Brief Report

Tissue distribution of indium after repeated intratracheal instillations of indium-tin oxide into the lungs of hamsters

Akiyo Tanaka¹, Miyuki Hirata¹, Nagisa Matsumura¹ and Yutaka Kiyohara¹

¹Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Japan

Abstract: Tissue distribution of indium after repeated intratracheal instillations of indium-tin oxide into the lungs of hamsters: Akiyo Tanaka, et al. Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University—

Objectives: The aim of this study was to analyze the tissue distribution of indium after intratracheally instilling indium-tin oxide (ITO) into the lungs of hamsters.

Methods: Male Syrian hamsters received an intratracheal dose of 3 mg/kg or 6 mg/kg of ITO particles containing 2.2 mg/kg or 4.5 mg/kg of indium, twice weekly for 8 weeks. In parallel, control hamsters received only an intratracheal dose of distilled water. A subset of hamsters was euthanized periodically throughout the study from 8 up to 78 weeks after the final instillation. The distribution of indium in the lungs, liver, kidneys and spleen, as well as pathological changes in the liver, kidneys, and spleen, was determined.

Results: The contents of indium in the lungs in the two ITO groups gradually decreased over the 78-week observation period, with elimination half-lives of approximately 142 weeks for the 3 mg/kg ITO group and 124 weeks for the 6 mg/kg ITO. The indium concentrations in the liver, kidneys, and spleen gradually increased throughout the observation period. Although foci of the lesions were observed histopathologically in the extrapulmonary organs among the two ITO groups, the control group showed similar lesions.

Conclusions: The results clearly demonstrate that the clearance of indium from the body is extremely slow after intratracheal instillation in hamsters.

(J Occup Health 2015; 57: 189–192)

Key words: Hamsters, Indium, Indium-tin oxide, Intratracheal instillation, Lung clearance, Tissue indium concentration

Received May 29, 2014; Accepted Nov 4, 2014
Published online in J-STAGE Jan 10, 2015
Correspondence to: A. Tanaka, Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, EC Bldg 2F, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan (e-mail: atanaka@envmed.med.kyushu-u.ac.jp)

Indium is an essential rare metal that is commonly used in the electronics industry; the use of indium compounds, most notably indium-tin oxide (ITO), has risen sharply since the 1990s¹. Several case reports and epidemiological studies of workers exposed to ITO have heightened awareness of the potential hazards of occupational exposure to this metal²−⁴. Although evidence of pulmonary lesions has been reported after respiratory ITO exposure in animals and humans²−⁷, it is not clear whether indium can further distribute throughout the body. In our previous study, pulmonary toxicity in hamsters was demonstrated over a four-month period of repeated intratracheal instillation of 3 mg/kg or 6 mg/kg doses of ITO (2.2 mg/kg or 4.5 mg/kg as indium, respectively)⁶. The serum indium concentrations among the two test groups gradually increased throughout the observation period, and the severity of pulmonary pathologies increased over time. The present study evaluated the long-term peripheral organ distribution of indium from 8 to 78 weeks after repeated intratracheal instillation of ITO into the lungs of hamsters as reported in our previous study⁶.

Materials and Methods

ITO particles were prepared as previously reported⁶ and were obtained by donation from a corporate source. All animal studies were conducted in accordance with the Guidelines for Animal Experiments in the Graduate School of Medical Sciences, Kyushu University, and in compliance with Law No.105 and Notification No. 6 of the Government of Japan. Eighty-seven 6-week-old male Syrian hamsters were purchased from the colony of Japan SLC Inc. (Shizuoka, Japan) to be used in this study. The animals were housed in a specific pathogen-free environment at the Laboratory of Animal Experiments in the Graduate School of Medical Sciences, Kyushu University. The lighting, food supply and drinking water were maintained under the same conditions as described previously⁶. Intratracheal instillation of ITO began after a 2-week acclimatization period when
the animals were 8 weeks old.

