

In Vitro RPM Fibrogenic Potential Assay of Welding Fumes

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The fibrogenic potential of 11 different welding fumes and metallic aerosols, considered to be reference standard surrogates for the commonly used welding technologies and applications responsible for 70% of welders exposure, is screened by using the rat peritoneal macrophage (RPM) *in vitro* bioassay. Only one class of fumes, that from the manual metal arc welding of stainless steel, shows distinct fibrogenic potential. This fume, however, is not common to more than four or five of the heretofore 90 cases of pulmonary fibrosis reported among welders. Thus, although insoluble Cr(VI) is probably the active fibrogen in stainless steel fumes, an etiological factor common to all fibrogenic welding exposures must be sought; it is tentatively proposed to be NO_x , a potent experimental *in vivo* fibrogen copiously produced by certain welding processes and ubiquitous at low concentrations in the welding environment.

Introduction

For most individuals, the inhalation of welding fumes [which have a mass, median diameter of between 0.2 and 2.0 μm (1)] is a reversible process, whereby metal rich particles deposited and retained in the lower respiratory tract (2) collect in regular spherical nodules of sufficient opacity to be detected in thoracic X-rays as small round shadows (3,4), the prevalence of which regresses with time (5-7) due to various clearance mechanisms after exposure ceases. This clinical observation has been frequently recorded in the extensive literature of the past decades [recently reviewed (8)] and is usually referred to as welder's siderosis (or welder's lung).

For some small (but unknown) fraction of welders, the reaction to fume deposition becomes irreversible with the formation of fibrous tissue. In the reports of some 90 cases revealed by a three-generation citation search of the literature since 1950 (8), various terms have been applied to the tissue changes observed at biopsy or autopsy. These include: fibrosis (parenchymal, diffuse, interstitial, conglomerative, alveolar, desquamative, perivascular, nodular, pulmonary) of fibrosclerosis, anthracosis, anthracosclerosis, anthracosiderosis and/or parenchymal sclerosis. All such observations are characterized by microscopic changes, including increased

deposition of collagen in the pulmonary alveolar septa and along lymphatic routes of clearance, and are frequently accompanied by reparative and/or atypical proliferation of the alveolar epithelial lining cells. The existence of these cases among welders raises the question of possible causality between welding fume exposure and pulmonary fibrosis.

The lung is capable of only a limited repertoire of responses to injury. Many different agents can produce the same pathologic response, e.g., hemorrhage, necrosis, scar, tumor or cancer. Conversely, exposure to the same agent may result in one of several different reactions, depending on factors such as concentration or duration, and for particulates on form, morphology, size, etc. The response may also depend on host factors such as tobacco use, pre-existing disease, genetic traits or coexposures. The exact role played by welding fumes in determining any eventual tissue pathology is therefore not easily determined.

A number of reports have recently appeared which document exposure to welding fumes by direct microscopic analysis of individual particles either *in situ* or isolated from lung tissue (9-12). The association of welding fumes with irreversible tissue reactions does not prove causality: the identification of particulates having the characteristics of welding fumes can only be taken as noncontroversial evidence of exposure to welding fumes. The observation of welding fume particles in a nontrivial number of such cases strongly suggests the need for further studies to define the cytotoxic and fibro-

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genic potential of well-characterized welding fume components to strengthen or dismiss the possibility of risk due to occupational exposures within the welding industry.

The absolute level of risk is impossible to determine, although it can be expected to vary considerably with age distribution and exposure of each cohort; from the British data (13) the absolute incidence (cumulative) for welders having less than the average exposure (17 years) is of the order of 10^{-3} . The reported cases may be random throughout the industry, or associated with high risk hot spots.

Although there is a possible excess fibrogenic hazard to welders as demonstrated above, identification of the fibrogenic agent(s) is still to be addressed. Because of the complex nature of welding exposures, it is impractical to try to establish the etiological factors involved by clinical methods alone, and therefore the possible use of *in vitro* techniques must be considered.

