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Comparison of fetal toxicity of various multi-wall carbon nanotubes in mice

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ABSTRACT

The fetal toxicities of multi-wall carbon nanotubes (MWCNTs) with various sizes were compared in CD1(ICR) mice. MWCNTs were suspended in 2% sodium carboxymethyl cellulose solution in phosphate-buffered saline. On day 9 of gestation, dams were administered a single intraperitoneal dose of MWCNTs (4 mg/kg body weight), while dams in the control group were administered vehicle (10 mL/kg body weight). The rectal temperatures of the dams were monitored 2 h after administaration to asses statuses of the dams. The dams and fetuses were examined on day 18 of gestation. The number of live fetus per dam decreased in some MWCNTs-administered groups. The mean percentages of live fetuses in total implantations in the MWCNTs-administered groups markedly varied from 0% to 95%, and the highest mean percentage of live fetuses in the MWCNTs-administered group was equivalent to that of the control group. The decrease in live fetuses depended on an increased number of early dead fetuses. In the groups with markedly lowered rectal temperature after administration, the fetal loss were evident. The blood levels of interleukin-6 and/or monocyte chemoattractant protein-1 in dam 2 h after administration of MWCNTs markedlyr increased, especially in the goups with significant decrease in live fetuses. These results indicated a relationship between inflammation in the dam, which probabely depended on the particular length of the MWCNTs, and the fetal toxicioty of MWCNTs in mice.

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1. Introduction

Recently, rapidly developing nanotechnologies have produced many kinds of nanomaterials that are appealing new resources that may make our life more convenient. Critical risk assessments have been conducted of the possible adverse effects of these materials in order to ensure the safety of humans, and domestic and companion animal. Among those, many investigators have paid close attention to carbon nanotubes, a very anticipated nanomaterial, because the size and structure of carbon nanotubes resemble those of asbestos [16,1,4,14,13,18,10]. First, Takagi et al. [19] reported the induction of mesotheliomas by the intraperitoneal administration of multi-wall carbon nanotubes (MWCNTs) to male *p*53-deficient mice. Subsequently, Sakamoto et al. [17] found the induction of mesotheliomas by MWCNTs in intact (not genetically modified) rats. In addition, during the course of various toxicological evaluations of MWCNTs in our laboratory, we revealed the teratogenicity of MWCNTs that were intraperitoneally or intratracheally administered to mice [5]. Currently, MWCNTs are a focus of attention in exogenous fibrous toxicity [10,12,2,21], because both mesothelioma and teratogenicity are induced by MWCNTs at lower dose levels than those of asbestos that cause the same effects [17,5,6]. Difference abilities for inducing inflammation and formation of mesothelial granulomas and finally inducing mesotheliomas had been shown to markedly vary in some MWCNTs products, and this is thought to possibly depend on the differences in the length of MWCNTs [16,4,12,18]. However, at the present, the differences in the effects on embryos or fetuses among MWCNTs products with different sizes remain unclear. The present study was conducted to clarify the relationship between the sizes of MWCNTs and the effects on fetuses or dams that result in the fetal toxicity in mice.

Abbreviations: MWCNTs, multi-wall carbon nanotubes; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1.

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Table 1
The size, purity and Fe content of the multi-wall carbon nanotubes (MWCNTs) utilized in the present study.

MWCNTs	Length (μm)	Diameter (nm)	Purity (%)	Fe content (%)
WS	0.5–2	40–70	–	0.046
N	1–4 ; 50.9% 5–20 ; 27.5% Mean 3.5	50–80 ; 94.8%	>98	0.046
M	1–4 ; 38.6% 5–20 ; 58.3% 20- ; 3.1% Mean 3.3	50–80 ; 97.2%	>95	0.35
WL	0.5–10	85-200	–	6.504
SD-1	Mean 6	Mean 150	>99.95	<0.005
T	several 10 to several 100	20–100	85–94	3.822

2. Materials and methods

2. Ethical consideration of the experiment

The experimental protocol was approved by the Experiments Regulation Committee and the Animal Experiment Committee of the Tokyo Metropolitan Institute of Public Health prior to its execution. It was monitored at every step during the experimentation for its scientific and ethical appropriateness, which included concern for animal welfare, with strict obedience to the National Institutes of Health Guideline for the Care and Use of Laboratory Animals, the Japanese Government Animal Protection and Management Law, Japanese Government Notification on Feeding and Safekeeping of Animals, and other similar laws, guidelines, rules provided domestically and internationally.