The hamsters were randomly divided into 3 groups: a control group (n=29), a 3 mg/kg ITO treatment group (2.2 mg/kg as indium; n=29) and a 6 mg/kg ITO treatment group (4.5 mg/kg as indium; n=29). There was no significant difference in body weight among the groups at the start of the study. A vehicle or test agent was instilled into the trachea of ether-anesthetized hamsters twice weekly over 8 weeks for a total of 16 doses. The control group received 1.0 ml/kg of distilled water. Six to eight surviving hamsters in each group were euthanized using carbon dioxide gas and autopsied at 8, 16, 40 or 78 weeks after their final dose. The indium concentrations were measured in the organs of the ITO-treated hamsters, as were the total organ weights, and histopathological examination of the liver, kidneys and spleen was also completed for all hamsters. To measure lung indium content, one apical lobe was soaked in 10 ml of 68% ultrapure nitric acid (TAMAPURE-AA-100, Tama Chemicals Co., Ltd., Kawasaki, Kanagawa, Japan) overnight, and 0.1 ml of lung soak solution, 0.02 g of liver tissue, 0.1 g of kidney, 0.1 g of spleen tissue or 1 ml of serum was digested with 6 ml of 68% ultrapure nitric acid (TAMAPURE-AA-100, Tama Chemicals Co., Ltd., Kawasaki, Kanagawa, Japan) and 0.5 ml of 35% ultrapure hydrogen peroxide (TAMAPURE-AA-100, Tama Chemicals Co., Ltd., Kawasaki, Kanagawa, Japan) using a microwave digestion apparatus (Multiwave 3000, PerkinElmer, Yokohama, Japan). Digested samples were then diluted into ultrapure water for a total volume of 20 ml and injected into an inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies, Tokyo, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University. Rhodium was used as an internal standard for indium measurements. The lower limit of quantitative detection for indium was 0.04 µg/g for the lungs, 0.005 µg/g for the liver, 0.001 µg/g for the kidneys and 0.001 µg/g for the spleen. In the cases where the indium concentrations were below the limit of detection, a value equal to one-half of the limit of detection was used for statistical calculations. The distribution of indium in the lungs was calculated as the concentration of indium in lung tissue.

Samples of the liver, kidneys and spleen were fixed in 10% neutral buffered formalin and processed in paraffin for histopathological examination, which included an evaluation of the severity of lesions as reported in a previous study. Briefly, histopathological findings in these organs were scored as present or absent; if they were absent, findings were expressed as 0. In the case of lesions, the severity of each of the lesions was graded on a 4-point scale ranging from slight to severe. Slight lesions were expressed as 1, mild lesions were expressed as 2, moderate lesions were expressed as 3, and severe lesions were expressed as 4.

For the statistical analyses of organ weights and indium content in the lung, indium concentrations in the liver, kidneys and spleen and score of the severity of the lesions, one-way analysis of variance followed by a Fischer’s least significant difference test was applied. In all the statistical comparisons, a p value of <0.05 was considered to represent a significant difference.

Results

Over the course of 8 weeks, hamsters received mean indium doses of 4.8 ± 0.4 mg (mean ± SD) and 9.5 ± 0.9 mg per animal in the 3 mg/kg and 6 mg/kg ITO treatment groups, respectively. The data collected in the course of the study for body weight, lung weight, mortality, serum indium concentration and lung lesions were presented in our previous study. The liver, kidney and spleen weights were not significantly different throughout the course of the study among the two ITO-treated groups and the control group. The indium content in the lungs decreased slowly from 8 to 78 weeks in both ITO-treated groups (Table 1). Although the concentrations of indium in the lung for the 6 mg/kg ITO group were slightly higher at 78 weeks than at 40 weeks, the clearance of indium from the lungs fit a biphasic exponential rate.

<table>
<thead>
<tr>
<th>Organ (mg/whole lung)</th>
<th>Group</th>
<th>Weeks after final instillation (weeks)</th>
<th>8</th>
<th>16</th>
<th>40</th>
<th>78</th>
</tr>
</thead>
<tbody>
<tr>
<td>In (mg/whole lung)</td>
<td>ITO 3 mg/kg</td>
<td>3.850 ± 1.425 (8)</td>
<td>3.183 ± 0.709 (8)</td>
<td>3.219 ± 0.440 (7)</td>
<td>2.587 ± 0.443 (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITO 6 mg/kg</td>
<td>6.633 ± 1.595 (8)</td>
<td>4.404 ± 1.750 (7)</td>
<td>3.690 ± 1.040 (7)</td>
<td>4.078 ± 0.636 (7)</td>
<td></td>
</tr>
<tr>
<td>% of In dose</td>
<td>ITO 3 mg/kg</td>
<td>82.6 ± 30.5</td>
<td>69.8 ± 18.1</td>
<td>66.5 ± 7.7</td>
<td>56.3 ± 8.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITO 6 mg/kg</td>
<td>64.5 ± 17.1</td>
<td>49.7 ± 18.9</td>
<td>39.4 ± 11.3</td>
<td>44.6 ± 7.3</td>
<td></td>
</tr>
</tbody>
</table>

The results are shown as means ± SD. a: The number of hamsters examined. b: Statistically different between the ITO 3 mg/kg group and the ITO 6 mg/kg group.
model for both ITO-treated groups. The elimination half-life of indium from the lungs was 142 weeks in the 3 mg/kg ITO group and 124 weeks in the 6 mg/kg ITO group. Indium was not detected in the lungs of the control group hamsters at any time during the observation period.

