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Comparative chronic toxicity, including tumorigenicity, of gallium arsenide and arsenic trioxide intratracheally instilled into hamsters

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Chronic toxicity, including tumorigenicity, of gallium arsenide (GaAs) and arsenic trioxide (As_2O_3) were studied using Syrian golden hamsters given intermittent intratracheal instillations. GaAs particles (0.25 mg × 15 times/animal) were likely to produce relatively severe lung damage and the survival of the animals was shortened significantly compared with a control group. The tumor incidence of each group examined was GaAs (3.3%), As_2O_3 (3.3%) respectively, at a dose of 3.75 mg total metal given during 15 weeks. In this experiment, both arsenic trioxide and gallium arsenide had no apparent carcinogenicity or tumorigenicity.

Keywords: Toxicity, tumorigenicity, gallium arsenide, arsenice trioxide.

INTRODUCTION

Epidemiological studies show that both environmental and occupational exposure to inorganic arsenic compounds are associated with increased skin and/or lung cancer in the exposed population.[1,2] Experimental studies showed that the carcinogenic potency of some arsenic compounds, such as arsenic trioxide (As_2O_3), calcium arsenate, arsenic trisulfide, differed.[3,4] However, there are few data on the evaluation of the carcinogenic potency of various arsenic compounds. The relation between human cancer and specific arsenic compound(s) is unknown. Gallium arsenide (GaAs) has been developed and used as a superior semiconducting material as it provides increased electron velocity.[5] The acute pulmonary toxicity of GaAs administered to rats was reported by Webb and co-workers,[6,7] but the chronic toxicity in long-term animal experiments was given little attention. In our present study, chronic toxicity, including tumorigenicity, of GaAs was studied using Syrian golden hamsters given intermittent intratracheal instillations, and the findings were compared with those of arsenic trioxide.

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EXPERIMENTAL

GaAs was obtained from Sumitomo Metal Mining Industry Co. Ltd, Japan. As_2O_3 (analytical grade) and phosphate buffer solution were obtained from Wako-Junyaku Co., Osaka, Japan. Benzo(*a*)pyrene was from Sigma (St Louis, MO, USA). Six-week-old male Syrian golden hamsters were separated into four subgroups of 30–33 animals each, and given a synthetic diet (Oriental NMF, Oriental Co., Japan) and drinking water *ad libitum*. Intratracheal instillation was carried out according to Ishinishi *et al.*[8] To each hamster anesthetized with diethyl ether, 0.2 cm^3 of GaAs and As_2O_3 suspended in phosphate buffer (pH 6.86) containing 0.25 mg of each metal was administered once a week during 15 weeks. Hamsters in the control group were given 0.2 cm^3 of phosphate buffer solution and those in the positive control group, 0.2 cm^3 suspension of 0.25 mg benzo(*a*)pyrene, in the same manner as for the treated groups.

All these hamsters were observed for two years. Those which died or were killed after a two-year observation period were autopsied, and detailed histopathological examinations were performed of the larynx, trachea, lungs, liver, spleen, gastric tract, kidney and bladder as well as of other tissues showing macroscopic abnormalities.

The survival curve of each group examined was assessed by the Kaplan–Meier method[9] and the Logrank test[10] was used for statistical analysis.

