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Effects of GaAs and Ga_2O_3 on Magnetometric Behavior of Iron Oxide Particles in Rabbit Lungs

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Intratracheal instillation of a GaAs suspension has been histopathologically shown to induce a diffuse pulmonary response. In the present study, magnetometry was used to evaluate the effects of intratracheally instilled GaAs and Ga_2O_3 on the behavior of externally magnetized iron oxide (Fe_3O_4) particles instilled in rabbit lung. Magnetometric evaluation of the effects of GaAs in rabbits dosed with 30 mg or 300 mg per animal showed a significantly decreased relaxation of iron oxide particles at 1, 3, 7, 14, 21 and 28 days following instillation compared with the controls. On the other hand, in the rabbits exposed to Ga_2O_3, significantly reduced decay constants were observed only on the first and third days following instillation.

Relaxation indicates a rapid decrease of remanent magnetic field following magnetization of the lungs due to random rotation of phagocytosed iron oxide particles in macrophages.

Clearance of the iron oxide particles was measured by serial determinations of the remanent magnetic field at the end of magnetization estimated from relaxation curves. Clearance was significantly impaired at 14, 21 and 28 days after instillation in rabbits exposed to both doses of GaAs. Slightly delayed clearance was also observed in rabbits exposed to Ga_2O_3.

Histological examination of lungs instilled with GaAs indicated active phagocytosis of GaAs and iron oxide particles by pulmonary macrophages, as well as pneumonocytes hyperplasia with marked thickening of the alveolar walls. Minimal histological changes with retention of iron oxide particles were found in the lungs exposed to Ga_2O_3.

Keywords: Magnetometry, gallium arsenide, gallium oxide, clearance, relaxtion, microelectronics

INTRODUCTION

Gallium arsenide (GaAs) is a crystalline intermetallic compound possessing semiconductor properties superior to those of more common materials, such as silicon. GaAs has led to the development of electro-optical devices such as light-emitting diodes and semiconductor lasers. Exposure to airborne GaAs in the semiconductor industry is a possible occupational risk, since slicing and polishing GaAs ingots to obtain the desired wafers generates GaAs particles.[1-3]

Several reports point to pulmonary exposure to GaAs dust as a potential occupational health hazard. *In vivo* solubility and toxicity have been demonstrated after both intratracheal and oral administration of GaAs.[4-7] Elevated blood arsenic concentrations, body weight loss, pulmonary toxicity, effects on porphyrin metabolism[4,7] and immunotoxicity[8-11] have been observed in animals exposed to GaAs.

A rapid decrease in the magnetic field after magnetization of the thorax of animals intratracheally instilled with ferrimagnetic particles has been noted and designated as 'relaxation'.[12] The results of *in vitro* and *in vivo* experiments indicated this to be due mainly to rotation of magnetized particles in the phagosomes of alveolar macrophages.[13,14] Remanent magnetic strength immediately after magnetization is an indicator of the amount of magnetized particles retained in the lungs. Clearance of magnetic particles can be assessed by serial magnetometric measurement. Cigarette smoking[15] and silica[16,17] have been reported to affect relaxation and clearance of iron oxide particles from the lung. This study was conducted to evaluate the effect of GaAs on the magnetometric behavior of iron oxide particles in rabbit lungs.

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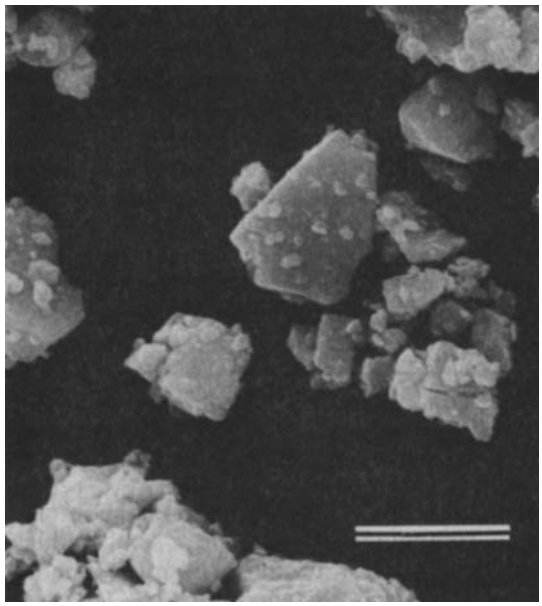

Figure 1 Scanning electron microscopic appearance of GaAs particles. The bar represents 1 μm.

