THE EFFECTS OF SILICON DIOXIDE AND CURCUMIN ON MALONDIALDEHYDE FOUND IN LUNG TISSUE

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Abstract: *Chronic inflammation found in silicosis probably implies the presence of an excess of free oxygenated radicals (FOR) with an effect shown through biological markers, such as malondialdehyde (MDA). Our purpose is to evaluate MDA in the lung tissue of Wistar rats, intratrachealy instillated with crystalline SiO_2 and protected with curcumin. Results: MDA levels rise significantly early on, 30 days after instillation and remain elevated throughout the entire observation period, the difference in values (measured in nmol/mg protein) recorded at 30 days (0.374±0.06), at 90 days (0.441±0.13) and at 120 days (0.440±0.08) being statistically insignificant. Curcumine induces a decrease in MDA at 90 days, reaching values close to the ones observed in the untreated group at 120 days. Conclusions: The prominence of oxidative stress in silicosis benefits from malondialdeyde in lung tissue as a valuable biomarker and curcumin has a positive effect, lowering MDA in the initial phase of inflammation.*

Cuvinte cheie: *silicoză experimentală, silice liberă, malondialdehidă, stres oxidativ, curcumină*

Rezumat: *Inflamaţia cronică din silicoză presupune cu multă probabilitate prezenţa unui exces de radicali oxigenaţi liberi (ROL), al căror efect poate fi evidenţiat prin markeri biologici precum malondialdehida (MDA). Scopul cercetării constă în evaluarea MDA în ţesutul pulmonar la şobolani Wistar, instilaţi intratraheal cu SiO_2 cristalin şi protejaţi cu curcumină. Rezultate: MDA creşte precoce, semnificativ, la 30 de zile după instilare şi se menţine crescută pe toată durata observaţiei, diferenţa valorilor (exprimate în nmoli/mg proteină) înregistrate la 30 de zile (0,374±0,06), la 90 de zile (0,441±0,13) şi la 120 de zile (0,44 ±0,08), fiind nesemnificativă statistic. Curcumina determină o scădere a MDA la 90 de zile, revenind la valori apropiate celor observate în lotul netratat, la 120 de zile. Concluzii: Evidenţierea stresului oxidativ în silicoza experimentală beneficiază de un biomarker valoros, malondialdehida în ţesutul pulmonar. Curcumina are un efect benefic, scăzând MDA în faza de promovare a inflamaţiei.*

INTRODUCTION

The research of the last two decades regarding the toxicity of free silica points out oxidative stress as an important link in the sequence of inflammatory mechanisms at the level of pulmonary parenchyma as well as the airways. The excessive production of FOR is possible thanks to the chemical properties related to the chemical reactivity on the surface of the quartz particles, but also because of their phagocytosis by the alveolar macrophages which, activated in this way, generate reactive oxygen species (ROS).(1,2) The silica availability in radicals is closely dependent on how freshly fractured the quartz crystal's surfaces are.(3) There is still research necessary, to confirm with certainty that FOR formed following free silica inhalation, own this key role in triggering and maintaining chronic inflammation, and thus the fibrous process in silicosis. Some studies support this assumption.(2,4,5,6,7,8,9)

The onset of oxidative stress matches the phase in which excessive production of FOR can no longer be neutralized by the antioxidant system (10,11), and ROS stimulate the secretion of proinflammatory cytokines and other mediators that maintain the inflammation and progress of pneumoconiosis.(12,13) FOR induce lipid peroxidation in the cellular membranes thus forming lipoperoxides and aldehydes, their level in serum or lung tissue allowing to appreciate the intensity of oxidative stress.(11,14)

Malondialdehyde (MDA) is a characteristic degradation product of polyunsaturated fatty acids under the influence of FOR and reflects, through its increase or decrease in concentration in the investigated biological environment, the augmentation, respectively the diminishing of the lipoperoxidation process.(14,15) It is thus recommended as an effective biomarker of FOR, very useful in investigating mechanisms involved in inflammation. The link between the formation of FOR and the morphogenesis and evolution of silicosis motivates the interest in evaluating proinflammatory effects of antioxidant agents in human and experimental silicosis.

PURPOSE

The aim of this study was to dynamically quantify the concentration of MDA in the lung tissue of Wistar rats, that have been previously instilled intratrachealy with crystalline SiO_2, and to assess the positive effects obtained by administering a natural antioxidant, curcumin.

METHODS

The experiment was carried out on male Wistar rats, 180 days old and weighting an average 320grams. We made use of a suspension of standardized crystalline silicon dioxide produced by Berkeley Springs, West Virginia (USA). The administration of silica was performed intratrachealy with a

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dosage of 0.3ml from the suspension containing 30mg/ml in saline solution, after the animals have been anesthetized with a mixture of Ketamine 10% and Xylazine 2% in a 2:1 ratio, using a quantity of 0,1ml/100g. The control groups were instillated at the same date with an identical quantity of saline solution (two control groups: one sacrificed 30 days after instillation – M_{30}, the other 120 days after – M_{120}). We formed 2 control groups in order to more accurately compare the results, taking into consideration the fact that the intensity of oxidative stress depends on the age. Regarding the time elapsed until date of sacrifice, the animals were divided in 7 groups: control (M_{30} and M_{120}), instilled with SiO_2 and sacrificed after 30 days (SiO_2.30), after 90 days (SiO_2.90) and after 120 days (SiO_2.120). Another two groups, instilled with SiO_2, were treated with curcumine and sacrificed after 90 days (SiO_2.90+C), respectively 120 days (SiO_2.120+C).

