Short Communication

Pulmonary Toxicity of Indium-Tin Oxide and Indium Phosphide after Intratracheal Instillations into the Lung of Hamsters

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Indium belongs to Group III A in the periodic table and is mainly used in the making of thin-film transistor liquid crystal displays (LCDs) for television screens, portable computer screens, pocket telephone displays and video monitors, mainly through the utilization of indium-tin oxide (ITO). ITO is a sintered alloy containing a large portion of indium oxide and a small portion of tin oxide⁴. On the other hand, indium phosphide (InP) belongs to the III–V semiconductor compounds that are widely used in the semiconductor industry⁵). In 2000, the total domestic indium use in Japan was comprised 83% ITO and 4% InP⁶). Due to the increasingly frequent industrial use of ITO and InP, the potential occupational or environmental exposure to indium compounds has attracted much attention. Although some animal studies concerning the lung toxicity of indium-containing semiconductor materials, such as indium arsenide (InAs) or indium phosphide (InP), have been conducted²–⁷), there are no available data concerning the pulmonary toxicity of ITO. In our previous studies⁶,⁷), we reported severe pulmonary damage caused by InAs or InP when given to hamsters in intermittent intratracheal instillations. From those studies, we considered that indium accounted for most of the lung lesions. Since the primary constitutive element of ITO is indium, there is a fair chance that the lung toxicity of ITO will appear when exposure to ITO is via the trachea.

In this study, we evaluated the pulmonary toxic effect of ITO when instilled repeatedly into the trachea of hamsters. The same dose of InP particles was also used to compare the toxicity of indium compounds.

Materials and Methods

ITO particles were obtained from a company and contained 74.4% (wt%) indium and 7.8% tin, the remainder being oxygen. InP particles, over 99.99% pure and purchased from Mitsuwa Chemicals (Osaka, Japan) were finely pulverized in an agate mortar. The mean count diameter for ITO and InP was 0.95 µm [σ g (geometric standard deviation): 2.42] and 1.06 µm [σ g: 1.80]. Both particles were analyzed in an energy dispersive X-ray fluorescence element analyzer (MESA-500, Horiba, Ltd., Kyoto, Japan). The ITO particles contained 0.16% zirconium and 0.02% silicon, and the InP particles contained 0.01% zirconium and a trace amount of yttrium. These compounds were suspended in sterile distilled water just prior to each instillation. Weanling male 4-week-old Syrian golden hamsters were purchased from Japan SLC Inc. (Shizuoka, Japan) and housed under conditions of temperature controlled between 22 and 25°C. All the animals were maintained on a cycle of 12-h lighting conditions in a specific pathogen-free laboratory room of the Laboratory of Animal Experiments, Faculty of Medicine, Kyushu University. Five or 6 hamsters were housed in one stainless steel cage with a commercial diet (CE-2 pellets, Clea Japan Inc., Tokyo, Japan) and tap water available ad libitum. The intratracheal instillations were started on 8-week-old hamsters. Twenty-one 8-week-old hamsters, with a mean ± SD weight of 107.4 ± 7.8 g, were randomized into 3 groups: the control group (n=6), the ITO group (n=10) and the InP group (n=5). There was no significant difference in body weight between the three groups at the start of the experiment. Each material was suspended in 1.0 ml/kg distilled water and instilled into the trachea of the anesthetized hamsters with ether once a week, for a total of 16 times. Each instillation per animal comprised 6.0 mg/kg as ITO (4.5 mg In/kg) or 6.0 mg/kg as InP (4.8 mg In/kg), this being twice the quantity of the dose used in our earlier study⁶). The control hamsters received 1.0 ml/kg of distilled water only. All the surviving hamsters were euthanized by carbon dioxide gas on the day subsequent to their final instillation, and then autopsied. Resected lungs were weighed and fixed in 10% neutral buffered formalin and processed in paraffin. Specimens were cut at a thickness of 6 µm and each section was stained with hematoxylin-eosin. These sections were examined by light microscopy. Histopathologic findings in the lung were scored as present or absent. If absent, it was expressed as negative. In the case of pulmonary lesions, the severity of each lung lesion was graded on a scale of slight to severe, indicating the approximate fraction of the lung or structure judged to be involved (slight = 1–10%, mild = 11–24%, moderate = 25–50%, and severe = 51–100%).

Fischer’s least significant difference procedure was
used after one-way analysis of variance. In all the statistical comparisons, a p value less than 0.05 indicated significant difference.

These experiments were conducted according to the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University.

**Results**

The mean total dosage received per animal was 12.4 ± 1.2 mg of ITO particles and 13.0 ± 1.5 mg of InP particles, containing 9.3 ± 0.9 mg and 10.4 ± 1.2 mg of indium, respectively.

Although one InP-treated hamster died of emaciation during the instillation period, no hamster died in the other two groups.

Fig. 1 shows the body weight change in each group during the instillation period. Intratracheal instillations of InP led to a marked suppression of body weight gain at the 13th instillation and the difference in trends of body weight change between the ITO group and the InP group was significant (P<0.01). There was no significant difference in the trends in body weight gain between the ITO group and the control group.