Materials and Methods

Welding Fumes

The positive identification of a fibrogenic agent or agents in the fumes from a specific welding process or class thereof, or the demonstration that welding fumes are not fibrogenic in general (perhaps with well-defined exceptions) would be of significant benefit in understanding the possible origins of the fibrotic reactions observed in welders. The availability of an *in vitro* test for fibrogenic potential, which is known to respond to acknowledged fibrogenic materials in the form of insoluble dust particles, presents an opportunity for screening welding fumes to determine the prevalence of fibrogens (14-16). Although it has been estimated that there are of the order of 5000 different welding fumes (17), a survey of the range of composition of aerosols from a wide variety of technologies and their applications has shown that the range of chemistries and materials responsible for 70% of welders exposure can be represented by a small number of fumes which can be considered as surrogates for the common industrial exposures.

Fumes are produced in a welding robot, collected on large paper filters from which they are immediately scraped, placed in glass vials and stored at room temperature. Fumes are divided into four classes as follows: (1) mild steels (MS) and aluminum (AL); (2) manual metal arc welding (MMA) on stainless steel (SS); (3) metal inert gas welding (MIG) on stainless steel; (4) nickel welding consumables. The general characteristics of the four fume classes are as follows:

Class 1. The MMA/MS fumes (1a, 1b) have complex chemistries due to the presence of the flux-forming material, and therefore have very different toxicities. MIG fumes can be nonferrous (such as MIG/AL (1c) which is primarily aluminum oxide) or ferrous (MIG/MS) (1d), in which case the major composition is a spinel (magnetite), a pure sample of which (1e) is included for comparison. The mass median diameter (MMD) for the MIG fume is of the order of $0.2 \mu\text{m}$, although they require ultrasonic dispersion to ensure homogeneous suspension in the media used.

Class 2. MMA fume from stainless steel welding contains Cr(VI), mostly as a sodium or potassium chromate in the large aerosol (MMD = $2 \mu\text{m}$) produced by the arc jet impinging on the molten slag pool and also from the melting of the electrode coating.

Class 3. MIG welding of stainless steel produces a fume which is an iron oxide spinel with various amounts of chromium as a substitutional atom. Also it contains Ni in about the same proportion, while the relative composition is dependent on the welding parameter (3a, 3b).

Class 4. MIG welding on cast iron with pure Ni wire produces a fume which is completely crystalline and which is an Ni/NiO mixture, the relative Ni excess depending on the choice of welding parameter (4a, 4b). Under some circumstances, the fume also contains Fe as well; an Fe-rich fume should contain a mixed Fe/Ni spinel. This process can be replaced by one which uses a MMA electrode having a pure nickel core wire with a coating of BaCO_3 , which produces a fume composed almost entirely of BaO encapsulating a small amount of Ni metal and oxide (4c).

Nominal chemical analysis of the fumes is given in Table 1.

In Vitro Fibrogenic Activity Assay

In the rat peritoneal macrophage assay (RPM) (14-16), cells harvested by a lavage procedure are precipitated by centrifugation and resuspended at about $10^6/\text{mL}$ in serum-free medium (containing heparin) to which is added a stock suspension of fume to give final concentrations of $0.5 \text{ mg}/10^5$ cells. After incubation at 37°C for 2 hr, an aliquot is removed, mixed with an equal volume of trypan blue (0.5% w/v) dissolved in phosphate buffered saline, and examined in a microscope with phase contrast optics. This assay consists of examining random fields for those cells which (when alive) exclude or (when dead) include trypan blue, and which contain or do not contain dust particles. The index of fibrogenic-

Table 1. Nominal chemical analysis of welding fumes.

