NANOTECHNOLOGIES

Evaluation of Genotoxicity and Reproductive Toxicity of Silicon Nanocrystals

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Silicon crystal 2-5 nm nanoparticles in the form of 1-5-μ granules in water suspension were injected intraperitoneally in a single dose to male F_1(CBA×C57Bl/6) mice or to outbred albino rats on days 1, 7, and 14 of gestation. Silicon crystal nanoparticles in doses of 5, 25, and 50 mg/kg exhibited no cytogenetic activity in mouse bone marrow cells after 24-h exposure and in doses of 5 and 25 mg/kg after 7 and 14-day exposure. A 24-h exposure to silicon nanoparticles in a dose of 5 mg/kg significantly increased DNA damage (detected by DNA comet assay) in bone marrow cells. In a dose of 50 mg/kg they considerably increased DNA damage in bone marrow and brain cells after exposure of the same duration. Silicon nanoparticles in doses of 5 and 50 mg/kg caused no genotoxic effects in the same cells after 3-h and in a dose of 5 mg/kg after 7-day exposure. Silicon crystal nanoparticles in a dose of 50 mg/kg caused death of 60-80% mice after exposure >24 h. Injected in a dose of 50 mg/kg on days 1, 7, and 14 of gestation, silicon crystal nanoparticles reduced body weight gain in pregnant rats and newborn rats at different stages of the experiment, but had no effect on other parameters of physical development of rat progeny and caused no teratogenic effects.

Key Words: *silicon nanoparticles; genotoxic effects; teratogenesis*

Silicon nanocrystals (nc-Si) can be dissolved in aqueous medium with the formation of orthosilicon acid and hence, are presumably biodegraded. This gives us grounds to regard nc-Si-based materials as functional additives for cosmetology and food industry [5]. Due to pores, nc-SI can serve as drug carriers.

In vitro studies revealed a cytotoxic effect of nc-Si presumably caused by photosensitized singlet oxygen [2,3]. However, possible toxic effects of nc-Si *in vivo* remain unclear.

We studied possible genotoxic and reproductive toxicities of nc-Si in experiments on mice and rats.

MATERIALS AND METHODS

Specimens of nc-Si were prepared by electrochemical etching of the p-type monocrystal silicon plates with specific resistance of 10-15 Ω×cm in an electrolyte based on hydrofluoric acid and ethanol (1:1) at the etching current density of 60 mA/cm^2 for 1 h. Using

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this procedure, we obtained layers of the so-called porous silicon, a bulk of nc-Si particles 2-5 nm in size [6]. After etching process was over, the layers of porous silicon were separated from the sublayer, dried, and subjected to mechanical fragmentation by the method described previously [2,3]. The resultant powder contained individual nanocrystals and their agglomerations (granules) no more than 1-5 μ in size. The powders were stored from preparation until injection for no more than 30 days.

In order to prepare injection mixtures, nc-Si aliquots were thoroughly grinded in an agate mortar. Water suspension of nc-Si in doses of 5, 25, and 50 mg/kg was injected intraperitoneally. The choice of doses and duration of exposure were based on experimental data obtained for chrysotile asbestos fibers [8].

Genotoxicity studies were carried out on F_1(CBA× C57Bl/6) mice (20-22 g), divided into groups (5 animals per group), by evaluation of chromosome aberrations in mouse bone marrow cells [12] and by evaluation of DNA damage by alkaline electrophoresis of isolated cells (DNA comet assay; [1]) on the bone marrow and brain cells.

Cytogenetic preparations of the femoral bone marrow were made by the standard air drying method [12]. The animals were sacrificed by cervical dislocation 24 h, 7 and 14 days after a single injection of nc-Si.

Cytogenetic analysis (Standart-25 microscope, ×1000) was carried out by the common method. The data were statistically processed (φ-test) by comparing the percent of abnormal cells in the control and experimental groups. Four or five animals were used in each variant of the test; 100 metaphase plates were analyzed from each animal.

