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Brief Report

Pulmonary Toxicity in Mice by 2- and 13-week Inhalation Exposures to Indium-tin Oxide and Indium Oxide Aerosols

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Abstract: Pulmonary Toxicity in Mice by 2- and 13-week Inhalation Exposures to Indium-tin Oxide and Indium Oxide Aerosols: Kasuke NAGANO, *et al.*, Japan Bioassay Research Center, Japan Industrial Safety and Health Association—Objectives: Inhalation toxicities of indium-tin oxide (ITO) and indium oxide (IO) in mice were characterized in comparison with those previously reported in rats. **Methods:** B6C3F$_1$ mice of both sexes were exposed by inhalation to ITO or IO aerosol for 6 h/day, 5 day/wk for 2 wk at 0, 0.1, 1, 10 or 100 mg/m^3 or 13 wk at 0, 0.1or 1 mg/m^3. **Results:** ITO and IO particles were deposited in the lung, mediastinal lymph node (MLN) and nasal-associated lymphoid tissue. Alveolar proteinosis, infiltrations of alveolar macrophages and inflammatory cells and increased lung weight were induced by 2- and 13-week exposures to ITO and IO, while alveolar epithelial hyperplasia occurred only in the 2-week exposures. Thickened pleural wall, hyperplastic MLN, extramedullary hematopoiesis in the spleen and increased levels of erythrocyte parameters were induced by 13-week exposure to ITO. The ITO- and IO-induced pulmonary lesions were milder in mice than those previously reported in rats, and the fibrotic lesions were different between these two species. Indium levels in the lung and pooled blood were analyzed in the mice exposed to ITO and IO for 13 wk. In the 13-week inhalation exposure of mice to ITO, alveolar proteinosis and significantly increased lung weight were induced at the same exposure concentration as the current threshold limit value for indium and its compounds.
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Serious concerns have been raised over workers' health in the plants where indium-tin oxide (ITO) is manufactured and processed. Fatal case studies[1, 2] and epidemiology studies of workers[3–6] have demonstrated that inhalation of indium is a potential cause of occupational lung disease and increases the risk of interstitial lung damage. Experimental toxicology studies have shown that an intratracheal administration of ITO powder induces persistent inflammation in the lung without any significant fibrotic response in rats[7], and elicits pulmonary inflammatory response with diffuse alveolar or bronchiolar cell hyperplasia and interstitial fibrotic proliferation in hamsters[8, 9]. Our previous study[10] showed that 2- and 13-week inhalation exposures of rats to ITO or indium oxide (IO) aerosol induce pulmonary fibrosis, alveolar proteinosis and macrophage infiltration.

The present studies were intended to characterize inhalation toxicities of ITO and IO aerosols in mice in comparison with those reported in rats[10].

Materials and Methods

The present studies were performed with the approval of the ethics committee of the Japan Bioassay Research Center. The animals were cared for in accordance with a guide for the care and use of laboratory animals. All these related documentations were cited in our previous rat studies[10].

ITO and IO powders were the same as those used in our previous rat studies[10], which were kindly supplied by JX Nippon Mining & Metals, Corp (Tokyo, Japan).

B6C3F$_1$/Crlj mice of both sexes were obtained at the age of 4 wk from Charles River Japan, Inc (Kanagawa, Japan). The animals were quarantined and acclimated for 2 wk before the start of experiment. The mice were individually housed in stainless-steel wire hanging cages

(112 W × 212 D × 120 H mm), and placed in stainless steel inhalation exposure chambers. Environment in the exposure chamber, lighting, and supply of food and drinking water were maintained under the same conditions as described in our previous rat study[10].

In the 2-week study, groups of 5 mice of both sexes were exposed to ITO or IO aerosol at a target concentration of 0.1, 1, 10 or 100 mg/m^3 for 6 h/day, 5 day/wk for 2 wk. In the 13-week study, groups of 10 mice of both sexes were exposed to ITO or IO aerosol at a target concentration of 0.1 or 1 mg/m^3 for 6 h/day, 5 day/wk for 13 wk. Groups of 5 or 10 mice of both sexes were exposed to clean air for 2 or 13 wk, and served as respective controls.

The aerosol generation and exposure system and the methods for measurements of the exposure concentrations and size distributions of ITO and IO aerosols, and for clinical and pathological examinations and analysis of indium in the lung and blood as well as statistical analysis were the same as those described in our previous rat study[10].

Results

Chamber concentrations and size distributions of ITO and IO aerosols

Chamber concentrations of ITO and IO aerosols were controlled precisely within less than 10% in the variation coefficient and accurately within less than 10% deviation from the target concentrations. Mass median aerodynamic diameters (MMADs) of ITO aerosol in the exposure chamber ranged from 2.4 to 3.8 μm in the 2-week study, and from 2.3 to 2.6 μm in the 13-week study, and geometric standard deviations (GSDs) ranged from 1.6 to 2.4. MMADs of IO aerosol ranged from 1.9 to 2.3 μm, and GSDs ranged from 1.5 to 2.1.

