pulmonary vessels compared to the controls, which was statistically significant already after 14 days ($p<0.05$ for both bronchioles and vessels, Sil14 vs Sal14), and this trend aggravated after 28 days of the treatment delivery ($p<0.01$ for bronchioles and $p<0.001$ for vessels, Sil28 vs Sal28) (Fig. 4). The dexamethasone treatment prevented the increase in smooth muscle mass in

bronchioles and vessels after 14 days of the treatment delivery ($p<0.01$ for bronchioles and $p>0.05$ for vessels, Dex14 vs Sil14), and the effect was even more obvious after 28 days of the treatment delivery ($p<0.001$ for both bronchioles and vessels, Dex28 vs Sil28) (Fig. 4).

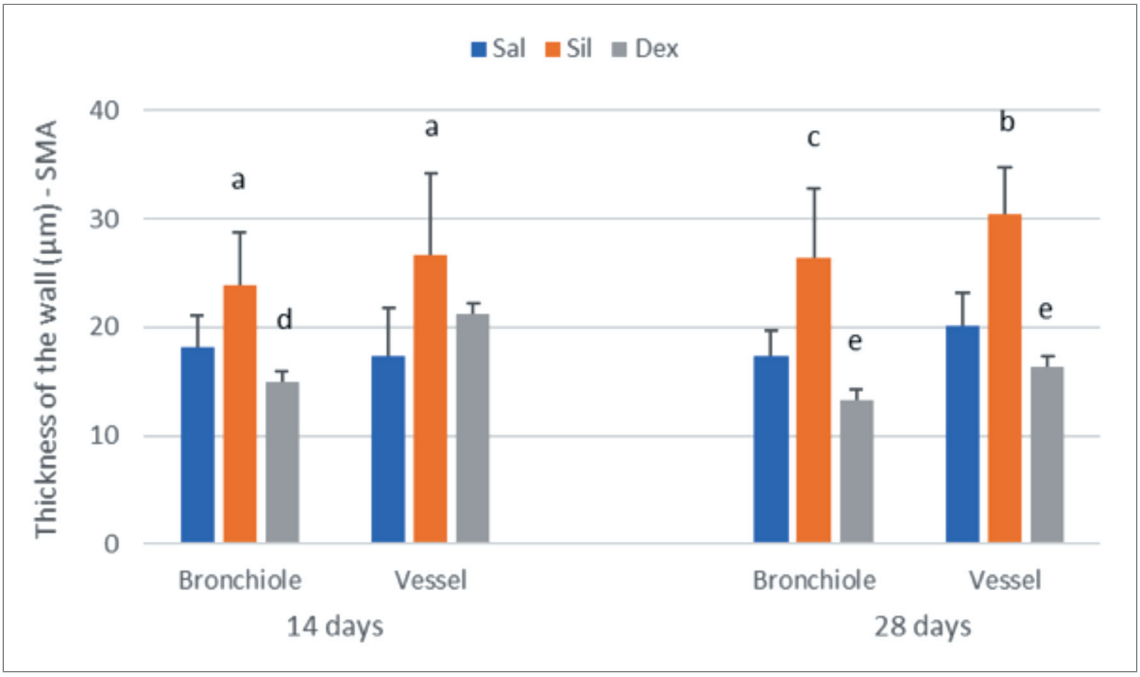


Fig. 4. Thickness of the wall of bronchioles and pulmonary vessels (in µm) – staining for presence of smooth muscle actin (SMA) by antibody against SMA in the control groups with saline instead of silica (Sal), in the silica-injected and non-treated animals (Sil), and in the silica-injected and dexamethasone-treated animals (Dex) after 14 or 28 days of the treatment delivery.

Statistical differences among the groups: [a] $p<0.05$ for Sil14 vs Sal14, [b] $p<0.001$ for Sil28 vs Sal28, [c] $p<0.01$ for Sil28 vs Sal28, [d] $p<0.01$ for Dex14 vs Sil14, [e] $p<0.001$ for Dex28 vs Sil28.

DISCUSSION

Inhaled silica particles may interact with pulmonary epithelial cells and resident macrophages, but also with dendritic cells and other immune cells contributing to both innate and adaptive immune responses. Our pilot study demonstrated that significant mobilization of leukocytes and first fibrotic changes in the walls of bronchioles and pulmonary vessels can be found already 14 days after a silica instillation into the rat lung. Early administration of dexamethasone mitigated the above mentioned changes suggesting its potential use in the treatment of silicosis.

Inhaled silica immediately reacts with immune cells, which causes their activation and subsequent overproduction of ROS and massive generation of proinflammatory cytokines *via* an increased expression of transcription factors including nuclear factor (NF)-κB (3,13,14). Our results demonstrated that the inflammatory changes detected after the silica instillation were associated with an increased total count of leukocytes in the blood and elevated absolute numbers of circulating lymphocytes and slightly increased counts of neutrophils

and eosinophils. Higher absolute numbers of lymphocytes, neutrophils, and eosinophils were also found in the BAL fluid of the silica-instilled compared to the saline-instilled animals after both 14 and 28 days of the experiment. The decreased percentage of circulating monocytes in the blood of the silica-instilled animals compared to the saline-instilled controls could indicate that these cells migrated from the circulation into the injured lung. However, the percentage of macrophages in the BAL fluid even decreased compared to the saline-instilled group. This may indicate that a part of macrophages could be destroyed by a direct cytotoxic action of silica. The macrophages which survived their contact with extremely toxic silica can move to the lung interstitium where they can transform to activated interstitial macrophages and participate in the progression of lung fibrosis (15), which may partially explain their shortage in the alveolar compartment and lumen of the airways from which the BAL fluid is extracted.

