Original Article

Evaluation of DNA damage in Mice Lymphocytes by exposure to Silica Dust

Gholami Abdollah[1], Golbabaei Farideh[1]*, Kianmehr Mojtaba[2], Yaseri Mehdi[3]

[1] Dept. of Occupational Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. [2] Dept. of Medical Physics, Faculty of Medicine, Gonabad University of Medical Sciences, Gonabad, Islamic Republic of Iran. [3] Dept. of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Correspondence: Golbabaei Farideh. Dept. of Occupational Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Occupational exposure to silica dust can cause various complications. Genetic side effects are possible complications raised about exposure to silica. The aim of this study was to assess the rate of DNA damage using Comet assay in lymphocytes of mice following exposure to silica dust. In total, 72 male BALB/c mice were selected and divided into five groups of 12 animals with respiratory exposure to pure silica dust at concentrations of 1.3, 3, 8, 12 and 17 mg/m3, as well as a non-exposed control group. The mice were exposed for 8 hours a day, 6 days a week for 1, 2, 3 and 4 months. Then, blood samples were taken from the mice to determine the level of lymphocyte DNA damage using Comet assay under fluorescence microscope and comet score software. The obtained results were analyzed using SPSS version 19 at a significance level of less than 0.05. The highest level of Tail length was found in the fourth month and in the Group 1. The results of DNA damage showed no significant difference among any of the groups in the first month compared with the control group ($p > 0.05$). After the second month, there was a significant difference only in the Groups 1 and 2, which were exposed to high concentrations of silica ($p < 0.05$). In the third and fourth months after exposure, DNA damage revealed significant differences in all groups compared to control ($p < 0.05$). More than two months exposure to silica dust even at low concentrations can cause significant DNA damage in lymphocytes of BALB/c mice compared to the control.

Keywords: DNA damage, lymphocyte, comet assay, silica dust, mice

Introduction

Silica is the main mineral on Earth, a material that there is the possibility of exposure to it in different industries, including casting, ceramic tile factories, production of washing powders and detergents, production of chemicals and glazed ceramic tile, manufacture of crystals, pharmaceutical, underground and open-pit mines [1]. The silica appears in two major forms as crystalline that includes quartz, cristobalite and tridymite depending on the formation temperature, and amorph that has different molecules with dissimilar spatial connection relative to each other; various forms of crystalline silica can convert into each other under certain temperature and pressure [2]. For this reason, different crystalline forms including quartz, cristobalite and tridymite can be seen in various processes with respect to temperature and pressure [2]. Chemical, physical and biological factors involving genotoxic agents in the living and working conditions cause variety of DNA damages such as single-stranded and double-stranded breaks in the DNA, mutagenesis and chromosomal damage, which would lead to mutations in the genes if the cells were unable to repair them. The mutations can cause various diseases like cancers [3-8]. The effects of silica on DNA have been investigated in many studies; for example, a study on foundry and pottery workers indicated that the rate of DNA damage in these workers was higher in comparison with the control group, as well as it was found that smoking could damage to DNA [9]. In another research on mice isolated epithelial cells, DNA damage increased significantly after three days of exposure to quartz, i.e. acute exposure [10]. Shi et al. demonstrated the DNA damage caused by silica as well as lipid peroxidation due to the release of radicals and stated that DNA damage can lead to the appearance of fibrosis and cancer [11].

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Gholami Abdollah, Golbabaei Farideh, Kianmehr Mojtaba, Yaseri Mehdi. Evaluation of DNA damage in Mice Lymphocytes by exposure to Silica Dust. J Adv Pharm Edu Res 2018;8(S2):97-100.
Source of Support: Nil, Conflict of Interest: None declared.