3. Test chemicals

The MWCNTs were provided from Mitsui Chemicals Inc., Tokyo Japan (MWNT-7) (M), Nikkiso Co., Ltd. (Tokyo, Japan) (N), Wako Pure Chemicals Industries Ltd. (Osaka, Japan) (WS and WL), Toda Kogyo Corporation (Hiroshima, Japan) (T) and Showa Denko K. K., (Tokyo, Japan) (SD-1). The size and purity of each MWCNTs are summarized in Table 1. The MWCNTs were suspended in 2% sodium carboxymethyl cellulose (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) /phosphate-buffered saline by vigorous stirring and sonication for uniform suspension. Those suspensions were autoclaved (120 °C, 30 min.) to ensure sterility before use.

4. Animals

Specific pathogen free male and female Crlj: CD1 (ICR) mice were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan) and they were housed individually in plastic cages with pulp bedding (ALPHA-dri®, Shepherd Specialty Papers, Inc., Watertown, TN, USA). The animals were allowed free access to the standard diet CE2 (CREA Japan, Inc., Tokyo, Japan) and water. The animal room was maintained at 23–-25 °C with a relative humidity of 50–60%, 10 ventilations per hour (fresh air was drawn in through a 0.3-μm HEPA filter with 99.9% efficiency), and a 12-h light/dark cycle. When the mice were 8–12 weeks old, they were mated by housing one male and one female in cage overnight. The next morning, females with vaginal plug were considered at day 0 of gestation.

5. Experiment

On day 9 of gestation, the dams were randomly allocated to seven groups of 10–15 mice each and the groups were administered a single intraperitoneal dose of MWCNTs (4 mg/10 mL/kg body weight). This dose has been shown to induce fetal toxicity (fetal death) in the previous teratogenicity study on M [5]. The control mice were administered vehicle. Before and 2 h after administration, the rectal temperatures of the mice were monitored with an electrical thermometer (Citizen Systems Japan Co., Tokyo, Japan). In addition, 2 h after the administration of MWCNTs, 6–13 μL of peripheral bloods were collected from tail vain by puncturing it with a needle. Blood smears were prepared from a part of the collected blood, and the sera from the remaining blood were stored at −20 °C. On day 18 of gestation, the dams were anesthetized with isoflurane, and blood samples were collected in ethylene diamine tetraacetic acid-2K anticoagulant tubes from the femoral vein. The white blood cell counts were examined with an automatic blood analyzer (SYSMEX KX21-NV; SYSMEX Corporation, Kobe, Japan). Blood smears at 2 h after administration and at the time of necropsy were stained by Diff-Quik (SYSMEX Corporation), and the subtypes of white blood cells were counted under the light microscopy. The livers, spleens, kidneys, thymuses, and ovaries were weighed. The uteri were opened and the numbers and positions of implantation site, early dead fetuses (defined as a case showing the implanted site and amorphous mass), late dead fetuses (defined as a case showing head and limbs), and live fetuses were examined. The blood levels of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) were measured in stored serum at 2 h after administration with an ELISA Kit (EM2IL6 and EMMCP1, respectively, Thermo Scientific Inc., Waltham, MA, USA).

6. Statistical analysis

Scheffe's multiple comparisons and the Chi square test were utilized to analyze the differences between the control and MWCNT-administered groups.

7. Results

The effects of MWCNTs on general status of dams are shown in Table 2. In the groups given M, N, WL, or SD-1, the rectal temperatures 2 h after the administration were significantly lower than that of the control group, while those in the groups given WS or T were not significantly changed. The percentage of lymphocytes was decreased and the percentage of neutrocytes was increased 2 h after the administration in the group given WL, to compare with that of the control. Similar tendencies were observed in the other groups given MWCNTs; however, the changes were not statistically significant. One–three days after the administration, bleeding from vagina was noted in the groups given M or N. Two dams in the group given SD-1 died at 1 and 3 days, respectively, after the administration. Necropsies that were performed in those dams cleared the extensive bleeding in uterine. At day 18 of the gestation, the spleen weight of the group given M or N were significantly increased to compare with that of the control group, while those of the group in the groups given WL or SD-1 were slightly increased and those given WS or T were equivalent to the control value. The total white blood cell counts at the necropsy in the group given M was significantly increased, while those in the groups given N or WL were slightly increased. The total white blood cell counts in the groups given WS, T or SD-1 were similar to or lower than the control value. The neutrocyte counts in the groups given M or N were significantly higher than that of the control group, while those of the group given WS, WL, T or SD-1 were similar to or lower than the control value. The eosinocyte counts of the group given M or WL were significantly higher than that of the control group. The monocyte counts in the group given M was significantly higher than that of the control group.