A significant increase in relative lung weight was observed in both the ITO and the InP groups compared with the control group; it was almost 2.6 or 4.7 times greater than the controls in the ITO- and InP-treated hamsters, respectively. Moreover, there was a significant difference between that in the ITO and the InP group.

The severity of pathogenic lung lesions in each group was evaluated according to five grades, as shown in Table 1. Foci of slight to severe inflammatory responses were present in both the ITO and the InP groups. These changes were increased in severity among the InP-treated hamsters. In the InP group, extensive inflammatory foci were scattered throughout the lungs and consisted of alveolar septae diffusely lined with hyperplastic alveolar epithelium or regenerative epithelium with mild cellular atypia, and infiltration of inflammatory cells. Inflammatory cells mostly consisted of neutrophils, lymphocytes and plasma cells, which were seen in the alveolar spaces, alveolar septum, bronchiolar lumen and peribronchiolar or perivascular tissue, and the alveolar walls had become markedly thickened. Although alveolar macrophages were scarcely seen in the alveolar spaces, numerous exudations had infiltrated the alveolar spaces including necrotic cell debris, which may have comprised necrotic alveolar macrophages. Abundant black InP particles were present within the alveolar septae, alveolar spaces or bronchiolar lumens, and mild cholesterol clefts were seen in the alveolar spaces. There was moderate interstitial fibrosis in the alveolar septae around these inflammatory lesions. Moreover, localized alveolar or bronchiolar cell hyperplasia with squamous cell metaplasia was seen involving the alveoli or bronchioli of the lung in one of the InP-treated hamsters (Fig. 2), but not in the ITO group.

In the ITO group, the inflammatory response was weaker than that in the InP group. Mild inflammatory foci were seen in the lung, and quite a number of alveolar macrophages with an expanded cytoplasm either containing or not containing brown ITO particles, necrotic cell debris, and a few neutrophils were present within the alveolar septae, alveolar spaces or bronchiolar lumens (Fig. 3). Alveolar walls and pleura were mildly thickened and alveolar cell hyperplasia occurred in the foci of

**Table 1.** Inflammatory lesions in the lungs of ITO- and InP-treated hamsters

<table>
<thead>
<tr>
<th>Group</th>
<th>Inflammatory cell infiltration</th>
<th>Exudation</th>
<th>Thickening of alveolar wall</th>
<th>Accumulation of alveolar macrophages</th>
<th>Diffuse alveolar cell hyperplasia</th>
<th>Cholesterol cleft</th>
<th>Fibrotic proliferation</th>
<th>Thickening of pleura</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITO</td>
<td>±</td>
<td>2+</td>
<td>+</td>
<td>2+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>InP</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>±</td>
<td>3+</td>
<td>+</td>
<td>2+</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>±</td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>–</td>
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</tr>
</tbody>
</table>

The severity of the lung lesions was evaluated according to five grades. –, negative; ±, slight; +, mild; 2+, moderate; 3+, severe.
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Discussion

In this study, we evaluated the pulmonary toxicity of these ITO and InP, comparing the toxicity of indium compounds, in hamsters because of a lack of toxicity data for this new material; in fact this is the first report to assess the toxicity of ITO. Previously, we reported the severe systemic and pulmonary toxicity of 4 mg/kg InAs and 3 mg/kg InP particles by repeated intratracheal instillations to hamsters twice a week for 8 weeks, and InAs revealed greater systemic effects than InP, nevertheless an equimolar amount of the two indium compounds was given. In this study, but because the instillation period was lengthened from 8 to 16 weeks, with administration once a week instead of twice a week, indium compounds were given at twice the dose used in the earlier studies.

The present results clearly demonstrated that ITO particles caused pulmonary damage, although the adverse health effects of the ITO particles appeared to be less than those of InP. The same dose of the two indium compounds was instilled intratracheally in this study. Weight reduction in the late administration period, pulmonary inflammation and lung weight gain were more severe in the InP group than in the ITO group. Furthermore, pulmonary localized hyperplastic lesions were observed in the InP group, but not in the ITO group. This finding in the InP group was consistent with the results of our previous study. Phosphorus in InP is biologically essential, and the toxicity of inorganic tin, including tin oxide, after inhalation and ingestion is low, causing benign pneumoconiosis only after many years of exposure to this dust among tin workers. Since ITO contains a high percentage of indium, but little tin, which in the body may be partly associated with pulmonary toxicity, the main cause of lung lesions is not tin but indium. The serum indium concentration in the InAs-treated hamsters was about double that in the InP-treated hamsters, even though an equimolar amount of InAs and InP particles was given in the previous study. Although, we have no data on serum indium concentrations in either group, the greater toxicity and localized hyperplastic lesions caused by InP may be due to differences in the clearance of these particles from the lung. Further clarification is needed regarding the pulmonary clearance of these particles and their distribution in the extra pulmonary tissues where the accumulation of indium would result in toxicity.

In conclusion, ITO and InP particles are toxic when repeated intratracheal instillations are given to hamsters, and InP has greater systemic or pulmonary toxic effects than does ITO. Acute or chronic effects on humans must be monitored after exposure to these particles. An additional study is now in progress to evaluate the pulmonary toxicity, clearance, distribution and systemic toxicity of ITO after intratracheal instillations.

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References