DNA damage was evaluated by DNA comet asay in the alkaline version according to previous recommendations [1]. Bone marrow and brain cell suspensions (60 μl), prepared 3, 24 h, and 7 days after injection of nc-Si, were added to tubes with 240 μl 1% easy-melted agarose and applied onto slides precoated with 1% universal agarose, covered with slides, and put on ice. After agarose hardening, the slides were put into lysing buffer (10 mM hydrochloric acid Tris-HCl (pH 10), 2.5 M NaCl, 100 mM EDTA-Na_2, 1% Triton X-100, 10% DMSO) and incubated for at least 1 h at 4°C. After lysis, the preparations were transferred to a vessel with buffer for electrophoresis (300 mM NaOH, 1 mM EDTA-Na_2, pH>13) and incubated for 20 min. Electrophoresis was carried out for 20 min at field voltage of 1 V/cm and current ~300 mA. After electrophoresis, the preparations were fixed in 70% ethanol (20 min), dried, and stored at ambient temperature.

The preparations were stained with SYBR Green I (Invitrogen, 1:10,000 in TE buffer). Analysis was carried out in a Micmed-2 12T epifluorescent microscope (LOMO) fitted with a high resolution digital camera (VEC-335, EVS) at ×200. Images of DNA comets photographed from micropreparations were analyzed using CASP 1.2.2 software [9]. A total of 100 cells of each organ from each animal were analyzed. The percentage of DNA in DNA comet tail served as the measure of DNA damage.

The reproductive toxicity of nc-Si was evaluated on outbred albino rats (200-250 g). The day of spermatozoon detection in vaginal smears of females was considered as pregnancy day 1. Pregnant females were kept 4-5 per cage. Silicon nanocrystals were injected intraperitoneally in a dose of 50 mg/kg on days 1, 7, and 14 of pregnancy. According to published data, these days are critical for the rat intrauterine development [7].

Pregnant rats were divided into 2 groups. Group 1 animals were sacrificed by cervical dislocation on day 20 of gestation. After autopsy, the corpora lutea in the ovaries, number of resorptions, dead and live fetuses were counted. The fetuses were examined, weighed, external abnormalities were recorded, and the craniocaudal size was measured. Microanatomical study of visceral organs was carried out some embryos; in others, abnormalities of the bone skeleton, mean number of ossification foci per embryo in the metacarpus, metatarsus, spine, and sternum, and the cranial abnormalities were counted.

Parameters of physical development and behavior in the open field test were evaluated in the progeny of group 2 rats.

All procedures for evaluation of the reproductive toxicity were carried out in accordance with the standard protocols [4].

The results were statistically processed using Student's *t* test and Pierson's χ^2 test. All embryos obtained in experiment were included in the study.

RESULTS

Cytogenetic analysis of bone marrow cells from mice treated with nc-Si in doses of 5, 25, and 50 mg/kg showed no increase in the levels of abnormal metaphases in any variant of the experiment. Chromosome aberrations in the control and experimental groups were represented by only achromatic gaps and solitary fragments. More complex aberrations were not detected (Table 1).

Analysis of DNA damage after 3-h exposure with nc-Si in doses of 5 and 50 mg/kg showed virtually identical levels of DNA damage in bone marrow and brain cells of control and experimental animals (Table 2).

Prolongation of exposure to 24 h resulted in a significant increase in the levels of DNA damage in the bone marrow cells of animals injected with 5 mg/kg nc-Si. Injection of nc-Si in a dose of 50 mg/kg resulted

TABLE 1. Cytogenetic Activity of nc-Si in Bone Marrow Cells of F_1(CBA×C57Bl/6) Males

nc-Si dose, length of exposure	Metaphase	Per 100 cells				Percentage of abnormal metaphases	Significance levels
		gaps	single fragments	paired fragment	exchanges		
Control	500	0.4	0.6	0	0	1.0±0.4	
5 mg/kg, 24 h	500	0.4	0.8	0	0	1.2±0.5	>0.05
50 mg/kg, 24 h	500	0	0.8	0	0	0.8±0.4	>0.05
5 mg/kg, 7 days	500	0.6	0.6	0	0	1.2±0.5	>0.05
25 mg/kg, 7 days	500	0.2	1.2	0	0	1.0±0.4	>0.05
5 mg/kg, 14 days	500	0.6	0.2	0	0	0.8±0.4	>0.05
25 mg/kg, 14 days	500	0.2	1.2	0	0	1.4±0.5	>0.05

in a significantly greater increase of the level of DNA damage in the bone marrow and brain cells. This effect was not observed after 7-day exposure to nc-Si in a dose of 5 mg/kg (Table 2).

In a dose of 50 mg/kg nc-Si caused death of 60-80% animals on days 2-3 of exposure, and hence, evaluation of the genotoxic potential of particles in this dose was impossible for the delayed periods after injection. Pathomorphological findings indicate that the mortality was most likely caused by kidney involvement.