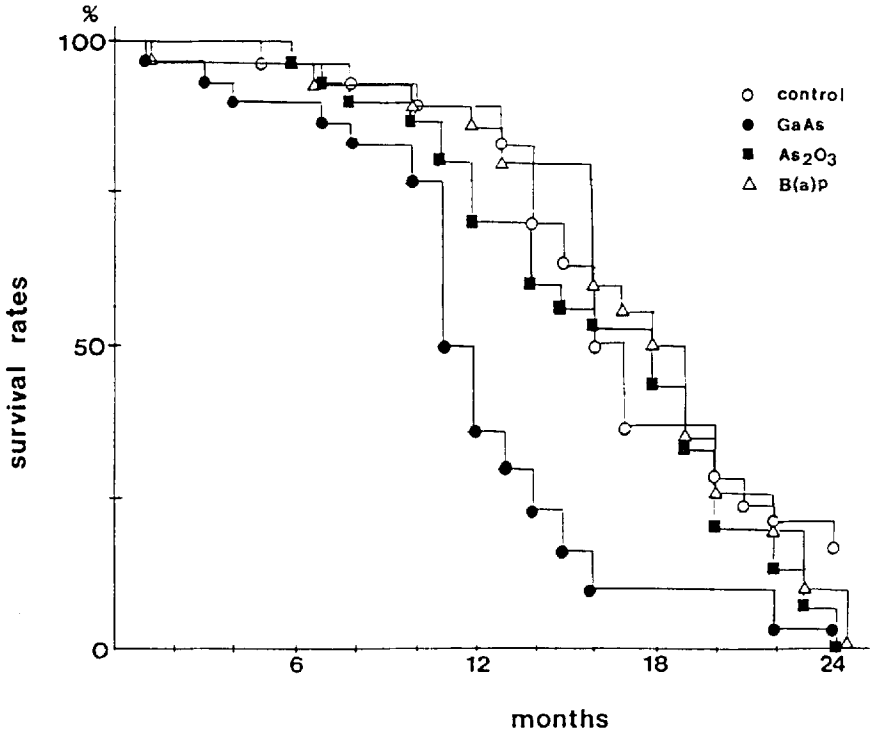

Figure 1 Survival rate of different groups of hamsters given 15 weekly intratracheal instillations of As_2O_3, GaAs and the vehicle solution.

RESULTS AND DISCUSSION

The intratracheal instillation of material examined did not significantly suppress cumulative weight gain in the hamsters. The survival rates in the different treatment groups are shown in Fig.1. There was a similar mortality during the first six months, including the 15-week treatment period in the four groups. However, thereafter a higher mortality rate was observed in the GaAs group during the first year. The mean survival time of control, GaAs, As_2O_3 and benzo(*a*)pyrene groups was 517, 399, 536 and 576 days, respectively. The difference in the survival rates between GaAs and the control group was statistically significant (Logrank test, $P<0.005$).

Table 1 shows the tumor incidence in the different groups. One liver tumor (malignant lymphoma, lymphoblastic type, see Fig. 2) was detected in both the GaAs and the As_2O_3 group, but there was no tumor in the control group. Both of the arsenic compounds showed no statistically significant incidence of tumors. In the benzo(*a*)pyrene group as positive control, two lung tumors, one liver tumor and one subcutaneous tumor were detected (the total tumor incidence was

Table 1 Tumor incidence in hamsters given 15 weekly intratracheal instillations of As_2O_3, GaAs or benzo(*a*)pyrene [B(*a*)p], and in a control group.

Compound	No. of survivors after 15 weeks treatment	Observation period (days)	No. of hamsters examined	Tumor			Tumor incidence (%)
				Lung	Liver	Subcutaneous	
Control	33/33	153–730	30	0	0	0	0
GaAs	32/33	111–730	30	0	1	0	3.3
As_2O_3	30/30	199–730	30	0	1	0	3.3
B(*a*)p	30/30	325–730	29(1)[a]	2	1	1	13.3[b]

[a] Number of animals eaten by other animals. [b] Statistically significant; the Fisher's exact probability was 0.05.

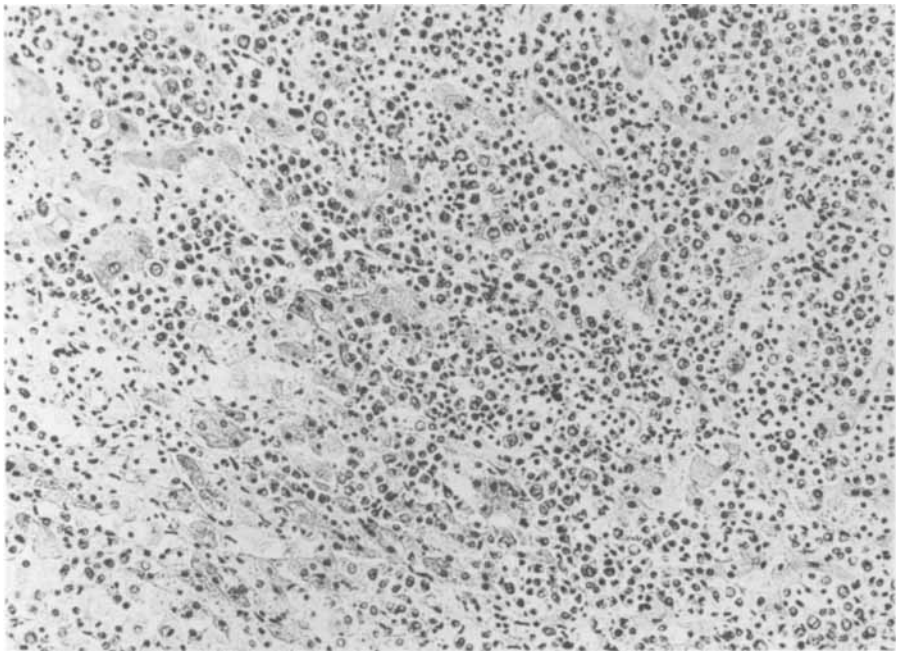

Figure 2 Liver tumor in a hamster which died on the 209th day after intratracheal instillation of GaAs. H & E stain. × 198.