Yamano and colleagues in a study on mice lung cells exposed to silica dust observed significant increase in DNA damage and elevated 8-Hydroxy-2'-Deoxyguanosine [12]. In a study of mouse and human lung epithelial cells in Germany, DNA damage was found along with increasing OH group in these cells and it was determined that DNA damage is associated with the concentration of hydroxyl ion [13]. Moreover, the presence of free radicals in cells exposed to the quartz has been proven in many studies. Promoted free radicals have been associated with an increase in 8-Hydroxy-2'-Deoxyguanosine according to in vitro studies [7, 10, 12]. In a survey carried out by Carl fanizza et al. in Canada, human lung cells were exposed to different concentrations of silica (25, 50 and 100µg/ml), which there was a significant relationship between the level of oxidation caused by silica and the rate of DNA damage in all concentrations within 2 and 4 hours [14].

The Comet assay is a rapid, sensitive and suitable technique for determining DNA damage among various methods, which provides the possibility of direct observation of DNA damage in individual cells. The Comet assay is a very sensitive test to identify the types of damage to DNA. This method is able to direct examination of DNA single-strand and double-strand breaks in individual cells. The Comet assay investigates genotoxic effects of compounds and protective impacts of natural compounds on DNA according to in vivo and in vitro experiments [4-8]. This approach evaluates the effects of environmental genotoxic substances, the rate of repair and the repair kinetics of DNA damaged by several factors. One application for this procedure is to evaluate DNA damage in people exposed to genotoxic agents due to occupational or environmental conditions [3, 4, 7].

The present study examined the effect of silica processed at silica mines of Iran, which is applied by the miners and silica consumer industries, on the lymphocytes from mice exposed to silica.

Material and Methods

Silica used in this study were prepared from 99% pure silica processed at silica mines of Iran, which is often applied as foundry sand and raw materials in ceramic tile industries and glass factories. In total, 72 male BALB/c mice with 6 weeks old were obtained from Experimental Medicine Research Center at Birjand University of Medical Sciences, Iran, and were divided into five groups (I to V) of 12, respectively, exposed to silica dust concentrations of 1.3, 3, 8, 12, 17 mg/m3, as well as a non-exposed control group. The average body weight was 25 grams on the day of exposure. The mice were exposed in the certain box where a blower pump drove dust to it from a container and control valves regulated the density of dust in the box. An outlet from each box was connected to direct sensing device to measure the concentration of dust and then the concentration of dust was recorded, of which was the same 99% pure silica. Finally, the mean concentration was calculated during the exposure period. The mice were exposed for 8 hours a day, 6 days a week and different periods of 1, 2, 3 and 4 months. The mice were anesthetized by ether at the time of sampling, and blood samples were collected from the heart. The blood lymphocytes were separated using Ficoll (Sigma Co.) by centrifugation at 3300 rpm for 15 minutes. The isolated lymphocytes were washed with PBS at 4°C, centrifuged again at 1400 rpm for 15 min at 4°C for separation, then mounted on slides and a layer of gel, and placed at 4°C for 1 hour in lysis solution for removing the membrane and proteins. In the next phase, electrophoresis solution for 20 min at 4°C broke the hydrogen bonds between the two strands, and then electrophoresis was performed for 20 minutes at a voltage of 0.65 volts per centimeter length of the tank. The samples were placed in a neutral buffer solution for 10 minutes, fixed by methanol, and stained with ethidium bromide for 10 minutes. After washing with water and putting cover slip, photos were taken from the samples using a fluorescence microscope model Nikon 50i, Japan. Two slides were prepared for each sample. Altogether, 100 cells were examined, and the damage rate was determined using Comet Score software (Version 1.5).

Results

The silica processed at the silica mines of Iran were used in this study, which miners and workers of ceramic tile, glass and foundry factories are exposed to this kind of silica. The concentration and particle size distribution of total silica dust have been introduced in Table 1.