Table 2
The effects of intraperitoneal dose of various MWCNTs on the dams.

	Control	MWCNTs administered (4mg/kg b.w.)					
	(CMCNa)	WS	N	M	WL	SD-1	T
Female mated	11	10	10	15	10	10	11
Female died	0	0	0	0	0	2	0
Female gestated	11	9	9	12	7	8	9
Rectal temperature							
Before the administration	38.4 ± 0.3	38.6 ± 0.2	38.2 ± 0.2	38.1 ± 0.3	38.5 ± 0.3	38.4 ± 0.4	38.5 ± 0.3
2 h after administration	37.4 ± 0.5	36.8 ± 0.7	33.3 ± 0.9**	33.1 ± 0.7***	34.4 ± 1.2***	34.8 ± 0.5***	36.9 ± 0.8
White blood cell differentiations							
2 h after administration							
Lymphocyte (%)	64.3 ± 7.1	55.7 ± 10.5	48.9 ± 10.2	48.6 ± 12.3	42.1 ± 6.8*	53.4 ± 13.6	52.6 ± 12.1
Neutrocyte (%)	29.7 ± 6.1	37.0 ± 10.0	44.7 ± 9.2	42.5 ± 11.2	50.3 ± 9.3*	41.2 ± 13.4	40.0 ± 12.8
Eosinocyte (%)	1.3 ± 1.0	2.0 ± 1.4	2.6 ± 1.7	3.0±1.6	2.1 ± 1.5	1.3 ± 1.3	1.9 ± 1.8
Monocyte (%)	4.7 ± 2.4	5.3 ± 1.7	3.9 ± 1.8	5.9 ± 3.0	5.3 ± 3.9	4.1 ± 2.3	5.4 ± 1.4
Organ weight							
Liver (g)	2.86 ± 0.30	3.15 ± 0.35	22.77 ± 0.55	0.94 ± 0.30	2.92 ± 0.70	3.36 ± 0.30	3.21 ± 0.25
Kidney (mg)	2.86 ± 0.30	464 ± 41	467 ± 85	454 ± 52	460 ± 43	476 ± 34	469 ± 27
Spleen (mg)	182 ± 36	154 ± 33	474 ± 110***	360 ± 104***	246 ± 34	476 ± 34	169 ± 36
Thymus (mg)	24.7 ± 7.2	29.3 ± 8.8	37.2 ± 9.3	25.8 ± 7.8	25.5 ± 6.0	26.4 ± 9.0	26.5 ± 7.9
Adrenals (mg)	9.1 ± 1.4	9.7 ± 1.5	10.3 ± 1.9	10.4 ± 1.2	9.6 ± 0.5	26.4 ± 9.0	10.0 ± 1.6
Ovaries (mg)	19.0 ± 2.6	24.7 ± 4.2	29.8 ± 2.4	5.0 ± 4.4	123.6 ± 4.3	26.3 ± 2.4	26.5 ± 3.2
White blood cell count (x10^2/μL)							
Total	62.7 ± 13.4	48.4 ± 20.9	82.1 ± 15.7	108.9 ± 37.0**	76.3 ± 13.5	76.3 ± 13.5	76.3 ± 13.5
Lymphocyte	29.2 ± 8.7	26.4 ± 10.7	218.5 ± 4.6	7.9 ± 8.7	27.9 ± 3.5	23.9 ± 6.6	24.5 ± 9.2
Lymphocyte	28.3 ± 10.3	19.8 ± 13.6	47.0 ± 8.9*	48.8 ± 19.6*	27.9 ± 3.5	24.0 ± 5.0	18.9 ± 8.2
Eosinocyte	1.0 ± 0.5	0.7 ± 0.6	9.9 ± 2.7	22.1 ± 13.7***	15.3 ± 4.0**	7.8 ± 3.3	0.5 ± 0.5
Eosinocyte	3.7 ± 2.0	0.7 ± 0.6	6.6 ± 2.7	10.0 ± 6.7*	5.0 ± 1.8	3.4 ± 1.5	2.4 ± 2.2

Values are the means ± standard deviations for numbers of the dam in each group. The asterisks represent that the values are significantly different from the corresponding control values (*, ** or *** indicating $p < 0.05$, 0.01 or 0.001, respectively).