| Sample | Al, % | Mg, % | F, % | Si, % | K, % | Ca, % | Ti, % | Cr, % | Mn, % | Ni, % | Fe, % | Cu, % | As, % | Ba, % | Zn, % | Mo, % |
|------------------|-------|-------|------|-------|------|-------|-------|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1a OK48.15 | 1.5 | 3 | 8.9 | 0.3 | 14 | 13 | 0.8 | 0.01 | 3.3 | — | 25 | 0.03 | — | — | — | — |
| 1b OK46.16 | 0.3 | 0.3 | 0.1 | 10 | 7 | 0.9 | 3.5 | 0.02 | 5.2 | — | 36 | 0.12 | — | — | — | — |
| 1d Autorod 12.51 | — | — | — | 0.8 | — | — | — | — | 3.4 | — | 72.9 | 0.2 | — | — | — | — |
| 2a P316R | | | | | | | | | | | | | | | | |
| Water-soluble | — | — | 11.3 | — | 19.5 | — | 0.4 | 3.6(VI) | 0.25 | 0.06 | — | — | — | — | — | 0.17 |
| Total | — | — | 14.7 | 7.3 | 22 | — | 1.4 | 3.8(VI+ III) | 2.7 | 0.53 | 36 | 0.03 | — | — | — | 0.12 |
| 3a 3RS17 | | | | | | | | | | | | | | | | |
| Water-soluble | — | — | — | — | — | — | — | 0.5(VI) ^a | 0.2 | 0.7 | — | — | — | — | — | 0.43 |
| Total | — | — | — | 3.2 | — | — | — | 9.8(III) ^b 0.5(VI) | 10.4 | 4.1 | 37 | 0.1 | — | — | 0.4 | 2.3 |
| 3b 3RS17 | | | | | | | | | | | | | | | | |
| Water-soluble | — | — | — | — | — | — | — | 0.05(VI) ^a | 0.6 | 0.25 | — | — | — | — | — | 0.018 |
| Total | — | — | — | 1.0 | — | — | — | 13.8(III) ^b 0.5(VI) | 7 | 6.5 | 38 | 0.13 | — | — | 0.6 | 0.36 |
| 4a Ni61 | | | | | | | | | | | | | | | | |
| Water-soluble | — | — | — | — | — | — | — | 0.004 | — | 0.06 | — | — | — | — | — | — |
| Total | — | — | — | — | — | — | — | 0.04 | — | 60.8 | 3 | 0.002 | 0.2 | — | 0.2 | — |
| 4c Grincast 1 | | | | | | | | | | | | | | | | |
| Water-soluble | — | — | — | — | — | — | — | — | — | 0.003 | — | — | — | 5.9 | — | — |
| Total | — | — | — | — | — | — | — | 0.014 | — | 1.3 | 3 | — | 0.17 | 40.2 | 1 | — |

^aUnstable.^bContains some metallic Cr.

ity (F) is defined to be the percentage of dust-bearing dead cells, i.e.,

$$F = \frac{\text{Number of dead cells with dust}}{\text{Number of cells with dust}} \times 100$$

The uptake of particles by cells is generally high. In the cases treated here, more than 80% of all cells examined contained optically visible particles.

Results

The average values of the fractions of dead dust-bearing cells, measured in two samples, for each of the eleven fumes, together with a positive control (α -quartz) and a negative control (Bioglass) are shown in Table 2. In addition, the assay was performed on several samples which had been washed three times overnight in physiological saline, to determine whether the surface activity could be removed by this procedure.

Further samples of fumes (2a and 3b) were suspended in 1% (w/v) aqueous sodium carbonate solution and incubated at 60°C overnight. The samples were then recovered by centrifugation, washed three times by brief resuspension in double-distilled water and resuspended in physiological saline. Tests were performed on these suspensions on the day of preparation. This procedure is designed to

Table 2. Fibrogenic activity of welding fumes in RPM test.

| Sample | F , % dust-bearing cells dead | | |
|-----------------------------------------|---------------------------------|----------------------|-------------------------|
| | Unwashed sample | Saline-washed sample | Carbonate-washed sample |
| α -Quartz (South African sample) | 63 ± 14 | | |
| Bioglass (negative control) | 2 ± 3 | | |
| Welding fumes | | | |
| 1a | 9 ± 5 | 4 | |
| 1b | 13 ± 6 | 3 | |
| 1c | 5 ± 4 | | |
| 1d ^a | 2 | | |
| 1e ^a | 0 | | |
| 2a | 68 ± 14 | 26 ± 11 | 43 ± 5 |
| 3a | 3 | | |
| 3a ^b | 26.5 ^b | | 21.5 ^b |
| 3b | 23 ± 1 | 4 | 29 |
| 4a | 0 | | |
| 4b | 2 | | |
| 4c | 4 ± 2 | | |

^aAlso unequivocally negative in a trial use of the P338D, test as described elsewhere (62-64).

^bAfter 18 months storage.

minimize reduction of Cr(VI) by the washing process (18).

The results of RPM assay on a number of other metal dusts with industrial use are shown in Table 3 for comparison. They exhibit little uptake or activity.

