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CASE STUDY

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Indium kinetics in an indium exposed worker before and after bilateral lung transplantation

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Abstract

Background: A male worker with indium-tin oxide (ITO)-induced pneumoconiosis underwent bilateral lung transplantation (LT).

Methods: Post-LT histopathological investigations of the isolated lungs and hilar lymph nodes were performed and indium concentration in serum (In-S) and serum Krebs von den Lungen-6 (KL-6) were tracked for 122 weeks.

Results: He has attained the ultimate treatment goal of > 2-year survival. The main histopathological characteristics were pan-lobular emphysematous change, interstitial fibrosis, and lymphocytic infiltration in the peribronchiolar/perivascular portions, and numerous cholesterol clefts and giant cells containing brown particles. These findings support the conclusion that the lung injury was caused by the inhalation of ITO. Metal element mapping and indium in the isolated lungs revealed that inhaled ITO particles in humans migrate to the lymph nodes. In-S remained at remarkably high levels (≥30 ng/mL) and showed wide fluctuation with bimodality until 46 weeks after LT, but KL-6 remained in the normal range for almost the entire period. The indium concentration in the donor's resection lung at 10 weeks after LT was 143.5 ng/g wet-weight, which was only one one-thousandth of the recipient's lung (161 μg/g wet-weight). After 48 weeks of LT, the recipient's In-S had gradually decreased; the biological half-life was 1.2 years. These results clearly suggest that indium remaining in the recipient's tissues did not adversely influence the transplant donor's lungs.

Conclusions: The transplanted donor's lungs were not influenced by indium in the recipient's organs. Bilateral LT is thus an effective treatment option in severe indium lung disease cases.

KEYWORDS

Emphysema, Indium, Indium kinetics, ITO, Lung transplantation, Pneumoconiosis

Abbreviations: In-S, indium concentration in serum; ITO, indium-tin oxide; KL-6, Krebs von den Lungen-6; LT, lung transplantation; SP-A, surfactant protein A; SP-B, surfactant protein B; SP-D, surfactant protein D.

1 | INTRODUCTION

Indium is a rare metal that is used mainly in the transparent electrodes in flat-panel displays. Indium lung disease is a new occupational pneumoconiosis with unknown appropriate therapy. The first fatal case was disclosed in 2003.[1] The inhalation of hardly soluble dusts of indium compounds such as indium-tin oxide (ITO) and indium oxide causes interstitial pneumonia[2,3] and emphysema.[4,5] Indium phosphide and ITO were shown to be lung carcinogens in rats and/or mice,[6,7] but the evidence of their carcinogenicity in humans is inconclusive.[8] Herein we describe indium kinetics of a male indium lung disease with severe emphysema and pulmonary hypertension who the first bilateral lung transplantation (LT) in the world and followed for 122 weeks after LT.

2 | METHODS

This study was approved by the Ethics Committee of the School of Medicine, Keio University (approval no 20 110 268) and the Kyushu University Institutional Review Board for Clinical Research (approval no 27-321). Written informed consent for clinical data was also obtained from the subject of this report (approval no 30-69) at the Kyushu University Institutional Review Board for Clinical Research.

2.1 | Quantitative analysis of indium and identification of metal elements

To measure In-S, 0.5 ml of serum was digested with 4 ml of 68% ultra-pure nitric acid (TAMAPURE-AA-100, Tama Chemicals Co., Japan) and 2 ml of 35% ultra-pure hydrogen peroxide (TAMAPURE-AA-100) by a microwave digestion apparatus (Multiwave Pro, Anton Paar, Japan) and diluted to 20 ml with ultra-pure water. To measure indium in the isolated recipient's organs, small samples were removed from segments S_2, S_5, and S_{10} of the right lung, segments S_{1+2}, S_4, S_5, S_9, and S_{10} of the left lung, and the bilateral hilar lymph nodes, and kept at -80° C until the analysis. Organ samples of 0.1 g were digested in the same way that serum digestion was conducted and diluted 10 000 times with 13% nitric acid. The pretreated samples were introduced into an inductively coupled plasma mass spectrometer (Agilent 7500c, Agilent Technologies Japan, Ltd., Japan) at the Center of Advanced Instrumental Analysis, Kyushu University.