Effects of MWCNTs on the pregnant status of dams are shown in Table 3. The number of early dead fetus and the percentage of the early dead fetus in the implantation were significantly higher in the groups given M than those of the control group. In the group given N, the number of dam with one more live fetus was zero, and the number of early dead fetus was equal to the number of the implantation, which resulted in 100% of the early dead fetus in the implantation. In the groups given WL or SD-1, the number of early dead fetus had a tendency to be increased, but these differences were not statistically significant. The incidence of the dam with early dead fetus in the groups given M, N, WL, T or SD-1, was significantly higher than that of the control. The number of late dead fetus or the percentages of late dead fetus in the implantation was not changed in any of the groups given MWCNTs. Resulting from increased number of the dead fetus in early stage, the number of live fetus and the percentage of live fetus in the implantation in the group given M were significantly lower than those of the control group. And the number of live fetus and the percentage of live fetus in the implantation in the group given N were zero. The body weight of live fetus (male and female) was not changed in the groups given MWCNTs.

Blood levels of IL-6 and MCP-1 in the dams 2 h after administration of MWCNTs are shown in Fig. 1. In the groups given M or N, significant increases in the blood levels of both IL-6 and MCP-1 were found. In the groups given WL or SD-1, those cytokine levels were somewhat increased, but these differences were not statistically significant to compare with those of the control.

8. Discussion

The fetal toxicity of MWCNTs markedly varied among the products tested. Administration of M (main length 5–20 μm) and N (main length 1–20 μm) significantly decreased the number of live fetuses. Administration of WL (length 0.5–10 μm) and SD-1 (mean

Table 3
The effects of intraperitoneal dose of various MWCNTs on pregnancy status.

Reproductive parameters	Control	MWCNTs administered (4 mg/kg b.w.)					
	(CMCNa)	WS	N	M	WL	SD-1	T
Female gestated	11	9	9	12	7	8	9
Female with>1 live fetus	11	9	0***	8	6	8	9
Implantations/litter[a]	13.3 ± 2.4	14.6 ± 2.2	14.6 ± 1.8	14.5 ± 1.6	13.6 ± 3.3	16.8 ± 1.7	15.4 ± 2.4
Number of early[b]dead fetus[a]	0.1 ± 0.3	0.4 ± 0.5	14.6 ± 1.8***	8.8 ± 6.3***	3.6 ± 5.2	3.8 ± 2.9	0.9 ± 0.8
(% of implantations)	1 ± 2	3 ± 4	100 ± 0***	59 ± 41***	24 ± 35	10 ± 9	6 ± 5
Female with early dead fetus	1	4	9***	10***	6**	7**	6*
Number of late[b]dead fetus[a]	0.3 ± 0.5	0.2 ± 0.4	0	0.3 ± 0.6	0.1 ± 0.4	0.4 ± 0.5	0.3 ± 0.5
(% of implantations)	2 ± 4	1 ± 3	0	2 ± 4	1 ± 3	2 ± 3	2 ± 3
Female with late dead fetus	2	2	2	0	1	3	3
Live fetus/litter[a]	12.9 ± 2.5	13.9 ± 2.2	0***	5.5 ± 5.6**	10.0 ± 5.1	12.6 ± 3.9	14.2 ± 2.0
	97 ± 4	95 ± 4	0***	39 ± 40***	76 ± 35	88 ± 6	92 ± 7
Body weight of live fetus (g)[a]							
Male	1.398 ± 0.14	1.367 ± 0.137	—	1.360 ± 0.146	1.320 ± 0.125	1.340 ± 0.123	1.435 ± 0.058
Female	1.352 ± 0.111	1.316 ± 0.142	—	1.228 ± 0.068	1.306 ± 0.034	1.252 ± 0.140	1.376 ± 0.073

The percent resorption (early or late dead fetus) and fetal body weight were obtained by averaging the value for each litter. The asterisks represent that the values are significantly different from the corresponding control values (*, ** or *** indicating $p < 0.05$, 0.01 or 0.001, respectively).

[a] Values are the means ± standard deviations.

[b] 'Early' was defined as a case showing the implanted sites and amorphous mass, while 'Late' was defined as a case showing the head and limbs.