MATERIALS AND METHODS

Preparation of particles

Ga_2O_3 (Wako Pure Chemical Industries, Japan) was sieved through a 38 μm mesh microsieve. A sample of GaAs was finely pulverized in an agate ball mill and the powder was sieved manually through a 38 μm mesh microsieve. The GaAs used was manufactured by Furukawa Electric Inc. in Japan, and kindly provided by Mr M. Kudoh. Particle size and shape were determined by scanning electron microscopy. The geometric diameter of the particles in this study ranged from 0.07 to 1.50 μm, and averaged 0.43 μm. Scanning electron microscopy showed the GaAs particles to be spherical, cubic and conical in shape (Fig. 1). Ga_2O_3 particles appeared columnar in shape by scanning electron microscopy (Fig. 2). Average short and long diameters are 0.64 and 2.15 μm, respectively. The Fe_3O_4 particles were manufactured by Toda Industry Inc., Japan. Their geometric diameter ranged from 0.08 to 0.57 μm, and averaged 0.26 μm. The number of polystyrene microspheres (Polybead, Polysciences Inc., USA) was adjusted to $2.2 \times 10^6\ \mu l^{-1}$, this being identical to GaAs (300 mg in 2 cm^3 of saline). The diameter of the polystyrene microspheres was uniformly 1 μm.

Intratracheal suspension instillation

A suspension prepared by dispersing 20 mg of Fe_3O_4 particles in 1 cm^3 of saline was instilled into the trachea of eight Japanese white rabbits in each group, using a silicon rubber catheter. A suspension of 30 or 300 mg, or 20 mg Ga_2O_3 per animal in 2 cm^3 of saline was then immediately instilled into the trachea of each rabbit. For the controls, 2 cm^3 of saline or polystyrene microsphere suspension (latex) was instilled into eight rabbits. Their body weight at the time of instillation ranged from 2.92 to 3.99 kg, and averaged 3.26 kg.

Magnetometry

After instillation, a 500 G magnetic field was applied to the thorax of each anesthetized rabbit positioned between a pair of enameled copper wire coils driven by a DC power supply. Each animal was exposed to this field for 15 s for magnetization. After removal of the external magnetic field, the remanent magnetic field in the chest was measured with a fluxgate magnetometer (Magnetoscop, Institut Dr Foerster, Germany) for at least 40 min. Time after magnetization was counted from zero when the magnetization was stopped, because iron oxide particles are thought to align completely in response to an external magnetic field and to start to become misaligned

Figure 2 Scanning electron microscopic appearance of Ga_2O_3 particles. The bar represents 1 μm.

as soon as it is removed. Magnetization and measurement of the magnetic field were repeated for four weeks after installation.

A five-minute period of relaxation was fitted to the exponential function

$$B = B_0 \exp(-\lambda t)$$

where B is the field strength at time t, B_0 the field strength at time $t=0$, and λ the relaxation rate (decay constant) for 5 min.[18] Linear regression of the natural logarithms of the field strengths at t by the least-squares method was used to fit the line. B_0 was estimated by extrapolating the function back to time zero. The data for clearance were calculated using the expression (B_0 of a rabbit each day)/(B_0 of the same rabbit immediately after instillation) × 100%.

Histological examination

Microscopical examination of the lungs was performed one and eight weeks after instillation. Both of the rabbits ($n=2$) were anesthetized and exsanguinated from the femoral artery, and a portion of each lobe of the lungs was fixed and stained with hematoxylin and eosin and with Berlin blue.

Statistical analysis

Statistical evaluation was conducted using a SAS package. Differences in the means of the four groups were assessed by analysis of variance and Tukey's test. $P<0.05$ was considered significant.

RESULTS

Relaxation

The means of remanent magnetic fields at three days following the instillation of GaAs, Ga_2O_3, iron oxide and latex particles are plotted in Fig. 3. An analogous curve was obtained for the controls. Relaxation curves of the rabbits exposed to 30 and 300 mg GaAs were markedly delayed compared with the controls on days 1–28 after instillation. A dose-dependent delay of relaxation was observed. The relaxation curves of animals exposed to Ga_2O_3 appeared equivalent to the controls.