Table 1: Mean concentration and particle size distribution of total silica dust in different exposure boxes

	Mean concentration				
Number of box	PM1	PM2.5	PM5	PM10	Total
1	6.8	7.8	11.7	15.5	17
2	4.9	5.56	8.32	10.5	12
3	3.1	3.7	5.74	6.59	8
4	1.42	1.9	2.013	2.7	3
5	0.82	1.01	1.22	1.23	1.3

The evaluated parameters in Comet assay were cell length, tail length and diameter of the head. The study results during 1, 2, 3 and 4-month periods have been shown in Tables 2, 3, 4 and 5.

Table 2: Parameters determining DNA damage in the first month after exposure

Parameter Group	Cell length (µm)	Diameter of head(µm)	Tail length (µm)	Tail DNA (%)
Control	21.1 ± 1.79	29.8± 3.9	1.3± 0.09	0.9± 0. 3
Group 1	21.1 ± 1.79	29.8± 3.9	1.3± 0.09	0.9± 0. 3
Group 2	20.4 ± 2.1	30. 3± 3.7	1.7± 0.08	1.2± 0.4
Group 3	21.3 ± 1.8	30.5± 3.9	1.1± 0.08	1.3± 0.4
Group 4	20.7 ± 1.8	29.9± 5.3	0.9± 0.8	0.8± 0.2
Group 5	21.1 ± 2.21	28.9± 5.5	0.57± 0.85	1.3± 0.75

Statistical parameters	P<0.001	P<0.001	P<0.001	P<0.001

Table 3: Parameters determining DNA damage in the 2 month after exposure

Parameter Group	Cell length (µm)	Diameter of head(µm)	Tail length (µm)	Tail DNA (%)
Control	21.7 ± 1.83	30.6 3± 4.6	1.8± 0.08	1.8± 0. 4
Group 1	22.9 ± 2.1	29.6 3± 3.4	2.1± 0.2	2.8± 0.2
Group 2	22.3 ± 1.86	29.6± 2.7	1.9± 0.09	2.1± 0.6
Group 3	21.9 ± 1.63	31.6 3± 3.2	1.9± 0.09	2.4± 0.2
Group 4	21.5 ± 1.78	29.9± 2.6	1.9± 0.07	1.6± 0.08
Group 5	21.3 ± 1.64	30.9± 2.9	1.7± 0.07	1.4± 0.03
Statistical parameters	P<0.001	P<0.001	P<0.001	P<0.001

Table 4: Parameters determining DNA damage in the 3 month after exposure

Parameter Group	Cell length (µm)	Diameter of head (µm)	Tail length (µm)	Tail DNA (%)
Control	21.6 ± 1.92	30. 3± 4.9	0.9± 0.08	1.9± 0. 4
Group 1	33.9 ± 4.2	21.2± 5.35	12.3± 1.2	41.7± 3.2
Group 2	30.3 ± 2.98	22.3± 4.6	10.9± 1.2	37.7± 2.6
Group 3	27.2 ± 2.89	25.6± 4.8	8.7± 0. 9	28.6± 2.2
Group 4	25.2 ± 3.23	27.4± 4.3	7.6± 0.8	19.1± 3. 8
Group 5	24.2 ± 2.78	28.9± 4.8	5.2± 0.8	16.8± 3. 3
Statistical parameters	P<0.001	P<0.001	P<0.001	P<0.001

Table 5: Parameters determining DNA damage in the 4 month after exposure

Parameter Group	Cell length (µm)	Diameter of head(µm)	Tail length (µm)	Tail DNA (%)
Control	21.3 ± 1.36	31. 3± 5.2	0.78± 0.2	1.7± 0. 4
Group 1	36.9 ± 4.2	19. 3± 2.9	15.3± 2.1	62.9± 3.2
Group 2	32.7 ± 4.3	20.3± 2.9	13.2± 2. 9	58.7± 3.7
Group 3	31.4 ± 2.33	21.33± 2.9	1.8± 2.1	46.8± 4.2
Group 4	30.3 ± 2.38	23.4± 3.8	10.7± 1.9	38.6± 3.7
Group 5	29.3 ± 2.78	25.7± 3.7	9.7± 1.4	30.8± 3.3
Statistical parameters	P<0.001	P<0.001	P<0.001	P<0.001