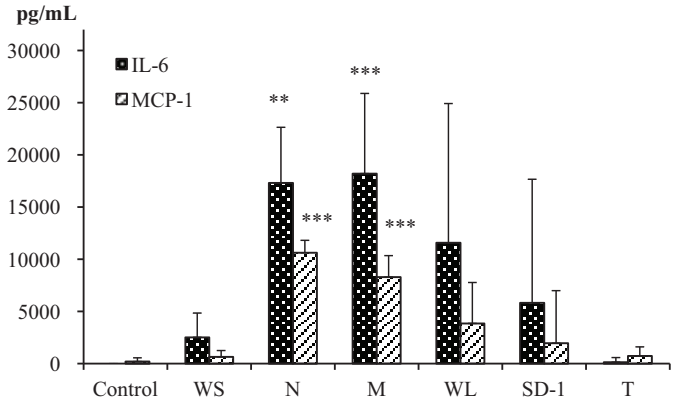

Fig. 1. Blood cytokine levels in dam 2 h after administration of multi-wall carbon nanotubes (MWCNTs). Each column and bar represents the mean ± standard deviation for numbers of the dam in the groups given MWCNTs or the control group. The asterisks represent that the values are significantly different from the corresponding control values (** or *** indicating $p < 0.01$ or 0.001, respectively).

length 6 μm) resulted in slight but non-significant increase in fetal loss. Administration of WS (length 0.5–2 μm) and T (length several 10 to several 100 μm) did not decrease the number of live fetuses in dam but the incidence of dam with early fetal death in T was significant from that of the control group. These results indicated that a moderate length of MWCNTs (those that are not short and not long), such as 5–20 μm, may be necessary to induce fetal toxicity in mice. It had been concerned that the oxidative stress by catalytic action of Fe contained in the MWCNTs may be possibly the mechanistic pathogenesis in mesothelioma induction [14]. However, the same theory can't be adaptive to the fetal toxicity, because T that had the second high Fe content in the presently utilized MWCNTs had the minimum fetal toxicity and N that had the same Fe content as WS had marked fetal toxicity to compare with WS with no fetal toxicity in the present study. Also, it was likely that the diameter of MWCNTs had no important relation on the fetal toxicity, because WS with diameter of 40–70 nm had no fetal toxicity to compare with N and M with diameter of 50–80 nm. It may be possible, however, that the high diameter reduces the fetal toxicity, because WL and SD-1 with relatively high diameter than N or M appeared slight and non-significant fetal effects in the present study. These results in the present study on fetal toxicity did not have contradiction to the report by Nagai [13] in which thin MWCNTs (diameter −50 nm) showed cytotoxicity and inflammogenicity than thick MWCNTs (diameter −150 nm). Also, it was shown that the fetal toxicity of MWCNTs did not depend on the particular manufacturer, because both WS with no fetal toxicity and WL with slight (significant incidence of dam with early fetal death) fetal toxicity were provided by the same manufacturer.

We conducted the experiment of administration in intraperitoneal cavity to avoid the stress to the dam such as anesthetizing and intratracheal administration, because the previous study [5] indicated that intraperitoneal administration of MWCNTs had equal effects on fetus to that of intratracheal administration. It had be shown that intraperitoneally administered asbestos fibers were engulfed in phagocytes Winkler and Rüttner, 1982 and we observed the grayish-colored and swollen lymph nodes beside the thymus in the thoracic cavity of dams given MWCNTs or asbestos, in the previous studies [12,13] and the present study (data not shown), indicating that those fibrous materials might be carried by lymphatic or blood flow from peritoneal cavity. As the asbestos fibers had been provided on the transplacental distribution form dam to fetus in human and experimental animals (Haque et al., 2001) [3,7–9], the same route to fetus might be speculated in the MWCNTs. However, the existence of MWCNTs in fetus has yet not proved at present. And it is unclear whether the cytotoxicity, the oxidative stress and the genotoxicity of single wall carbon nanotubes [23] or MWCNTs [1] observed in the cultured cell lines can be applied on the mechanism of the case of fetal toxicity in mice.