Table 3. Fibrogenic activity of other metal particles.

| Industrial metal powders | | <i>F</i> , % dust-bearing cells dead, unwashed sample | % Cells with dust |
|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|-------------------|
| 5 | Flame/plasma spraying powder (Metco 41F Stainless); (Cr = 1%, Ni = 12%, Mo = 2.5%, Si = 1%, Fe = remainder) | 11 | 29 |
| 6 | Metco 439 tungsten carbid-cobalt flame spray (WC/Co = 50% (Co = 12%), Cr = 6%, Al = 7%, Fe = 1%, Si = 1%, B = 1%, C = .05%, Ni = remainder) | 11 | 36 |
| 7 | Nickel (Goodfellow) Goodfellow metal of Cambridge 4-7 μ m, Cat. No. Ni 006021/2 | 10 | 57 |
| 8 | Stainless steel (Goodfellow) Goodfellow metal of Cambridge 45 μ m, Cat. No. Fe 246020 | 13 | 58 |
| 9 | Cu/Ni (Goodfellow) Goodfellow metal of Cambridge Cu/Ni = 90%/10%; 37 μ m, Cat. No. 126100 | 15 | 61 |
| 10 | α -Quartz (South African) | 53 | 88 |

Discussion

In the general application of the RPM test to industrial mineral dusts, values of *F* between 0 and 20% are judged to be nonfibrogenic, *F* between 20 and 30% doubtful, and those dusts with *F* greater than 30%, as probably fibrogenic, as all fibrogenic material so far tested (as verified by *in vivo* testing) has fallen within this (arbitrary) category. The system is usually not prone to false negatives, but false positives do occur. On the basis of the results shown in Table 2, it can be concluded that fumes 1d and 1e are essentially inert. Samples 4a, 4b, 4c are inert as well. Sample 2a is a strong candidate for possessing fibrogenic potential, since the active material is not completely removed from the surface by overnight washing in saline, as verified by two independent pairs of assays.

Note that the presence of active material in the initial particulates is not necessarily an indication of fibrogenic potential. A water-soluble component present on the surface of the particles might lead to acute effects, but could be expected to be cleared in a period of time (typically days or weeks) short compared to that necessary for the average induction of fibrosis.

The immediate conclusion to be drawn from this survey of fibrogenicity of welding fumes based on the RPM assay is that positive activity is restricted to those particles which are produced through the welding of stainless (or high alloy) steels. These fumes are characterized by the presence of Cr and Ni, which do not appear either in the fumes from mild steel welding or in aluminum welding where Cr has not specifically been used as an alloying metal. Since the (relatively) pure Ni fumes (4a, 4b) were negative, the potential source of fibrogenic activity is not Ni, but is most likely Cr(VI), although the

chemistry of the respective chromium compounds in MIG and MMA fumes is very different, especially with respect to solubility and bioavailability. This hypothesis is strengthened by the results from the samples washed in sodium carbonate solution. As expected, the MIG samples retain their activity in this process, reinforcing the view that the activity can be explained on the basis of the Cr(VI) content as this washing process would be expected to minimize the electrolytic reduction. The MMA sample shows a reduced activity even over that seen in the saline-washed sample, again suggesting that the relative solubility of components is important as the elevated temperature of the carbonate wash would be expected to solubilize (and hence remove) more material from the sample. The observation of enhanced activity in MIG sample 3a after 18 months storage probably reflects the slow conversion of soluble Cr(VI) to insoluble Cr(VI) previously observed (18).

The stainless steel fumes have been assayed for genotoxicity in several screening tests and are found to be mutagenic in bacteria *in vitro* (19-21) mutagenic and cytotoxic in cultured mammalian cells *in vitro* (22-24) and embryotoxic *in vivo* (25, 26). The origin of the genotoxicity has been shown to be the presence of water-soluble Cr(VI) in these fumes.

The observation that fibrogenic activity is restricted to a single class of welding fumes known to contain Cr(VI), together with the evidence of the mutagenicity of these same fumes, gives rise to the possibility that exposure to similar fumes could be responsible for a "hot spot" for respiratory disorders in welders: typical welding process-dependent concentrations of various fume components are given in Table 4 (17). Such a hypothesis can only be verified by means of epidemiological studies specif-