Discussion

The silica dust is present in many workplaces, so numerous studies have examined the effects of silica dust on human beings; genetic complications and DNA damage by creating oxidative effects are regarded as potential side effects of the silica dust. The Comet assay is a method of evaluating genetic side effects among the techniques used to assess DNA damages, originally a highly sensitive, easy, inexpensive and quick method to measure DNA strand breaks in individual cells [5-8]. The results of this study demonstrated that the silica dust processed at the silica mines of Iran could cause DNA damage in lymphocytes of mice. The damage was associated with the silica dust concentration and duration of exposure. These results are consistent with the findings of a study conducted on mice epithelial cells isolated after quartz acute exposure [10]. Shi and colleagues demonstrated silica-based damage on DNA and lipid peroxidation caused by forming radical and concluded that DNA damage can proceed to the emergence and development of cancer and fibrosis [11]. Yamano and colleagues in a study on mice lung cells exposed to silica dust observed a significant increase in DNA damage and promoted levels of 8-Hydroxy-2'-Deoxyguanosine, in line with the results of our study [12]. The research on mouse and human lung epithelial cells in Germany suggested that DNA damage was observed along with the increase of OH group in these cells, and they found that DNA damage is associated with the concentration of hydroxyl ions, similar to the results of present work [13]. In addition, the presence free radicals has been proven in the cells exposed to quartz during a lot of in vitro studies, which enhanced free radicals have been associated with an increase in 8-Hydroxy-2'-Deoxyguanosine; the free radicals can lead to DNA damage [14-18]. In the research presented by Carl fanizza et al. in Canada, human lung cells were exposed to different concentrations of silica (25, 50 and 100µg/ml), which there was a significant relationship between the level of oxidation caused by silica and the rate of DNA damage in all concentrations within 2 and 4 hours. Silica-induced genetic complications seen in this study was too short in duration than the present study [14]. In the present study, we used total silica dust containing all sorts of silica and the results showed that this type of silica could cause DNA damage in mice lymphocytes as well as the dust concentration and duration of exposure increased cell damage. There was no considerable damage within one month after exposure in any of the groups even at high concentrations, but DNA damage were statistically significant in all groups in the third and fourth months even at low concentrations. Thus, we can conclude that duration of exposure is also important parameter in creating damage apart from the condensation of dust. Similar results were observed in a study conducted by carl J.JOHNSTON at 13 weeks after exposure to crystalline silica [19]. An important difference between this study and other similar works mentioned is that the previous and similar investigations have used same type of amorphous silica with a specific crystalline, but we used silica processed at mines of Iran that many workers are in contact with the silica in mines and consumer industries. The findings of this study can be used practically in determining the genetic complications resulting from this type of silica dust among workers of miners, ceramic tile and glass industries, casting etc. that use this type of silica within the production process.

Conclusion

The results obtained from the present study indicated that the silica dust processed at silica mines of Iran could cause DNA damage in mice lymphocytes, which the duration of exposure and the concentration of dust increased the damage. In addition, long-term exposure even low concentrations could result in DNA damage.