Identification of the distribution of metal elements in the right segment S_2 and the hilar lymph node were mapped by a scanning electron microscope with an energy dispersive X-ray analyzer (SEM-EDX, SU3500, Hitachi High-Technologies GLOBAL, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University.

2.2 | Clinical history

A 53-year-old male smoker (10 cigarettes/day for 2 years) was a participant of our indium cohort study and was exposed to fine ITO grinding dusts for 9 years. In April 2015, BODE index was 7. Obstructive disorders, severe diffusive dysfunction, and secondary pulmonary hypertension also occurred. In July 2015, we performed our second follow-up study on baseline participants at the factory. In this case subject, we recorded an In-S of 37.6 ng/mL, KL-6 of 1,640 U/ml. Pulmonary function tests showed FVC of 3.52 l (83.9% of the JRS predicted value), FEV1 of 1.73 l (48.7% of the JRS predicted value), FEV1-to-FVC ratio of 49.2%, a single-breath carbon monoxide diffusing capacity (DLco) of 4.2 ml/min/mmHg (15.2% of the value predicted using Nishida's equation for Japanese adult males), and resting room air SpO2 of 87%, suggesting severe emphysema.[9] Details of his clinical history before the LT were published in our previous article.[9]

In January 2016, he was finally listed on the Japan Organ Transplant Network waiting list for candidate of cadaveric LT because of his severe emphysema and pulmonary hypertension caused by ITO exposure. After a year and 10 months of waiting, bilateral sequential LT was performed under cardiopulmonary bypass. Transplant procedure was done very smoothly, and post-operative course was generally uneventful. A sub-pleural mass (3cm) with cavity formation was found in the lower lobe of the right lung allograft by survey CT scan at a month after surgery. The mass was removed by thoracoscopic partial lung resection procedure 10 weeks after LT and diagnosed as “partial lung necrosis”. There was no sign of allograft rejection or specific bacterial or fungal infection. The patient was then discharged from the hospital and back to his normal family life, and finally returned to his former work until 6 months after the LT. He is working as a white-collar worker as of April 2020.

3 | RESULTS

3.1 | Pathological findings of isolated lungs

Grossly, surgical specimens of the recipient's bilateral lungs showed emphysematous and interstitial changes in all lobes. Emphysematous change was most prominent in the bilateral upper lobes. Microscopically, pan-lobular cystic change and interstitial fibrosis around the bronchioles and vascular tissues were observed (Figure 1A,B). Numerous multinucleated giant cells with cholesterol clefts and lymphocytic infiltration were found in areas of fibrosis and in alveolar air spaces (Figure 1C). Cholesterol granulomas and fibroblastic foci were also observed in part (Figure 1D). Entrapped air spaces in the fibrosis were focally filled with eosinophilic