Body weight increment decreased significantly by the 2nd injection in animals injected with nc-Si on day 1 of pregnancy. No differences in body weight gain between the experimental and control animals were detected before the 3rd injection. A significantly greater body weight increment was noted in experimental rats after the 3rd injection (on day 20 of pregnancy). Hence, the summary body weight increment throughout pregnancy was similar in the two groups (Table 3).

The increase in the postimplantation mortality was statistically negligible (p=0.05). No differences between the groups in the number of implantation foci, placental weight, mean weights and size of embryos were detected (Table 4).

Visual examination of fetuses from females injected with nc-Si showed 6.9% fetuses with hematomas, 17.2% with hemorrhages, and 39.1% with hyperemias, which virtually coincided with the values in the control (6.8, 6.8, and 37.5%, respectively).

Analysis of visceral abnormalities (by Wilson method) showed no differences between the fetuses in the control (43 fetuses) and experimental (51 fetus) groups. No abnormalities in the fetal bone system were detected (Dawson's method).

Newborn rats (male and female) exposed to nc-Si *in utero* were small for date from day 5 to day 45 of their lives. These differences in body weight between experimental and control animals disappeared only by the age of 60 days (Table 5).

No differences in physical development of little rats in the two groups were detected. The terms of pinnae detachment, hair growth, canine eruption, eye

TABLE 2. Evaluation of DNA Destructive Activity of nc-Si (in %) in F_1(CBA×C57Bl/6) Males

Length of exposure; group			Bone marrow	Brain
3 h	control		2.7±0.6	3.6±0.7
	nc-Si	5 mg/kg	3.7±0.6	3.7±1.2
		50 mg/kg	4.2±1.1	3.5±0.9
24 h	control		4.9±0.7	2.8±1.1
	nc-Si	5 mg/kg	22.1**±0.9	3.5±0.8
		50 mg/kg	24.2**±1.3	6.5*±0.6
7 days+	control		7.7±1.3	3.6±0.9
	nc-Si	5 mg/kg	7.3±1.3	3.8±1.0

Note. +For the dose of 50 mg/kg: animal death. *p<0.05, **p<0.001 compared to the control.

TABLE 3. Effect of nc-Si on Body Weight Gain (g) in Pregnant Rats

Group	Day of pregnancy			
	1-7	7-14	14-20	1-20
Control (*n*=15)	24.0±2.1	28.0±2.7	38.3±3.5	90.3±3.4
nc-Si (*n*=14)	9.7±2.7*	35.8±2.8	51.4±3.4*	96.9±4.7

Note. Here and in the Table 4 and 5: *$p \leq 0.05$ compared to the control.

opening, vagina opening, and descent of the testes corresponded to normal. No disorders in the formation of unconditioned reflexes by day 5 (turning on the plane, edge avoidance, and sticking to a horizontal cord) were observed.

Evaluation of behavior of 30-day-old rats in the open field test showed significantly better parameters of horizontal motor activity and exploratory activity (number of explored holes) and lower anxiety (estimated by the number of boluses) in experimental females compared to control females. Experimental males exhibited higher grooming and anxiety values (by the number of boluses). However, these variants of behavior were within the range of historical controls, and could not be attributed to the effect of nc-Si.

Available data on nc-Si capacity to induce oxidative stress suggested that genotoxic effects could be expected after long exposure [12,13], as was shown in studies of chrysotile asbestos fibers [8]. However, no cytogenetic effects of nc-Si were detected; a slight genotoxic effect in the DNA comet assay was detected only after 24-h exposure.

Disagreement in the results of the two widely used tests is in line with the general ambiguity of the results obtained in nanoparticles genotoxicity testing [10]. All these data confirm principal importance of choice of the test systems and methods, validation of doses, routes of administration, duration of exposure, and other methodological features of experiments in nanoparticles genotoxicology. Possible practical decisions concerning optimization of methodological approaches to studies of nanoparticles and adequate evaluation of their genotoxicity can be made only after accumulation of comprehensive empirical data. At present we have to agree with the opinion expressed by R. Landsiedel *et al.* that we have much more questions to ask about nanoparticles genotoxicology than answers to them [10].