The indium concentrations in the serum, which were reported in our previous study, liver, kidneys and spleen gradually increased during the observation period, from the concentrations at least 7-fold higher at 78 weeks than at 8 weeks for both ITO treatment groups. However, the accumulation ratio of indium in the liver, kidneys and spleen for the total dose instilled was very low; that is, it was less than 2% at 78 weeks (Table 2). No indium was detected in the organs of control group hamsters at any point during the observation period. There was one renal adenocarcinoma in the ITO 3 mg/kg group at 16 weeks and one cavernous hemangioma of the liver in the ITO 6 mg/kg group at 78 weeks. The pathological evaluations revealed some lesions in the organs that increased in severity as the study progressed. There were no significant differences between the ITO-treated groups and the control group (data not shown).

Discussion

In this study, the long-term tissue distribution of indium was assessed after repeated intratracheal administration of ITO in hamsters. This is the first study of long-term indium distribution following respiratory exposure. Indium was found to be absorbed and retained in the lungs for a long time. The half-life of indium elimination from the lungs was more than two years and was similar for the two different levels of ITO dosing. Although the indium accumulation ratio in the liver, kidneys and spleen for the total instillation dose was very low and quantity of indium excretion in feces and urine was not clear, indium accumulation in these organs indicated that translocation from the lungs occurred, but there was no gradual elimination from these organs during the observation period. These results are consistent with our previous study in which it was reported that ITO-induced lung lesions and that serum indium levels increased significantly after exposure. It may be that the low solubility of ITO particles leads to long-term deposition in the lungs.

To date, a few studies have assessed lung indium levels among workers who handle indium or its derivatives occupationally. These studies have reported indium levels of up to 31.2 µg/g among recycling workers or 29.3 µg/g in an ITO-handling worker. Furthermore, although there is some data assessing indium levels in peripheral organs at a single point in time, the data do not clarify whether there was long-term absorption of indium in tissue. In the present study, it was demonstrated that indium was significantly absorbed in peripheral organs after respiratory exposure and that the absorption continued to increase long after ITO instillation. Interestingly, Yamazaki et al. reported a gradual decrease in serum indium concentrations after the cessation of dosing with indium arsenide (InAs) or indium phosphide (InP). It may be that serum and peripheral organ absorption depend upon the species of indium compound to which the animals are exposed. Further clarification will be needed to elucidate a trend between absorption or excretion and the properties of various indium compounds.

Table 2. Indium concentrations in the liver, kidneys and spleen after the final instillation of ITO

<table>
<thead>
<tr>
<th>Organ</th>
<th>Group</th>
<th>Weeks after final instillation (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Liver</td>
<td>ITO 3 mg/kg</td>
<td>0.538 ± 0.163 (8)</td>
</tr>
<tr>
<td>(µg In/g)</td>
<td>ITO 6 mg/kg</td>
<td>1.130 ± 0.573 (8)</td>
</tr>
<tr>
<td>Kidney</td>
<td>ITO 3 mg/kg</td>
<td>1.435 ± 0.177 (8)</td>
</tr>
<tr>
<td>(µg In/g)</td>
<td>ITO 6 mg/kg</td>
<td>2.210 ± 1.110 (8)</td>
</tr>
<tr>
<td>Spleen</td>
<td>ITO 3 mg/kg</td>
<td>0.612 ± 0.260 (8)</td>
</tr>
<tr>
<td>(µg In/g)</td>
<td>ITO 6 mg/kg</td>
<td>1.067 ± 0.477 (8)</td>
</tr>
<tr>
<td>Serum</td>
<td>ITO 3 mg/kg</td>
<td>0.060 ± 0.019 (8)</td>
</tr>
<tr>
<td>(µg In/ml)</td>
<td>ITO 6 mg/kg</td>
<td>0.080 ± 0.022 (8)</td>
</tr>
<tr>
<td>% of In dose in the liver, kidneys, and spleen</td>
<td>ITO 3 mg/kg</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>ITO 6 mg/kg</td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

The results are shown as means ± SD. a: The number of hamsters examined. b: Statistically different between the ITO 3 mg/kg group and the ITO 6 mg/kg group. c: Data from this study were shown as a part of our previous study.
The degree of lesions observed in the liver, kidneys or spleen was not significantly different between the two dose levels of ITO and was similar to that of lesions observed in the same organs of the control animals in this study. This finding is consistent with a previous report that no exposure-related organ lesions were observed outside the lungs in mice and rats. It is conceivable that the toxic effect on extrapulmonary tissues is relatively weak with the instillation doses delivered herein.

In conclusion, the present study presents the first real evidence that indium is eliminated from the lungs very slowly and does accumulate in extrapulmonary organs over a long period of time after respiratory exposure to ITO.

Acknowledgments: This study was funded in part by a Grant-in-Aid for Scientific Research on Innovative Areas (24108009) and Grants-in-Aid for Scientific Research (B) (23390164) and (A) (23249033) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the New Energy and Industrial Technology Development Organization (NEDO) Incorporated Administrative Agency under the Ministry of Economy, Trade and Industry (METI).

References