Mortality and clinical signs, and hematology

Neither death, abnormal clinical sign nor growth retardation occurred in any group exposed to ITO, IO or clean air for 2 or 13 wk. In the 13-week study, red blood cell counts, hemoglobin concentration and hematocrit value were significantly increased in the 0.1 and 1 mg/m^3 ITO-exposed groups compared with the respective controls (data not shown). However, 13-week exposure to IO did not induce any hematological changes.

Lung and spleen weights

In the 2-week study (Table 1), the relative lung weights were significantly increased in the 1 mg/m^3 ITO-exposed female mice and in the 10 and 100 mg/m^3 ITO- and IO-exposed mice of both sexes compared with the respective controls. In the 13-week study (Table 2), the relative lung weights in the 0.1 and 1 mg/m^3 ITO-exposed mice of both sexes and in the 1 mg/m^3 IO-exposed mice of both sexes were significantly increased compared with the respective controls. The relative spleen weight was significantly increased in the 1 mg/m^3 ITO- and IO-exposed mice of both sexes in the 13-week study compared with the respective control.

Histopathological findings

In the 2-week study, ITO and IO particles were deposited separately as single particles in the lungs of almost all 1, 10 and 100 mg/m^3 ITO- and IO-exposed mice (Table 1). The particles of ITO and IO were pale brown and transparent, looked like amber, and were located primarily within alveolar macrophages. ITO and IO particles were also deposited in the mediastinal lymph node (MLN) of the 10 and 100 mg/m^3 ITO- and IO-exposed mice, and in the nasal-associated lymphoid tissue (NALT) of the nasopharyngeal duct of a few 10 and 100 mg/m^3 IO-exposed male mice. The most remarkable lung lesion found in the 2-week study was alveolar proteinosis, characterized by filling of the alveolar space with a granular, pale eosinophilic material, which was positively stained with a PAS reagent. This lesion was found in all the 10 and 100 mg/m^3 ITO- and IO-exposed mice. The severity score of alveolar proteinosis was higher in the 100 mg/m^3 ITO-exposed mice of both sexes than in the 100 mg/m^3 IO-exposed mice. Hyperplasia of alveolar epithelium and infiltration of inflammatory cells composed of neutrophils and lymphocytes were observed primarily in the 100 mg/m^3 ITO- and IO-exposed mice. Hyperplasia of the alveolar epithelium was characterized by the increased numbers of cuboidal cells which were assumed to be type II pneumocytes. Infiltration of alveolar macrophages was found in a few 100 mg/m^3 ITO-exposed mice and in one 100 mg/m^3 IO-exposed male mouse.

In the 13-week study, ITO and IO particles were deposited separately as single particles in the lungs of all the 0.1 and 1 mg/m^3 ITO- and IO-exposed mice (Table 2). Those particles were also deposited in the MLN of the 0.1 and 1 mg/m^3 ITO-exposed mice and the 1 mg/m^3 IO-exposed mice. Significantly increased incidences of alveolar proteinosis in the 0.1 and 1 mg/m^3 ITO-exposed mice and in the 1 mg/m^3 IO-exposed mice and infiltrations of alveolar macrophages and inflammatory cells in the 1 mg/m^3 ITO- and IO-exposed mice were noted. Swelling of cytoplasm was recognized in the alveolar macrophages engulfing the particles. Alveolar wall fibrosis was not found in any mouse exposed to either ITO or IO. Thickening of pleural wall and hyperplasia of the MLN occurred in the 1 mg/m^3 ITO-exposed mice. The thickened pleural wall was characterized by an increase in collagen-like connective tissue in the interstitium, and was located in focal lung areas. However, ITO particles were not found in the area of the thickened pleural wall. Hyperplasia of the MLN was characterized by increased numbers of lymphocytes resulting in increased MLN size and area of lymphoid follicles. Extramedullary hematopoiesis occurred in the red pulp of the spleen of the 1 mg/m^3 ITO-

Table 1. Relative lung weights and histopathological findings in the lung and lymph nodes of male and female mice exposed to ITO or IO at 4 different concentrations or clean air for 2 wk