TABLE 4. Effect of nc-Si on Fetal Development in Rats

Group	Number of corpora lutea per female	Number of implantation foci per female	Pre-implantation death, %	Number of viable fetuses per female	Postimplantation death, %	Mean fetal body weight, g	Mean fetal size, cm	Placental weight, g
Control (*n*=10)	9.9±0.5	9.0±0.5	9.1	8.8±0.0	2.2	2.58±0.06	2.99±0.05	0.59±0.01
nc-Si (*n*=9)	13.1±0.6*	10.2±0.7	21.4*	9.6±0.9	6.8	2.63±0.09	3.10±0.03	0.62±0.06

TABLE 5. Dynamics of Body Weight (g) in the Progeny of Control and Experimental Rats

Group	Rat gender	Day of life				
		5	15	30	45	60
Control (*n*=41)	Females (*n*=24)	13.0±0.3	30.2±0.8	94.7±2.9	167.8±3.9	215.5±4.7
	Males (*n*=17)	14.0±.0.4	32.4±1.1	101.9±4.6	195.2±9.3	284.1±9.3
nc-Si 50 mg/kg (*n*=48)	Females (*n*=19)	10.6±0.3*	24.8±0.5*	79.9±1.9*	147.7±4.3*	204.9±4.4
	Males (*n*=29)	11.7±0.2*	27.3±0.5*	85.9±1.4*	174.3±3.4*	270.7±4.9

The lethality of nc-Si in a dose of 50 mg/kg detected in mice was not confirmed in experiments on pregnant rats which received a total dose of 150 mg/kg. This presumably indicates species specificity of the toxic effect of the studied specimen. However, the general toxic effect of intraperitoneal nc-Si in rats is obvious: it manifested in impaired body weight gain in pregnant rats and their progeny at different stages of observation.

It is principally important that there were no teratogenic effects even in such a stringent experimental protocol as 3 intraperitoneal injections of nc-Si in a total dose of 150 mg/kg.

Hence, no cytogenetic and teratogenic effects of nc-Si were detected *in vivo*. Slight genotoxic activity of these particles in bone marrow and brain cells were detected. The general toxic effects manifested in deaths of some mice and impaired body weight gain in pregnant rats and their progeny at some stages of the experiment.

REFERENCES

1. A. D. Durnev, A. K. Zhanataev, E. A. Anisina, *et al.*, *Evaluation of Genotoxic Effects of Natural and Synthetic Compounds by the Alkaline Gel Electrophoresis of Isolated Cells. Methodological Recommendations* [in Russian], Moscow (2006).
2. A. A. Kudryavtsev, V. P. Lavrovskaya, L. A. Osminkina, *et al.*, *Fiz. Med.*, No. 2, 4-8 (2006).
3. V. Yu. Timoshenko, A. A. Kudryavtsev, L. A. Osminkina, *et al.*, *Zh. Eksper. Teoretich. Fiz.*, No. 9, 492-495 (2006).
4. N. M. Smol'nikova, B. I. Lyubimov, A. D. Durnev, *et al.*, *Manual of Experimental (Preclinical) Studies of New Drugs*, Ed. R. U. Khabriev [in Russian], Moscow (2005), P. 87-100.
5. L. T. Canham, *Nanoscale Semiconducting Silicon as a Nutritional Food Additive*, *Nanotechnology*, **18**, 185,704-185,710 (2007).
6. A. G. Cullis, L. T. Canham, and P. D. J. Calcott, *J. Appl. Phys.*, **82**, 909-965 (1997).
7. M. J. Derelanko and M. A. Hollinger, *Developmental Toxicology: Handbook of Toxicology*, 2nd ed., London, New York (2002), P. 498-509.
8. A. D. Durnev, N. O. Daugel-Dauge, L. G. Korkina, and S. B. Seredenin, *Mutat. Res.*, **319**, No. 8, 303-308 (1993).
9. K. Konca, L. Lankoff, A. Bansik, *et al.*, *Ibid.*, **534**, Nos. 1-2, 15-20 (2003).
10. R. Landsiedel, M. D. Kapp, M. Schulz, *et al.*, *Ibid.*, **681**, Nos. 2-3, 241-258 (2009).
11. E. J. Park and K. Park, *Toxicol. Lett.*, **184**, No. 1, 18-25 (2009).
12. R. J. Preston, B. J. Dean, S. Galloway, *et al.*, *Mutat. Res.*, **189**, No. 2, 157-165 (1987).
13. H. Yang, C. Liu, D. Yang, *et al.*, *J. Appl. Toxicol.*, **29**, No. 1, 69-78 (2009).