On the other hand, significant increases in neutrophils, eosinophils, and lymphocytes in the BAL fluid were also observed by other authors (16-19). For instance, a significant increase in total cell counts, but particularly in neutrophil counts, in the BAL fluid together with an increased count of neutrophils in the blood was found already in the first days of silica exposure in the silica-aerosol induced model of silicosis (13).

A persistence of inflammation because of an ineffective clearance of phagocyted material and a release of macrophage-derived products is associated with activation of profibrotic mechanisms, which finally results into irreversible fibrotization of the lung tissue (2,18,19) and pathological changes in the airways (17). The comparison of the silica-instilled and the saline-instilled animals in our study showed a significant accumulation of collagen and smooth muscle mass in the walls of bronchioles and pulmonary vessels in the silica-injured animals already after 14 days of silica exposure and the extent of fibrotization enlarged during the following 14 days of the experiment. This finding is in agreement with the study of Porter et al. (13) who found a progressive worsening of the lung inflammation and fibrosis with increasing time after the silica exposure. Surprisingly, slight fibrotic changes were also detected in the saline-instilled animals after 14 days when compared to 28 days of the experiment. We can hypothesize that the orotracheal instillation of sterile saline in the control group could initiate some local inflammatory response resulting into a slight increase in collagen formation which was detected only by Sirius red staining, but not by antibody against SMA. Our results are in agreement with other authors who found a significant but transient increase in annexin A5, a marker of apoptosis (20), or sligh but non-significant increases in lactate dehydrogenase, a marker of cell injury, and protein content in BAL fluid (21), or elevations in hydroxyproline, a component of collagen, and malonyldialdehyde, a marker of lipid peroxidation in the lung tissue (22) of the saline-instilled animals.

The therapeutic action of corticosteroids is mediated by complex genomically- and non-genomically-mediated mechanisms (23,24). These mechanisms result into effective suppression of inflammation *via* modulation of inflammatory cascades on various levels and influencing various cells including respiratory epithelium, endothelium, macrophages, eosinophils, neutrophils, and lymphocytes, as well as into the reduction of oxidative and fibrotic changes (25-27). However, the effects of corticosteroids may be largely non-homogenous depending on the type, dose, delivery route, and timing of the given therapy, as we previously published elsewhere (28). In this study, early treatment with dexamethasone decreased the total count of circulating leukocytes, showed a clear trend to reduce the mobilization of inflammatory cells into the BAL fluid and prevented fibrotic changes in the lung as expressed by a decrease in production of collagen and lower accumulation of smooth muscle mass in the walls of bronchioles and vessels in comparison to the non-treated Sil group. In agreement with our results, partial alleviation of inflammatory infiltrate, granulomatous response, and generation of collagen after dexamethasone treatment was demonstrated in other two studies in silica-instilled mice (19, 29). However, in the study by Rabolli et al. (19) the

reduction in inflammatory response after dexamethasone was not associated with antifibrotic effect. Variability in the antifibrotic response to given dexamethasone may be related to inter-species differences of laboratory animals which were used for preparing the model of silicosis. Thus, dexamethasone may reduce the pulmonary fibrotic response in rats (30), but it may omit such effect in mice (31). The decreased markers of inflammation and oxidative stress, mitigated expression of NF-κB, and lower total counts and neutrophil counts in the BAL fluid after dexamethasone treatment was demonstrated by other authors in the silica-instilled animals (32,33) but also by us in the models of acute lung injury (34,35). Dexamethasone-induced inhibition of chemotactic factors may result in a reduction of neutrophil margination and influx into the lungs, with their simultaneous increase in the blood (36,37) as also demonstrated in our previous studies (34,35). On the other hand, the potential of dexamethasone to reduce the numbers of eosinophils, neutrophils and lymphocytes in the blood and BAL fluid shown in our study is in agreement with the action of corticosteroids in the treatment of other respiratory diseases including bronchial asthma (38-40), chronic obstructive pulmonary disease (41-43), community-aquired pneumonia (44), or various types of acute lung injury (45).

Of course, we are aware of several limitations of our experimental study. In this phase of the project, only male animals were used. Female animals are more sensitive to induction of various forms of lung fibrosis including silicosis, which may be associated with more frequent complications and higher mortality (46,47), therefore, a majority of animal studies with modeling of lung fibrosis or silicosis use male rats or mice. Moreover, dynamics of progression of pulmonary silicosis in patients inhaling silica particles in higher or smaller concentrations is gradual and develops within the years. In this context, the distribution of the silica particles after an artificial instillation in the laboratory animals is different. However, as demonstrated in many previous studies (2,13,14,19,31), silica induces rather homogenic local response in the immune cells and the silica-induced inflammatory and fibrotic changes in the lung of laboratory animals are fully comparable with the changes observed in patients suffering from lung silicosis. The other objection may arise from the use of corticosteroids in the treatment of silicosis regarding their wide adverse effects in long-term use. In this study, the long-acting corticosteroid dexamethasone was used for only 28 days to evaluate its potential in administration in an early phase of the development of silicosis. Our results showed that dexamethasone given early may prevent later development of massive inflammatory and fibrotic changes in the lung. These findings may subsequently provoke the following research regarding the early or even preventive use of any anti-inflammatory agents (either synthetically produced or natural compounds) which may prevent the development and progression of irreversible fibrotic changes after silica inhalation.

CONCLUSIONS

Our pilot study has shown that early administration of dexamethasone exerted anti-inflammatory, anti-fibrotic and anti-remodeling effects in the rat model of lung silicosis suggesting a future perspective for the early use of anti-inflammatory drugs including corticosteroids in the treatment of lung fibrosis.

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