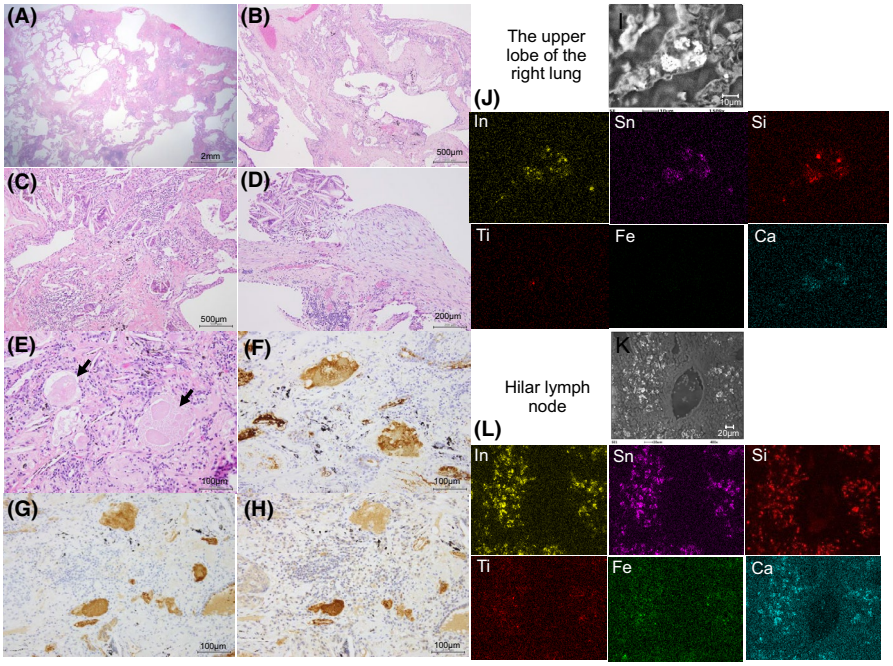

FIGURE 1 (A-H) Microscopic findings of the right upper lobe. (A) Low magnification of the right upper lobe shows pan-lobular emphysematous cystic spaces and interstitial fibrosis around the bronchioles and vascular tissues. (B) High magnification of the right upper lobe shows emphysematous change and interstitial fibrosis with multinucleated giant cells. (C) Multinucleated giant cells have cholesterol clefts and brown particles. (D) Cholesterol granuloma and fibroblastic focus. (E) Eosinophilic granular materials in entrapped air spaces (arrow). (F, G, H) Eosinophilic granular materials are positive for SP-A (F), SP-B (G), and SP-D (H) immunostaining, which is indicative of surfactant proteins. (I-L) Elemental analysis using SEM-EDX of particles deposited in the lung and hilar lymph node. (I,K) Backscattered electron image of particles shows a brighter contrast than the tissue in the right upper lobe (I) and hilar lymph node (K). (J,L) Elemental mapping image of In, Sn, Si, Ti, Fe, Ca in the right upper lobe (J) and hilar lymph node (L).

materials (Figure 1E). Immunohistochemistry using a monoclonal antibody against surfactant protein A (SP-A) (PE-10, DAKO Japan, Kyoto, Japan) and surfactant protein D (SP-D) (IIIH3, Hycult Biotechnology b.v., Uden, The Netherlands), and polyclonal antibody against surfactant protein B (SP-B) (Chemicon International, Inc, Temecula, USA) showed the presence of surfactant proteins in eosinophilic materials (Figure 1F-H).

3.2 | Indium concentration and distribution of metal elements in isolated organs

Figure 1I-L shows a metal element mapping image obtained by SEM-EDX in the recipient's right upper lobe and hilar lymph node. Several metal elements were revealed, and indium and tin were identified at the same spots in both organs. Indium was not detected in 30 deposited particles in the partially isolated donor's lung by SEM-EDX.

In-S just before LT was 60.4 ng/mL. Indium concentrations in the small fractions of right segments S_2, S_5, and S_{10} and the left segments S_{1+2}, $S_4 + S_5$, and $S_9 + S_{10}$ of the resected recipient's lungs were 54, 168, 211, 155, 207, and 173 μg/g wet-weight, respectively, and the average was 161 μg/g (In-Lung). The indium concentrations in each one of the right and left hilar lymph node were 643 and 805 μg/g wet-weight, respectively, and the average was 724 μg/g (In-Lymph). The ratios of In-Lung/In-S and In-Lymph/In-S were 2665 and 11 987, respectively. The indium concentration in the transplanted donor's resection lung was 143.5 ng/g wet weight, which was only one one-thousandth of the recipient's lung.

Figure 2 illustrates the chronological changes of In-S and KL-6 before LT and after LT. In May 2008, he was transferred from the grinding job site to an inspection work section, where indium concentration was very low.[9] After indium exposure stopped in December 2011, his In-S gradually decreased until LT. KL-6 also decreased, but it kept more than double of its reference value until LT. After LT, In-S remained at remarkably high levels (≥30 ng/mL) and showed wide fluctuation with 2 peaks until 48 weeks after the LT. In-S just after LT was 32.7 ng/mL, and it rapidly increased up to 71.3 (first peak) at 6 weeks after LT. It decreased to 40.9 at just before the partial lung resection (10 weeks after LT), rapidly increased up to 104.4 (second peak) at 22 weeks after LT, and rapidly decreased to 26.1 until 48 weeks. KL-6 has remained under the reference value except for one value (505 U/mL) since the LT.