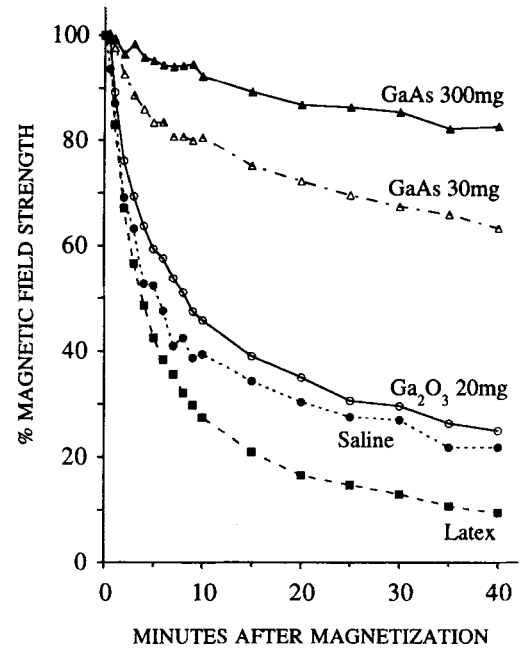

Figure 3 Relaxation curves of instilled iron oxide particles in the lungs of rabbits intratracheally exposed to 30 or 300 mg of GaAs, or 20 mg Ga_2O_3, per animal, latex or saline. The normalized means of remanent magnetic fields three days after instillation of iron oxide followed by administration of GaAs, Ga_2O_3, latex or saline are plotted.

A significant decrease in the decay constant for the first five minutes of relaxation was observed in animals exposed to GaAs, as shown in Fig. 4. Compared with controls, a significant decrease in relaxation was shown in animals exposed to GaAs during 28 days after instillation. No significant difference between the groups exposed to Ga_2O_3 and saline was detected from seven days after instillation onward.

Clearance

From day 14 after instillation onward, the mean percentage magnetic fields at time 0, normalized to the data on the day of instillation in the groups exposed to doses of either GaAs or Ga_2O_3, were higher than those of the controls, as shown in Fig. 5. There was a dose-dependent delay in the clearance of iron oxide particles from the lungs of rabbits exposed to GaAs. Mild but statistically significant delay of clearance was also observed in the animals exposed to Ga_2O_3. No significant difference between the groups exposed to saline and latex was detected.

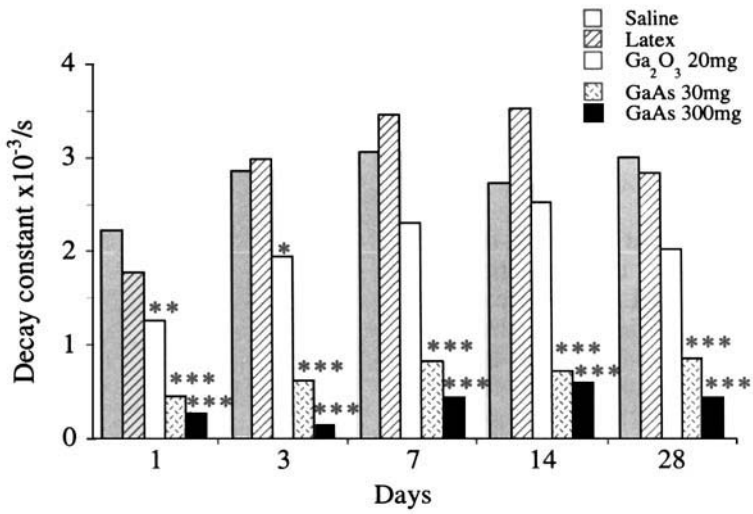

Figure 4 Decay constant for the first 5 min after magnetization. Decay constant (λ) is an initial relaxation rate calculated by the equation $B = B_0 \exp(-\lambda t)$. Asterisks indicate significant differences from animals exposed to saline as follows: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Histological examination

Microscopical examination of lung tissue from the rabbits at one week and eight weeks after instillation of iron oxide and 30 or 300 mg GaAs showed multifocal proliferative alveolitis, pulmonary edema and bleeding (Fig. 6). Thickening of the alveolar wall was due to pneumonocyte hyperplasia and interstitial pneumonia. Large clusters of GaAs and Fe_3O_4 could occasionally be observed. Histological examination of lung tissue showed essentially the same results in the rabbits exposed to 30 and 300 mg GaAs.