In the present study with intraperitoneal administration and the previous study with intraperitoneal and intratracheal administration [5], we observed the increase of the white blood cell counts, particularly those of the neutrocytes and eosinocyte, and significant increase of spleen weight in the groups given MWCNTs on day 18 of gestation, which indicated the presence of inflammatory change(s) in dams given MWCNTs. Because pregnancy depends upon the highly delicate and successful balance of immunological status, which exceptionally allows growing of the genetically different organism in dam, it is likely that perturbation in the balance of immunological status results in discontinuance of pregnancy, so-called rejection of fetus [15,24,11]. Yamaguchi et al. [22] reported the detailed inflammatory response after intraperitoneal administration of MWCNTs in female mice. However, those previous observation on inflammatory changes were at more than 1 week after [22] or at 9 days after (day 18 of the gestation) [5] administration of MWCNTs. As was clearly shown in the present study and the previous study [5], fetal loss induced by administration of MWCNTs depended on an increase of early fetal death, and not on the late fetal death. The inflammatory changes that occurred at early stage after administration of MWCNTs might be more important in the fetal toxicity that induced by MWCNTs than those that occurred at late stage of gestation. In addition, in the preliminary experiment, we observed the changes in the white blood cell differentiations in pregnant mice that occurred after administration of MWCNTs differed from those in non-pregnant females, in which the increase of eosinocyte counts was numerous than the increase of neutrocyte counts. The inflammatory response to MWCNTs in the pregnant female appeared to be different from the non-pregnant female or male mice. Thus, in the present study, we made observation on the white blood cell differentiations and analyzed the cytokine change(s) in dam soon after administration of MWCNTs, in conjunction with the comparison of fetal toxicity of MWCNTs with various sizes. In the white blood cell differentiations, percentages of neutrocytes were increased in the most groups given MWCNTs 2 h after their administration as well as at the necropsy. Regrettably, blood samples gained 2 h after administration were not enough (50 μL) to analyze total white blood cell counts. However, the tendencies to increase neutrocytes and eosinocytes was similar to the white blood cell differentiations at necropsy, in which case, increased white blood cell counts depended on the increases of neutrocyte counts and eosinocyte counts. It is probable that the total white blood cell counts 2 h after the administration of MWCNTs might increase as a result of the increase of neutrocytes and eosinocytes. These inflammatory changes that occurred soon after administration of MWCNTs in dams could affect fetuses. We thought that a more detailed analysis on the inflammatory changes in dam, for example, cytokine and/or chemokine measurement might be necessary, because the administration of IL-2 caused the abortion in mice [20]. In the preliminary experiment, serum levels of IL-6 and MCP-1 significantly increased soon after the administration of MWCNTs (M) in pregnant females, while serum levels of IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12 (total), the tumor necrosis factor alpha (TNFα) and the interferon gamma (IFNγ) were not significantly changed (data not shown). It was likely that IL-6 and MCP-1 levels in dam are important in the effect(s) of the MWCNTs on the fetuses. The result of the present study induced that (1) the MWCNTs (M and N) that caused marked fetal death also resulted in significantly increase in the level of IL-6 and MCP-1 in the dams, (2) the MWCNTs (WL and SD-1) that caused less fetal death resulted in slight increase in the level of those cytokines and (3) the MWCNTs (WS and T) that

did not affects live fetus numbers caused only negligible changes in those cytokines, supported the fore mentioned hypothesis.

In addition to the inflammatory changes, dam given MWCNTs (M) appeared remarkably less active from several hours to 1 day after administration in the previous study. Regrettably we were unable to measure blood pressure of mice, for the next best thing, we measured the rectal temperature of dams 2 h after administration with comparing that at immediately before the administration of MWCNTs. The MWCNTs (M and N) that caused marked fetal death also significantly lowered the rectal temperatures of dams, the MWCNTs (WL and SD-1) that caused mild fetal death slightly lowered the rectal temperatures and the MWCNTs (WS and T) that did not affects live fetus numbers caused only negligible changes in the rectal temperatures. Although main organ weights, except spleen, did not change, indicating less toxicity on dams by MWCNTs, something shock-like status by the administration of effective MWCNTs on fetus was suggested. The mechanism that caused this condition is currently unclear. Also, the relationship or the eventual order in the inflammatory change, the shock-like status and fetal death is not clear. A study to clarify the relationship between these changes and the fetal loss that induced by MWCNTs is now being conduction in our laboratory.

9. Conclusion

MWCNTs of mean length(μm) 1–20 and 5–20 showed severe fetal toxicity and those of length 0.5–10 and mean length 6 had mild fetal toxicity, while those of length under 2 and over several tens had negrigible fetal toxicity in mice. Diameter, purity or Fe content of MWCNTs seemed not related to the fetal toxicity. In dams given effective MWCNTs, white blood cell counts, spleen weight and blood levels of IL-6 and MCP-1 were increased. A relationship between inflammation in the dam, which probabely depended on the length of the MWCNTs, and the fetal toxicioty of MWCNTs in mice was indicated.

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