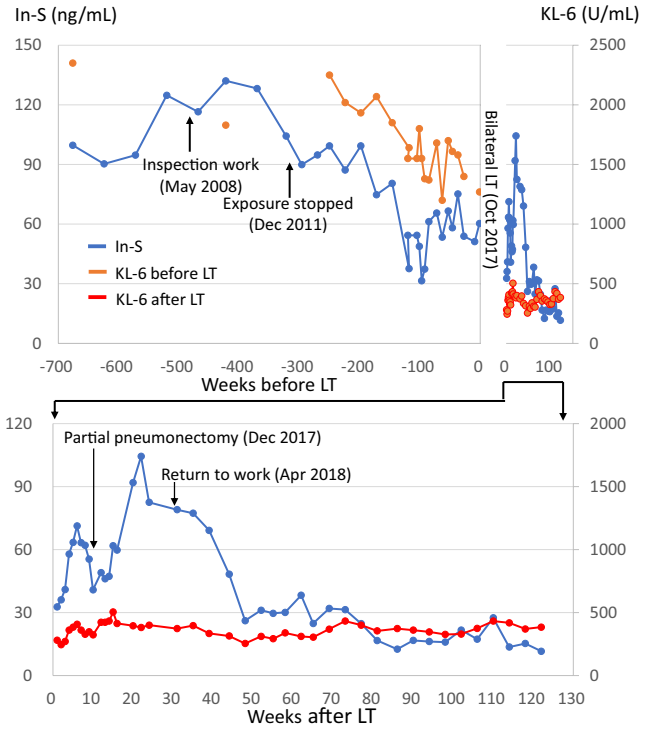

FIGURE 2 Chronological changes of In-S and KL-6 before and after LT

4 | DISCUSSION

This is the first case in which LT was used to treat indium lung disease in the world. This LT case has a great prognosis and has attained the ultimate treatment goal of a more than two-year-long survival time.

The histopathological characteristics of this case were: (a) pan-lobular emphysematous change; (b) interstitial fibrosis and lymphocytic infiltration which showed a patchy distribution of peribronchiolar and perivascular portions; (c) numerous cholesterol clefts and giant cells containing brown particles; and (d) focal eosinophilic exudates with immunoreactive SP-A, SP-B, and SP-D in entrapped air spaces in the fibrotic areas. Ten cases of indium lung disease histologically revealed intraalveolar eosinophilic exudates characteristic of pulmonary alveolar proteinosis (PAP), granulomas with cholesterol clefts, fibrosis with cholesterol clefts, and emphysema.[10] The present case also showed similar histological characteristics, except for the presence of PAP. Eosinophilic exudates containing surfactant proteins were focal and small in amount. Although the distribution of emphysematous change and fibrosis were not clear because of the disease progression, patchy peribronchiolar fibrosis with inflammatory infiltrates was identified. This finding suggests interstitial change caused by inhaled ITO particles. Interstitial and intraalveolar cholesterol granulomas are usually caused by the consequences of fibrosis associated with lipid-laden macrophages or endogenous lipids in lipoid pneumonia.[11,12] In the present case, the progressive destruction of lung tissue with chronic inflammation is evident. In contrast, the PAP-like change was focal and mild. From the histological findings, we speculated that the cholesterol-ester which caused the development of cholesterol granulomas was derived from the destruction of tissues or cells of the lung. These results support the proposed diagnosis of lung injury caused by the inhalation of ITO.[12] The PAP-like change was suggestive of a secondary phenomenon based on the lung injury.

Chest CT images of the recipient's lungs before the LT showed notable and severe emphysematous changes with septal destruction and several large bullae in the prominent upper lungs, and little of the interstitial area remained throughout the lungs.[9] The histopathological findings of the recipient's lungs showed combined interstitial fibrosis and pan-lobular emphysematous change. One hypothesis is that exposure to indium and smoking had increased the macrophages in the respiratory bronchioles and alveoli, causing desquamative interstitial pneumonia-like[13] interstitial changes, progressing to emphysema, and this condition was associated with pulmonary arterial hypertension.[14]

An analysis of metal element mapping showed indium and tin distributed at the same spots in both the isolated recipient's lungs and hilar lymph nodes (Figure 1I-L). ITO particles were in both the lungs and hilar lymph nodes. Furthermore, In-Lymph was ca. five-times higher than In-Lung. This is the first evidence in humans that ITO particles inhaled into the lungs can migrate to the lymph nodes.