Pulmonary exposure to Ga_2O_3 particulates

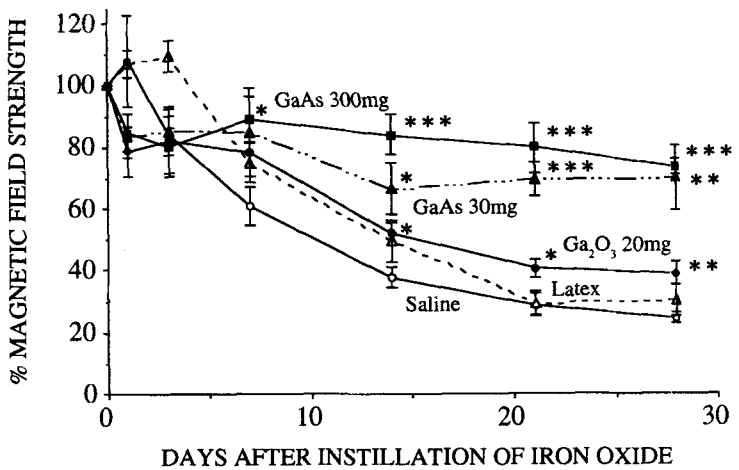

Figure 5 Clearance of iron oxide from the lungs measured with a magnetometer, for rabbits intratracheally exposed to 30 or 300 mg GaAs or 20 mg Ga_2O_3 20 mg per animal, latex or saline. Asterisks indicate significant differences from animals exposed to saline, as follows: * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Means and standard errors of the mean percentage magnetic field at time 0, normalized to the data on the day of instillation, are shown by the vertical bars.

resulted in minimum histopathological changes in the lung, while the alveolar walls were of normal thickness in most sections (Fig. 7).

DISCUSSION

Magnetic lung measurements have been used to evaluate the retention of the magnetizable ferrimagnetic fraction of occupational dusts, such as welding fumes[19,20] and asbestos fibers.[21] Magnetic measurement of ferrimagnetic particles in the lungs should thus facilitate determination of the amount of magnetizable dusts inhaled and retained in the lungs. Magnetopneumographic studies have shown remanent magnetic fields generated by external magnetization of ferrimagnetic particles in the lung to decrease rapidly with time.[14,18] This phenomenon, relaxation, is primarily attributed to the rotation of phagocytized ferrimagnetic particles in macrophages.[22] Since most of the particles are in phagosomes or secondary lysosomes, and since these organelles seem to be integrated into the cytoskeletal network, rotational forces responsible for relaxation are related to contractile forces in this filamentous network.[14] The speed of relaxation (the decay constant) is regarded as representing the speed of rotation of iron oxide particles in phagocytes.

In the previous study,[23] iron oxide particle relaxation was delayed by instillation of GaAs. Hence, we presume that GaAs interferes with the rotation of iron oxide particles in phagocytes. This phenomenon may be an indication of GaAs toxicity to phagocytes, since inert polystyrene particles did not affect relaxation. In the present study, the effects of Ga_2O_3 on the magnetometric behavior of iron oxide particles in the lungs were evaluated.

Particles deposited in alveolar spaces are cleared by the action of macrophages. Brain *et al.*[16] observed delayed clearance and fast relaxation in hamster lungs after intratracheal administration of various doses of silica. In contrast, we previously noted a dose-dependent delay in both clearance and relaxation in rabbit lungs following the administration of two doses of silica.[17]

In this study, two different doses of GaAs, 30 and 300 mg per animal, instilled into the trachea of rabbits, caused a dose-dependent delay in relaxation and clearance. On the other hand, Ga_2O_3 (20 mg per animal) instilled into the trachea of rabbits caused milder delay in relaxation and clearance in comparison with those of GaAs administration. As negative controls, in addition

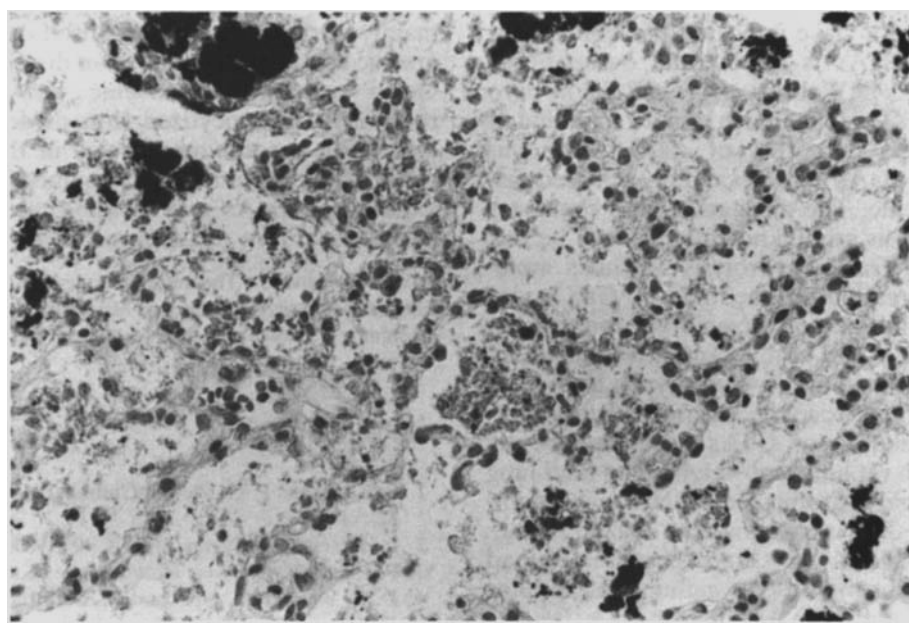

Figure 6 Rabbit lung seven days after the intratracheal instillation of 30 mg GaAs per animal. H & E ×200.