		ITO					IO				
Group name (mg/m^3)		Control	0.1	1	10	100	Control	0.1	1	10	100
No. of animals examined		5	5	5	5	5	5	5	5	5	5 a)
<Male>											
Relatve lung weigtht (%)	mean	0.69	0.65	0.79	0.98$^{\#\#}$	1.10$^{\#\#}$	0.60	0.63	0.70	0.91$^{\#\#}$	1.00$^{\#\#}$
	SD	0.05	0.04	0.06	0.11	0.08	0.07	0.04	0.03	0.14	0.10
Deposition of particles											
Lung		0	0	5	5	5	0	0	5	5	5
MLN		0	0	0	1	5	0	0	0	4	5
NALT		0	0	0	0	0	0	0	0	1	3
Histopathological findings											
Lung											
Alveolar proteinosis		0	0	0	5	5	0	0	0	5	5
					<1.0>	<1.8>				<1.0>	<1.0>
Infiltration of alveolar macrophages		0	0	0	1	2	0	0	0	0	1
					<1.0>	<1.0>					<1.0>
Infiltration of inflammatory cells		0	0	0	1	5	0	0	0	1	5
					<1.0>	<1.0>				<1.0>	<1.0>
Hyperplasia of alveolar epithelium		0	0	0	1	5	0	0	0	0	3
					<1.0>	<1.0>					<1.0>
<Female>											
Relative lung weight (%)	mean	0.74	0.76	0.94$^{\#\#}$	1.06$^{\#\#}$	1.34$^{\#\#}$	0.69	0.71	0.76	0.93$^{\#\#}$	1.12$^{\#\#}$
	SD	0.12	0.03	0.06	0.15	0.07	0.06	0.04	0.12	0.14	0.08
Deposition of particles											
Lung		0	0	4	5	5	0	0	5	5	4
MLN		0	0	0	3	5	0	0	0	1	4
NALT		0	0	0	0	0	0	0	0	0	1
Histopathological findings											
Lung											
Alveolar proteinosis		0	0	0	5	5	0	0	0	5	4
					<1.0>	<2.0>				<1.0>	<1.0>
Infiltration of alveolar macrophages		0	0	0	0	3	0	0	0	0	0
						<1.0>					
Infiltration of inflammatory cells		0	0	0	0	4	0	0	0	0	3
						<1.0>					<1.0>
Hyperplasia of alveolar epithelium		0	0	0	0	4	0	0	0	0	2
						<1.0>					<1.0>

Values indicate number of animals bearing lesions. The values in angle bracket indicate the average of severity grade index of the lesion. The average of severity grade is calculated with a following equation. Σ(grade × number of animals with grade) / number of affected animals. Grade: 1, slight; 2, moderate; 3, marked; 4, severe. Significant difference: $^{\#\#}$, $p \leq 0.01$ by Dunnett's test. MLN: Mediastinal lymph nodes. NALT: Nasal-associated lymphoid tissue. a): Number of female animals was 4, because one female accidentally died before the end of the 2-week exposure period.

Table 2. Relative lung and spleen weights, histopathological findings in the lung, lymph nodes and spleen and indium contents in the lung and blood of male and female mice exposed to ITO or IO at 0.1 or 1 mg/m^3 or clean air for 13 wk

		ITO			IO		
Group name (mg/m^3)		Control	0.1	1	Control	0.1	1
No. of animals on examined		10	10	10	10	10	10
<Male>							
Relative lung weight (%)	mean	0.51	0.65$^{\#}$	1.01$^{\#\#}$	0.56	0.57	0.82$^{\#\#}$
	SD	0.03	0.04	0.08	0.04	0.03	0.06
Relative spleen weight (%)	mean	0.19	0.20	0.33$^{\#\#}$	0.20	0.20	0.22$^{\#}$
	SD	0.01	0.02	0.07	0.01	0.03	0.02
Deposition of particles							
Lung		0	10	10	0	10	10
MLN		0	4	9	0	0	6
Histopathological findings							
Lung							
Alveolar proteinosis		0	10**	10**	0	0	10**
			<1.0>	<1.4>			<1.6>
Infiltration of alveolar macrophages		0	2	10**	0	0	10**
			<1.0>	<1.1>			<1.0>
Infiltration of inflammatory cells		0	0	10**	0	0	5*
				<1.5>			<1.6>
Thickening of pleura		0	0	3	0	0	0
				<1.0>			
Lymph nodes							
Hyperplasia of MLN		0	0	9**	0	0	0
				<1.0>			
Spleen							
Extramedullary hematopoiesis		0	0	4	0	0	0
				<1.0>			
Indium contents							
Lung (μg/g as In)		ND	11.5 ± 1.1	77.4 ± 12.2	ND	10.1 ± 1.1	183.3 ± 17.1
Blood (μg/*l* as In)	a)	ND	ND	0.58	ND	ND	ND
<Female>							
Relative lung weight (%)	mean	0.60	0.68$^{\#}$	1.12$^{\#\#}$	0.64	0.66	0.97$^{\#\#}$
	SD	0.04	0.02	0.09	0.05	0.05	0.11
Relative spleen weight (%)	mean	0.29	0.31	0.49$^{\#\#}$	0.32	0.33	0.38$^{\#}$
	SD	0.02	0.02	0.15	0.04	0.05	0.05
Deposition of particles							
Lung		0	10	10	0	10	10
MLN		0	2	8	0	0	9
Histopathological findings							
Lung							
Alveolar proteinosis		0	6*	10**	0	0	10**
			<1.0>	<1.6>			<1.8>
Infiltration of alveolar macrophages		0	0	10**	0	0	9**
				<1.0>			<1.0>
Infiltration of inflammatory cells		0	0	9**	0	0	6*
				<1.6>			<1.3>
Thickening of pleura		0	0	1	0	0	0
				<1.0>			
Lymph nodes							
Hyperplasia of MLN		0	0	5*	0	0	0
				<1.0>			
Spleen							
Extramedullary hematopoiesis		0	0	6*	0	0	0
				<1.0>			
Indium contents							
Lung (μg/g as In)		ND	7.8 ± 1.3	74.9 ± 10.0	ND	8.6 ± 1.1	166.6 ± 20.8
Blood (μg/*l* as In)	a)	ND	ND	0.90	ND	ND	ND

