Short Communication

Pulmonary Squamous Cyst Induced by Exposure to Indium Arsenide in Hamsters

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Indium arsenide (InAs) belongs to the group of semiconductor materials comprising III-V compounds1, 2). With the increasing industrial use of InAs, the question as to whether or not occupational exposure to this material is a potential health hazard has been attracting attention. To date, there is no information regarding adverse health effects on workers arising from their exposure to InAs particles.

In our previous study, we confirmed the development of proteinosis-like lesions or localized hyperplastic lesions in the lungs of hamsters which had been induced by exposure to InAs particles3–5). In particular, alveolar or bronchiolar cell hyperplasia with squamous cell metaplasia, squamous cell hyperplasia or squamous cell metaplasia with keratinization were observed when 7.7 mg/kg of InAs was instilled intratracheally, twice a week for a total of 14 times4). Accordingly, a further study was undertaken with only half the dose (4 mg/kg). As a result, neoplastic change was never evident5). We therefore considered that it was necessary to use a dose in excess of 4 mg/kg of InAs per instillation in order to give rise to a neoplastic response.

The aim of the present study was to confirm whether the localized hyperplastic lesions induced by the overloaded condition of InAs particles actually represented a neoplastic response, or not. In this study, 8 mg/kg of InAs was instilled and double the number of hamsters (n=16), compared with that (n=8) in the previous study4), received the particles.

Materials and Methods

InAs, which had a purity of more than 99.9999%, was obtained from Mitsuwa Chemicals (Osaka, Japan). The phosphate buffer solution (0.025 M, pH 6.9) used was purchased from Katayama Chemicals (Osaka, Japan). The mean diameter of InAs particles was 1.58 µm, og: 2.15, and the InAs sample contained 0.01% of zirconium and 0.01% of yttrium, which could have been due to adulation from the planet ball mill used in pulverization.

All the 23 male Syrian golden hamsters, from the colony of Japan SLC, Inc. (Shizuoka, Japan) were purchased at 4 weeks of age. The experimental conditions were described in our previous study4).

The hamsters were randomly distributed into 2 groups: the InAs group (16 hamsters) and the control group (7 hamsters). The average body weight at the beginning of the instillations was 111.9 ± 8.1 g (mean ± SD) in the InAs group and 112.7 ± 7.4 g in the control group. The intratracheal instillations were commenced on 8-week-old hamsters which had been anesthetized with diethyl ether gas in a desiccator. Each tested material was suspended in 1.0 ml/kg phosphate buffer solution and instilled into the trachea of the anesthetized hamsters twice a week, for a total of 14 times. The InAs particles used comprised 8 mg/kg of body weight per instillation, per animal. The control hamsters each received 1.0 ml/kg of phosphate buffer solution alone.

Body weight was measured at the time of each instillation. Two hamsters died after the 12th instillation of InAs. All the surviving hamsters were euthanized with carbon dioxide gas within 2 wk after their final instillation because of severe emaciation. All the hamsters, including the two InAs-treated hamsters which died during the instillation period were autopsied. For histological examination, sections were prepared as described in our previous study4). The InAs particles used comprised 8 mg/kg of body weight per instillation, per animal. The control hamsters each received 1.0 ml/kg of phosphate buffer solution alone.

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Data on body weight were analyzed by repeated measure-ANOVA. In all the statistical comparisons, a p value of less than 0.05 was used to indicate significant differences.

This experiment was conducted according to the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University and under the Law (No.105) and Notification (No. 6) of the Government of Japan.

Results

After the eighth instillation, a remarkably suppressed body-weight gain was observed in the InAs group compared with the control group. The average body
weight at the last instillation was 103.3 ± 18.0 g in the InAs group and 150.0 ± 7.1 g in the control group, and the difference in trends of body weight changes between the InAs group and the control group was statistically significant (p<0.01).