Table 2 Histopathology of the lung lesions

Lung lesion	Control	GaAs	As_2O_3	B(*a*)p
Multifocal macrophage	2	1	6	3
Alveolar cell hyperplasia	5	14	18	7
Pneumonia	8	11	13	12
Prulent pneumonia	2	0	0	0
Bronchitis	1	0	1	0
Congestion with hemorrhage	4	10	4	4

statistically significant by Fisher's exact probability, $P<0.05$).

Table 2 shows the histopathological incidence in the lungs of each group examined. In all the groups, there was a histopathological appearance of a high incidence for alveolar cell hyperplasia (Fig. 3), pneumonia, macrophage accumulation and congestion with hemorrhage (Fig. 4) in the lungs. The main pathological findings in examined hamsters dying before the mean survival time of each group are shown in Table 3. Findings of alveolar cell hyperplasia were high in the GaAs group (30%) and in the As_2O_3 group (23.3%) but low in both the control and benzo(*a*)pyrene groups (6.6%). The incidence of pneumonia was relatively high (20%) in both the GaAs and benzo(*a*)pyrene groups. Congestion with hemorrhage was at a high incidence in the GaAs group (20%).

The acute pulmonary toxicity of GaAs to rats was noted in cases of intratracheal instillation.[6,7] It was found that GaAs particles (100 mg kg^{-1}) produced a marked thickening of the alveolar wall due to alveolar cell hyperplasia and interstitial pneumonia at 14 days after a single administration.

We investigated the chronic pulmonary toxicity and tumorigenicity of GaAs in the case of a smaller dose, 0.25 mg per hamster (1.5 mg kg^{-1}), and compared the findings with those of arsenic trioxide at the same dose. The most important finding was that the survival rate of hamsters given GaAs was lower not only than that in the control group but also that in the As_2O_3 group. Histological data, as shown in Tables 2 and 3, suggest that a more severe lung damage including alveolar cell hyperplasia and congestion with hemorrhage in addition to pneumonia relates to a shortened survival time, since pneumonia alone occurred in the benzo(*a*)pyrene group with a survival rate similar to that of the control group. The severe pulmonary toxicity of GaAs may by due to a longer retention in the lung,[7] compared with a very short clearance time of intratracheally instilled As_2O_3.[11] In addition, Webb *et al.*[6] found that clearance and distribution of GaAs in the lung was accompanied by a significant increase in arsenic blood concentration while gallium was not detected in the blood. The long retention of GaAs and dissolved gallium in lung have a potential of leading

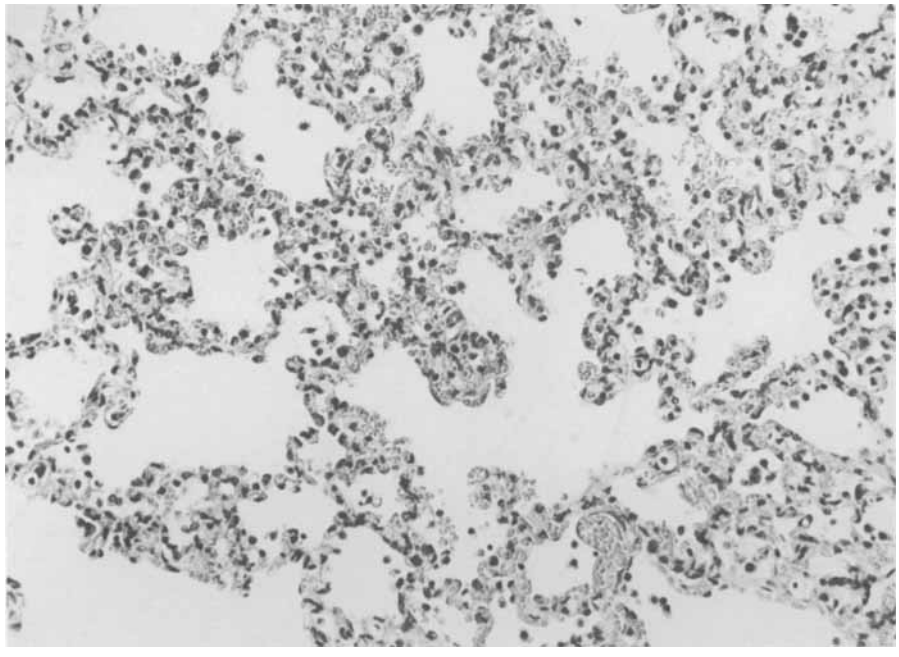

Figure 3 Alveolar cell hyperplasia seen in the lung in the GaAs group. H & E stain, × 99.