Values indicate number of animals bearing lesions. The values in angle bracket indicate the average of severity grade index of the lesion. The average of severity grade is calculated with a following equation. Σ(grade × number of animals with grade) / number of affected animals. Grade: 1, slight; 2, moderate; 3, marked; 4, severe. Significant difference: $^{\#}$, $p \leq 0.05$; $^{\#\#}$, $p \leq 0.01$ by Dunnett's test; *, $p \leq 0.05$; **, $p \leq 0.01$ by Chi-square test. ND: Indium contents were below the quantitative detection limits (lung: 0.006 μg/g tissue, blood: 0.5 μg/*l* whole-blood). MLN: Mediastinal lymph nodes. a) : The value was obtained from the pooled blood of 10 animals for the indium analysis.

exposed mice.

Lung and blood contents of indium

Lung concentrations of indium expressed as μg/g tissue were increased with an increase in the exposure concentrations (Table 2). The contents of indium in the 0.1 mg/m^3 ITO-exposed mice of both sexes were approximately equal to those in the 0.1 mg/m^3 IO-exposed mice of both sexes, but the indium contents in the 1 mg/m^3 ITO-exposed mice was lower by 60% than those in the 1 mg/m^3 IO-exposed mice. Pooled blood contents of indium from ten 1 mg/m^3 ITO-exposed male and female mice were found to be 0.58 and 0.90 μg/*l*, respectively.

Discussion

In the present study, incidences and severities of the pulmonary lesions were found to be higher after ITO exposures than after IO exposures. Higher susceptibility of mice to ITO than IO is consistent with the previously reported findings in rats[10]. Species differences in the toxicity are apparent in comparison with the previously reported rat toxicity[10]. First, the severity score of alveolar proteinosis and the incidence of alveolar macrophage infiltration were lower in mice than in rats. Our previous and present results are in sharp contrast to the NTP's findings[11] that mice are more susceptible to the pulmonary toxicity of indium phosphide particles than rats. Second, 4 cases of thickened pleural wall were recognized in the mice exposed to ITO for 13 wk, while only one female case of thickened pleural wall was observed in the ITO-exposed rats at the end of the 26-week post-exposure period[10]. On the other hand, the ITO-exposed rats exhibited a high incidence of alveolar wall fibrosis which occurred only at the end of the 26-week post-exposure period after cessation of the 13-week exposure to ITO[10]. ITO particles were not found in the area of the thickened pleural wall in the ITO-exposed mice, although we did find deposition of the particles in the MLN. Insoluble particles in the deep lung have been reported to translocate through the pleural surface of the pulmonary lymphatic pathway into the MLN[12, 13]. A pathogenic behavior of ITO particles in the pleural surface of mice and the pulmonary interstitium of rats to explain the species difference in the fibrotic response pattern remains to be solved. Third, the significant increase in the erythrocyte parameters and the increased incidence of extramedullary hematopoiesis in the spleen occurred only in the ITO-exposed mice, but not in the ITO-exposed rats[10] , in the 13-week studies. A plausible explanation for this is that ITO-induced lung inflammation and alveolar proteinosis might cause functional impairment of respiration, including possible reduction of blood oxygen saturation, resulting in an adaptive increase in the erythrocyte parameters and extramedullary hematopoiesis in the spleen.

A threshold limit value (TLV) of 0.1 mg/m^3 for indium and its compounds has been recommended by the American Conference of Governmental Industrial Hygienists (ACGIH)[14]. In the present 13-week study, alveolar proteinosis in the ITO-exposed mice was found to occur at the same exposure concentration as the ACGIH's TLV. Therefore, the present mouse study provides novel information about ITO-induced toxicity which leads to the re-consideration of the current occupational exposure limit value for indium.

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