Lung lesions in the InAs group are shown in Table 1. Three squamous cysts developed in the 16 InAs-treated hamsters, where keratinization had occurred with distorting alveolar architecture, with necrotic cell debris in the central area of the keratin mass, invasion to the normal pulmonary structures and moderate nuclear atypia (Fig.1), but no mitotic figures were seen. Localized hyperplastic lesions in the lung were evident in all the InAs-treated hamsters, but not in any of the control hamsters. The lesions were classified into 5 types, alveolar or bronchiolar cell hyperplasia, squamous cell hyperplasia, squamous cell hyperplasia with keratinization, mixed morphology of alveolar cell or bronchiolar cell hyperplasia together with squamous cell hyperplasia, and mixed morphology of alveolar cell or bronchiolar cell hyperplasia together with squamous cell hyperplasia plus keratinization. The most frequent localized hyperplastic lesion was alveolar or bronchiolar cell hyperplasia or mixed morphology of alveolar cell or bronchiolar cell hyperplasia together with squamous cell hyperplasia. Almost all the localized lesions were seen focally involving the alveoli or bronchi of the lung, and showing partial compression at the margin. Moreover, the progression of epithelium hyperplasia became papillary or multilayered with distortion of the pulmonary architecture. Some of the lesions contained eosinophilic, mucinous and amorphous exudates in part. Moderate nuclear polymorphism and cellular atypia were observed, but few mitotic figures were seen. More than one localized lesion developed within each lobe.

Proliferating activities were estimated with the PCNA labeling index (L.I.). The mean L.I. was 77.7 ± 9.9 (mean ± SD) in the localized hyperplastic lesions of the InAs group, whereas it was 1.6 ± 1.2 in the nuclei of the bronchiolo-alveolar cells of the control group.

In addition to the localized hyperplastic lesions, extensive inflammatory foci and severe interstitial fibrosis were scattered throughout the lung, and numerous neutrophils, necrotic cell debris and alveolar macrophages, sometimes containing InAs particles were present within the alveolar septa, alveolar spaces or bronchiolar lumens in all the InAs-treated hamsters. Neither inflammatory response nor interstitial fibrosis was evident in the control group.

<table>
<thead>
<tr>
<th>Number of animals examined microscopically</th>
<th>Control</th>
<th>InAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cyst</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Localized hyperplasia*</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Alveolar or bronchiolar cell hyperplasia</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Squamous cell hyperplasia</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Squamous cell hyperplasia with keratinization</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Mixed type of alveolar or bronchiolar cell hyperplasia</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Mixed type of alveolar or bronchiolar cell hyperplasia together with squamous cell hyperplasia</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Interstitium fibrosis</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

a: Different types of localized lesions were observed in each hamster.

Fig.1. Pulmonary squamous cyst of a hamster in the InAs group. Well-differentiated stratified squamous epithelium with a central keratin mass is evident and extension into the adjacent alveoli is apparent. ×95.
Discussion

In this study, squamous cysts and pulmonary localized hyperplastic lesions with keratinization became evident and PCNA protein expression was prominent in the pulmonary localized lesions in the InAs-treated hamsters. Furthermore, pulmonary hyperplastic lesions were found accompanying severe inflammation and interstitial fibrosis in all the InAs-treated hamsters. Except for the appearance of squamous cysts, this result supports the findings of our previous studies. While it remains obscure why squamous cysts developed in this study, but not in a previous study, it seemed to be attributable to the increased number of hamsters used in this study. Since pulmonary localized hyperplastic lesion was recognized in both the InAs- and InP-treated animals, but not in GaAs-treated animals in our previous studies, the main cause of squamous cyst induced by InAs exposure is not arsenic but indium, nevertheless InAs contains arsenic, a potent carcinogen. There is disagreement concerning squamous cysts among researchers, who variously consider squamous cyst to be either a benign tumor or exaggerated squamous metaplasia. Recently Gottschling et al. reported that InP particles caused lung tumors when Fischer 344 rats were exposed to the particles for 2 yr. Furthermore, they mentioned that non-neoplastic lesions, including alveolar squamous metaplasia, atypical hyperplasia, and squamous cysts were also induced, accompanying extensive, severe inflammatory lesions, in addition to lung tumor development, and that oxidative stress plays a major role in the development of lung tumors induced by InP exposure. It is likely that the presence of severe pulmonary inflammation caused by InP exposure correlates with the release of highly reactive oxygen and nitrogen species from inflammatory cells, and that oxidative damage progressing to development of lung tumor. Although the period of InAs treatment and the observation period of this study were shorter than those of the InP inhalation study reported by Gottschling et al., we observed both pulmonary squamous cysts and localized hyperplastic lesions with keratinization. Nevertheless, based on the evidence of carcinogenicity of InP, regardless of whether a squamous cyst actually represents tumorigenic change or not, it would seem that indium compounds probably induce neoplastic changes but further clarification is needed regarding the effects of indium dissolved from InAs particles.

In conclusion, it would seem that InAs particles give rise to squamous cysts when instilled intratracheally into hamsters, and that InAs has strong toxic effects on the lungs.

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References