Curcumin, an aromatic phenolic antioxidant, natural extract from the species Curcuma longa, originary from India and used as a colorant and preservative in the food industry, was administered orraly, with a dosage of 50mg/kgC, starting the 10^{th} day after the instillation of SiO_2, with a frequency of 3 administrations/week. The final configuration of the 7 groups and the number of animals for each group are illustrated in table no. 1. The sacrifice was performed by giving an overdose of anaestethic mixture (double the dose used for instillation). After sacrifice the trachea and lungs were harvested, from which samples were collected for processing. The animals were kept in the ordinary conditions of the biobase and fed ad libitum. The experiment was approved by the Ethics Comission of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca.

Table no. 1. The layout of the study groups and average MDA values

Group	No. of specimens	Mean±SD
M_{30}	10	0,252 ±0,04
SiO_2.30	9	0,374 ±0,06
SiO_2.90	8	0,44 ±0,14
SiO_2.90 + C	7	0,267 ± 0,04
M_{120}	8	0,281 ± 0,05
SiO_2.120	7	0,440 ±0,08
SiO_2.120 + C	7	0,341±0,10

The collected samples of lung tissue were homogenized in order to dose the MDA. The tissue homogenate was boiled for one hour with o solution of 2-tiobarbituric acid 10mM in K_2HPO_4 75mM at a pH of 3.

The product of the reaction was extracted in n-butanol, after abruptly cooling down the sample, and the MDA concentration was determined in organic phase, after separating and centrifuging, by using the synchronous fluorescence technique at a 14 nm wave length difference ($\Delta\lambda$) between excitation and emission. The values were expressed in nmol/mg protein.(16) The data analysis was performed using the MedCalc software.

RESULTS

The concentrations of MDA in lung tissue are significantly higher in the animals treated with silicon dioxide compared to the control groups (table no.1 and no. 2). The MDA level was significantly raised after 30 days, remaining constantly elevated at 90 and 120 days.

Figure no. 1. Development in MDA levels

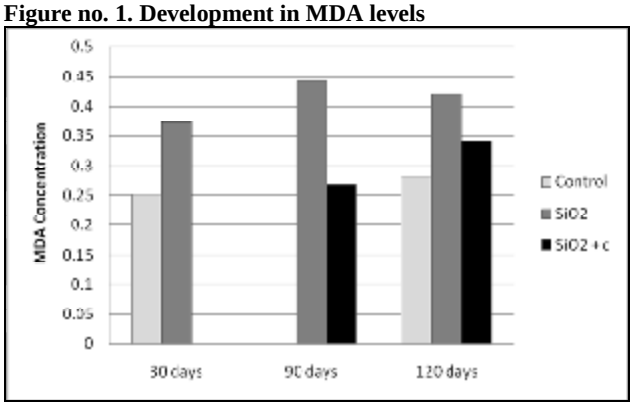

Table no. 2. The values of p for the correlation between groups (Student t test was used)

	M_{30}	SiO_2.30	SiO_2. 90	SiO_2.90+C	M_{120}	SiO_2. 120	SiO_2. 120+C
M_{30}	-	**0,0001**	**0,0055**	0,47	0,21	**<0,0001**	0,06
SiO_2.30	**0,0001**	-	0,22	**0,0011**	**0,005**	0,08	0,43
SiO_2. 90	**0,0055**	0,22	-	**0,007**	0,12	0,97	0,06
SiO_2.90+C	0,47	**0,0011**	**0,007**	-	0,58	**0,0002**	0,08
M_{120}	0,21	**0,005**	0,12	0,58	-	**0,0008**	0,18
SiO_2. 120	**<0,0001**	0,08	0,97	**0,0002**	**0,0008**	-	0,06
SiO_2.120+C	0,06	0,43	0,06	0,08	0,18	0,06	-

Administration of curcumin throughout the experiment proved to be efficient especially within the first 90 days after silica instillation, a phenomenon translated by the decrease of the MDA levels almost reaching the constant values noticed in controls (table no. 2 and figure no.1). Although the MDA values 120 days after instillation were decreased by curcumin, its efficiency did not reach the effect observed in the group sacrificed after 90 days.

DISCUSSIONS

FOR generated either by the surface activity of crystalline silica, or mediated by inflammatory cells through alveolar macrophages, are considered in a growing number of studies as important links in triggering and maintaining inflammation produced by silicon dioxide (2,9,17,18), oxidative stress being induced either by their excess, or by lack of antioxidant defensive potential. Langley and co.(6) noticed a overadjustment of the genes controlling the fibrous process and oxidative stress in experimental silicosis.

Our research confirms that the level of MDA in lung tissue is a valuable biomarker for evaluating oxidative stress in experimental silicosis, it's being the source of increase in serum concentration observed in subjects with silicosis.(19,20,21) The rise of MDA level in pulmonary tissue proved to be an early phenomenon and its steady risen levels over time, reflect the persistance of chronic inflammation. Zang and co. (10) noticed an increase in serum MDA not correlated with the stage of the disease, an aspect suggestive for the persistence of oxidative stress throughout the development of silicosis in humans.

The significant drop in lung tissue MDA levels under the influence of curcumin confirms its positive effect, probably through interfering in oxidative stress. Its antioxidant capacity was confirmed in cancer, diabetes, various cardiovascular diseases (22,23), but also in experimental research that used the model of bleomycin induced pulmonary fibrosis.(24)

Nevertheless, in our study, the significant efficiency of curcumin was present in the initial phase, of advancing inflammation, and the late persistency of elevated MDA concentration suggests a breakdown of the defensive potential.

CONCLUSIONS

Silicon dioxide, intratrachealy administered to Wistar rats, induced a statistically significant increase in MDA levels in lung tissue related to control animals. The growth in MDA levels was constant throughout the whole duration of the experiment, suggesting the persistency of oxidative stress.

The decrease in MDA levels under the influence of curcumin was observed in the first 90 days following instillation.

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