Because the recipient's lungs, which are a major indium storage organ, would be removed, we expected before the LT that In-S would rapidly decrease after the LT. Contrary to our expectation, however, In-S persisted at high levels ($\geq$30 ng/mL) and showed bimodal peaks until 48 weeks after the LT (Figure 2). Exposure to indium from the general environment via air, foods and beverages is negligible, and In-S is $\leq$ 0.1 ng/mL in almost all indium-unexposed workers. In animal experiments, Tanaka et al[15] disclosed accumulation of indium in the spleen, kidneys, and liver after intratracheal instillation of ITO in male hamsters. Nagano et al[7] reported an accumulation of indium in the spleen, kidney, liver, bone marrow, ovary, pancreas, testis, and epididymis after 26-week inhalation exposure to ITO in male and female rats. The origin of In-S after LT might be the accumulated mobilizable indium in the recipient's soft tissues and hardly soluble ITO particles in the residual recipient's lymph nodes around the lung hilum.

This is the first report to describe the chronological changes of In-S after LT, and no data concerning indium kinetics after organ transplantation in either human or animal organs are available. The temporal changes of In-S until 48 weeks after the LT indicated one possibility. It is that the mobilization process of indium from soft tissues to the blood

is modified by internal loads generated by surgical and/or medication interventions directly or via the alteration of unknown systemic conditions.

From 48 to 122 weeks after LT, In-S gradually decreased (Figure 2). This suggests that the indium kinetics in the soft tissues and residual lymph nodes were basically stable. When a natural log approximation model was applied, a biological half-life of In-S during the stable phase was 1.2 years. Using the same model, a biological half-life during his end of indium exposure until LT was 6.1 years. Amata *et al*[5] reported median biological half-life of In-S in the In-S > 10 ng/mL workers' group who stopped indium exposure was 8.95 years (Interquartile range: 7.49-12.21). Compared to the latter two biological half-lives, the biological half-life of 1.2 years was very rapid. This may indicate that the kinetics of indium are quite different whether the lungs accumulate indium or not.

Before the LT, KL-6 remained at extremely high levels, suggesting active and persistent interstitial changes in the recipient's lungs, although KL-6 gradually decreased after the cessation of indium exposure. From just after LT until 122 weeks, almost all KL-6 values were within the normal range in spite of the large fluctuation of In-S and the long-term continuation of high In-S levels (Figure 2). This is an extremely unnatural phenomenon from the viewpoint of the existing evidence on the dose-effect relationships between In-S and KL-6 that has been observed.[1-5] Next, the In-Lung/In-S ratio of the recipient at the time of the LT was 2665. The In-Lung/In-S ratio of the transplanted donor's lungs without inhalation exposure to indium at the partial lung resection was only one one-thousandth of the ratio of the recipient's lungs. Furthermore, we failed to detect indium in particles of the donor's lungs. These facts clearly show that indium did not adversely influence or re-accumulate in the transplanted donor's lungs.

5 | CONCLUSIONS

Bilateral LT was performed in a case of severe indium lung disease with emphysema and pulmonary hypertension in 2017. During the 122-week follow-up period, the transplanted donor's lungs were not influenced by the indium remaining in the recipient's organs, although In-S persisted at high levels and fluctuated greatly. We believe that bilateral LT is an effective treatment option in cases of severe indium lung disease with no possibility of disease recurrence.

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DISCLOSURE

Ethical approval and informed consent: This study was approved by the Ethics Committee of the School of Medicine, Keio University (approval no 20 110 268) and the Kyushu University Institutional Review Board for Clinical Research (approval no 27-321). Written informed consent for clinical data was also obtained from the subject of this report (approval no 30-69) at the Kyushu University Institutional Review Board for Clinical Research.

Registry and the Registration no. of the study/trial: N/A.

Animal Studies: N/A.

Conflict of Interest: The authors declare no conflicts of interest associated with this manuscript.

AUTHOR CONTRIBUTIONS

MN takes responsibility for the integrity and accuracy of the data and the drafting of the article. MN, HM, HM, TA, and OK wrote the primary draft of the article. HM contributed to the fieldwork management between the staff at the factory, the patient, and doctors. MN, HM, HM, and T.A are responsible for the design of the study. MN, HM, HM, TA, TS, and OK contributed to the analysis and interpretation of the data. All authors assisted in the study design, contributed to data collection, and assisted in the interpretation of data and to critical revision of the manuscript. All authors approved the final version of the manuscript.

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