Table 4. Typical average welding exposures.^a

| Process | % of welding population | Al ₂ O ₃ , mg/m ³ | Fe ₂ O ₃ , mg/m ³ | Cr mg/m ³ | Cr(VI) mg/m ³ | Ni mg/m ³ | Mn mg/m ³ | NO ₂ NO, ppm | O ₃ , ppm |
|---------|-------------------------|----------------------------------------------------|----------------------------------------------------|----------------------|--------------------------|----------------------|----------------------|-------------------------|----------------------|
| MIG/AL | 3 | 10 | 0.8 | 0.008 | — | — | — | 0.1 | 0.1 |
| MMA/MS | 60 | — | 2 | — | — | — | 0.5 | 0.02 | — |
| MMA/SS | 10 | — | 4 | 0.2 | 0.2 | 0.05 | 0.3 | 0.02 | 0.01 |
| MIG/SS | 2 | — | 1.2 | 0.2 | 0.1 | 0.1 | 0.3 | 0.2 | 0.01 |

^aFrom Stern (17).

ically designed to examine the process-dependent risk of delayed health effects (17) among selected cohorts of welders with well-defined exposures. Such verification is necessary in order to evaluate properly the usefulness of *in vitro* tests for metal particulate aerosols and their ability to predict human risk.

It should be noted that there is no indication that those welders who experience "fibrosis" have a prevalence of exposure to stainless steel fumes; such exposures are specifically noted for only 4 of the 90 cases reported. Thus, either one must postulate that there is a high and universal but unreported exposure among the welding population to either Cr(VI) or the fumes from stainless steel welding which is responsible for the incidence of reported fibrosis, or that there are other substances with high fibrotic potential present in the work environment, but which are not included in these surrogate fumes. There may also be such substances present but which escape detection in the RPM test: welding fumes may, on the average, have only slight fibrogenic potential which is then expressed only after massive exposure, or may show a synergistic effect with other atmospheric contaminants.

If the positive RPM test results are to be considered solely indicative of the possible origins of fibrogenic potential in the particulate fraction of welding fumes in general, then one can conclude that Cr(VI) is a suspected fibrogenic hazard, and that a widespread prevalence of low concentrations of Cr(VI) might be responsible for the resulting fibrosis: the individual susceptibility determining which workers are affected. Welders of stainless steel should, however, be considered at relatively high risk for pulmonary fibrosis, although they represent only 5-10% of the entire welding population (17).

The limited *in vivo* animal experiments (27, 28) support this possibility, as they also suggest a sensitization to and/or a weak fibrotic potential of Cr(VI) and other fume components. The human evidence does not exclude the possibility of a high interindividual variation in sensitivity to Cr(VI), although the effects of other sensitizing agents (e.g., Co and Mn or small particulates in general) must be considered as well.

With respect to the nonparticulate fraction of welding exposures, which is not tested in this RPM assay, there is an extensive literature demonstrating the toxicity and fibrogenic potency of NO₂ in a number of animal species. In addition to a wide range of functional changes, biochemical abnormalities and specific molecular reactions, fibrotic reactions have been demonstrated to result from exposures that ranged from one week to three years at 15 ppm in rats, mice, guinea pigs, cats, monkeys and dogs. Prolonged exposure to NO₂ is therefore known to result in interstitial fibrosis and centrilobular emphysema. Cigarette smoking produces similar effects (29, 30) through the common mechanism of the induction of α_1 -antitrypsin antiprotease deficiency. An extended review has been given by Guidotti (31). The possibility of a synergistic (or additive) effect of particulates has not been investigated and cannot be excluded.

There is ample evidence of acute effects of exposure to high concentrations of nitrous gases in welding and in allied occupations such as flame cutting (32-34), and it is indicated that 10% of all welders who utilize inert gas technologies are chronically exposed to NO_x levels (8 hr average) greater than 3 ppm. The average values for all welding exposure lie at approximately 0.5 ppm NO_x, or less. High O₃ concentrations are only found in the work environments of inert gas welders of aluminum and stainless steel (35).

It would appear that most welders have a common occupational exposure to at least one experimental fibrogen: nitrogen oxide. Since a wide range of interindividual sensitivity to NO₂ has been observed, one can propose that welders utilizing a limited number of technologies and applications which produce high concentrations of NO_x (e.g., inert and active gas welding of mild steel, stainless steel and aluminum) and flame cutters are at risk, together with a number of high sensitivity individuals in other trades: The contribution to the fibrogenic risk factor due to tobacco use must also be considered. The absolute risk level for welders remains unknown.

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