References

1. Lumens ME, Spee T. Determinants of exposure to respirable quartz dust in the construction industry. Annals of Occupational Hygiene. 2001;45(7):585-95.
2. Gholami A, Fani MJ, Sadeghi N. Occupational exposure determination of silica dust in an iron-stone ore and comparison with standard. International Journal of collaborative Research on Medicine and Public Health. 2012;4(6):1141-9.
3. Møller P. Assessment of reference values for DNA damage detected by the comet assay in human blood cell DNA. Mutation Research/Reviews in Mutation Research. 2006;612(2):84-104.
4. Poli P, de Mello M, Buschini A, de Castro V, Restivo F, Rossi C, et al. Evaluation of the genotoxicity induced by the fungicide fenarimol in mammalian and plant cells by use of the single-cell gel electrophoresis assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2003;540(1):57-66.
5. Gichner T, Patková Z, Száková J, Demnerová K. Cadmium induces DNA damage in tobacco roots, but no DNA damage, somatic mutations or homologous recombination in tobacco leaves. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2004;559(1):49-57.
6. Ladeira MS, Rodrigues MA, Freire-Maia DV, Salvadori DM. Use of Comet assay to assess DNA damage in patients infected by Helicobacter pylori: comparisons between visual and image analyses. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2005;586(1):76-86.
7. Menke M, Chen I-P, Angelis KJ, Schubert I. DNA damage and repair in Arabidopsis thaliana as measured by the comet assay after treatment with different classes of genotoxins. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2001;493(1):87-93.
8. Faust F, Kassie F, Knasmüller S, Boedecker RH, Mann M, Mersch-Sundermann V. The use of the alkaline comet assay with lymphocytes in human biomonitoring studies. Mutation Research/Reviews in Mutation Research. 2004;566(3):209-29.
9. Başaran N, Shubair M, Ündeğer Ü, Kars A. Monitoring of DNA damage in foundry and pottery workers exposed to silica by the alkaline comet assay. American journal of industrial medicine. 2003;43(6):602-10.
10. Knaapen AM, Albrecht C, Becker A, Hohr D, Winzer A, Haenen GR, et al. DNA damage in lung epithelial cells isolated from rats exposed to quartz: role of surface reactivity and neutrophilic inflammation. Carcinogenesis. 2002 Jul;23(7):1111-20. PubMed PMID: 12117767. Epub 2002/07/16. eng.
11. Shi X, Mao Y, Daniel LN, Saffiotti U, Dalal NS, Vallyathan V. Silica radical-induced DNA damage and lipid peroxidation. Environmental health perspectives. 1994;102(Suppl 10):149.
12. Yamano Y, Kagawa J, Hanaoka T, Takahashi T, Kasai H, Tsugane S, et al. Oxidative DNA damage induced by silica in vivo. Environmental research. 1995;69(2):102-7.
13. Schins RP, Knaapen AM, Cakmak GD, Shi T, Weishaupt C, Borm PJ. Oxidant-induced DNA damage by quartz in alveolar epithelial cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2002;517(1):77-86.
14. Fanizza C, Ursini CL, Paba E, Ciervo A, Di Francesco A, Maiello R, et al. Cytotoxicity and DNA-damage in human lung epithelial cells exposed to respirable α-quartz. Toxicology in vitro. 2007;21(4):586-94.
15. Nguyen T, Brunson D, Crespi C, Penman B, Wishnok J, Tannenbaum S. DNA damage and mutation in human cells exposed to nitric oxide in vitro. Proceedings of the National Academy of Sciences. 1992;89(7):3030-4.
16. Daniel LN, Mao Y, Umberto S. Oxidative DNA damage by crystalline silica. Free Radical Biology and Medicine. 1993;14(5):463-72.
17. Daniel LN, Mao Y, Wang T-CL, Markey CJ, Markey SP, Shi X, et al. DNA strand breakage, thymine glycol production, and hydroxyl radical generation induced by different samples of crystalline silica in vitro. Environmental research. 1995;71(1):60-73.
18. Lin W, Huang Y-w, Zhou X-D, Ma Y. In vitro toxicity of silica nanoparticles in human lung cancer cells. Toxicology and applied pharmacology. 2006;217(3):252-9.
19. Johnston CJ, Driscoll KE, Finkelstein JN, Baggs R, O'Reilly MA, Carter J, et al. Pulmonary chemokine and mutagenic responses in rats after subchronic inhalation of amorphous and crystalline silica. Toxicological Sciences. 2000;56(2):405-13.