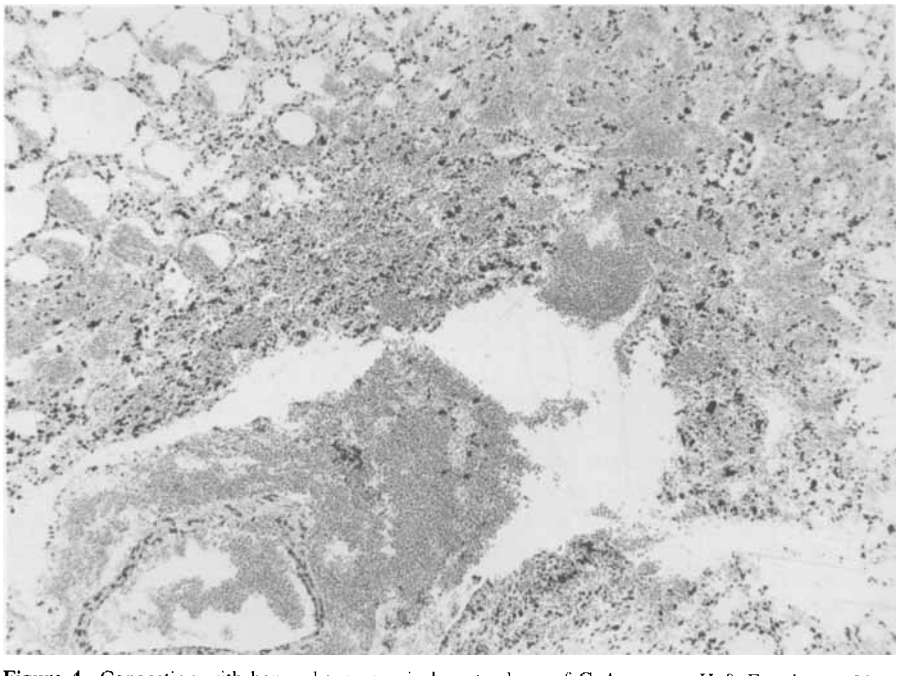

Figure 4 Congestion with hemorrhage seen in hamster lung of GaAs group. H & E stain, × 99.

to lung fibrosis, e.g. as silica, which is insoluble, shows a potent fibrogenicity in lungs of rats and guinea-pigs.[12,13] The present study suggests that much attention should be given to chronic exposure to GaAs particles.

GaAs has a similar but not lower carcinogenic potency compared with As_2O_3, although tumor incidence in both GaAs and As_2O_3 groups was low (3.3%, Table 1). Ishinishi *et al.*[3] reported a 10% adenoma incidence in female Syrian golden hamsters given

Table 3 Main histopathological findings in hamsters which died before each mean survival day

Lung lesion	Control	GaAs	As_2O_3	B(*a*)p
Multifocal macrophage accumulation	0(0%)[a]	0(0%)	1(3.3%)	0(0%)
Alveolar cell hyperplasia	2(6.6%)	9(30%)	7(23.3%)	2(6.6%)
Pneumonia	3(10%)	6(20%)	4(13.3%)	6(20%)
Congestion with hemorrhage	2(6.6%)	6(20%)	3(10%)	2(6.6%)

[a] Value in parentheses (%) is percentage relative to overall group size.

As_2O_3 at the same total dose (3.75 mg as arsenic), as in the present study, and 30% adenoma in hamsters given 5.75 mg. Pershagen and Björklund[4] detected one lung adenoma in 28 hamsters treated with arsenic trisulfide, and four adenomas in 35 hamsters treated with calcium arsenate. Yamamoto *et al.*[14] showed that calcium arsenate is significantly tumorigenic compared with a carried (vehicle) control. Considering that the retention of calcium arsenate was markely higher than that of arsenic trioxide and arsenic trisulfide,[15] the carcinogenic potency of arsenic compounds seems to relate to the retention time of arsenic in the lung. Although the retention of GaAs is higher than that of As_2O_3, the difference of retention time does not appear to affect carcinogenic potency between the two compounds in this study. It appears that the retention of GaAs is not so great as to enhance the carcinogenicity of arsenic, compared for example with that of calcium arsenate. On the other hand, the valence of arsenic might relate to the carcinogenicity of these compounds, since arsenic in calcium arsenate [As(V)] with a high carcinogenicity differs from that of the other arsenic compounds [As(III)].

On the other hand, it is interesting to note that one liver tumor was found both in the GaAs and the As_2O_3 groups, since liver tumor and more specifically hemangioendothelial sarcoma was often observed in humans exposed to arsenic compounds, such as vineyard workers and an individual using Fowler's solution for the treatment of psoriasis, etc.[2,16,17]

CONCLUSIONS

In vivo, gallium arsenide is likely to produce severe lung damage, in addition to the tumorigenicity and acute or subacute toxicity of arsenic generally. The pulmonary damage seems to cause a shortened lifespan of hamsters administered gallium arsenide. Therefore, much attention should be given to chronic exposure to GaAs particles, which might be volatile or suspended in the atmospheric environment in semiconductor production companies.

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