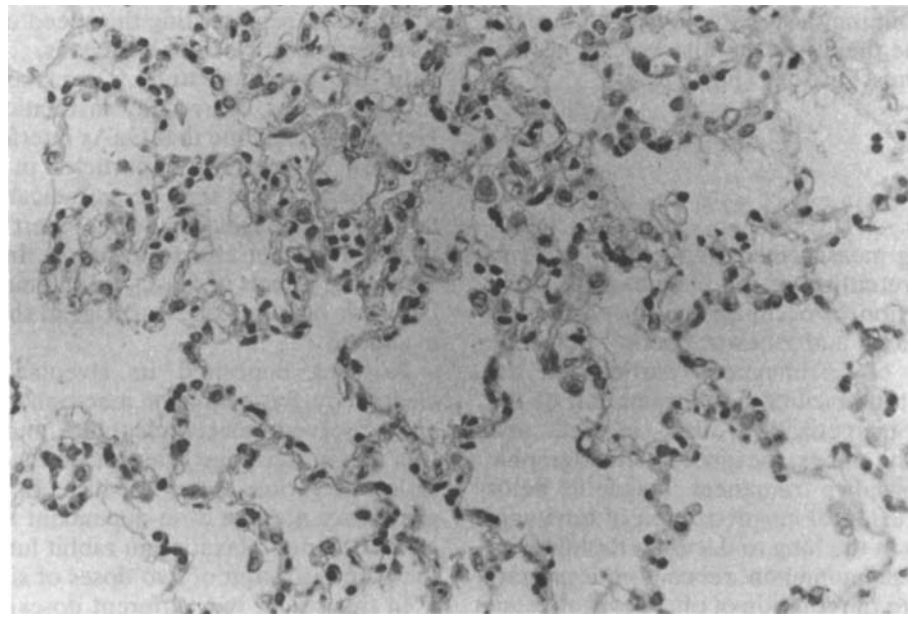

Figure 7 Rabbit lung seven days after the intratracheal instillation of 20 mg Ga_2O_3 per animal. H & E ×200.

to saline alone, a suspension of polystyrene microspheres was instilled intratracheally, since the phagocytosis of inert material itself may affect the relaxation and clerance of iron oxide particles. No marked differences in relaxation or clearance were observed between the groups exposed to saline and latex.

Several reports have indicated that pulmonary exposure to GaAs is a potential occupational health hazard.[2,4-6] Webb *et al.*[4] found the *in vitro* solubility of GaAs to be much greater than that of Ga_2O_3 but less than that of As_2O_3. Aqueous incubation of GaAs particles rapidly released gallium and arsenic. The mechanism and chemistry of this hydrolysis are unknown but it may be due to particulate GaAs, hydrolysis of GaAs molecules, or formation of the oxides, Ga_2O_3 and As_2O_3. Elevated blood arsenic concentrations, body weight loss and both quantitative and qualitative alterations in urinary porphyrins have been reported.[5,7] The toxicological effect of the intratracheal instillation of GaAs dose-dependently increased with rat lung wet weight. The total lung content of lipids, protein and DNA was significantly elevated in rats exposed to GaAs.[5] Lungs from rats exposed to GaAs particulates had retained 44% of the dose as gallium and 28% as arsenic at the end of the 14-day study.

Our own primary histopathological examinations one and eight weeks after intratracheal instillation of GaAs showed an inflammatory reponse and pneumonocyte hyperplasia, as also noted by Webb *et al.*[5] The intratracheal instillation of Ga_2O_3 evoked negligible pulmonary response according to the histological findings.

The delayed relaxation and clearance of iron oxide particles observed in this study may thus be mainly attributable to GaAs itself or to dissolved arsenic compounds rather than dissolved gallium. GaAs or dissolved arsenic may impair the rotation of phagocytized iron oxide particles in cells, with a consequent delay in the relaxation rate. The infiltration of pneumocytes in lungs exposed to GaAs may possibly induce delayed clearance of iron oxide particles from the lungs.

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