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A Rat Lung Model of Exposure, Dose and Response to Inhaled Silica

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The objectives of this study were: (i) to construct a physiologically based mathematical model to describe the retention and clearance of respirable crystalline silica (MIN-U-SIL 5), the inflammatory reaction and the development of fibrosis; (ii) to calibrate/validate the model using animal data from inhalation experiments with MIN-U-SIL 5. A physiologically based mathematical model was constructed with the following features: compartments to describe the retention and clearance of silica, critical lung burdens for initiation of inflammation and fibrosis and recruitment of neutrophils and alveolar macrophages. Data were available from two recent NIOSH studies of rats exposed by inhalation to respirable crystalline silica. Data from the first study were used to estimate the model parameters not obtainable from the earlier studies, using a numerical method combining non-linear least squares analysis and integration of differential equations. The second study was used for model validation by comparing the model predictions with the data. Once validated, the model was used to predict the outcome of a 2 yr inhalation experiment with DQ-12 quartz. Finally, the model was extended to describe the dose–response relationships, including oxidant dose, antioxidant reactions, transcription factor switching, cell damage and fibrosis. A good fit of the model to the data was obtained. The critical doses of MIN-U-SIL 5 were calculated for both inflammation and fibrosis. The model was able to simulate the retention and clearance of DQ-12 quartz. The model was also extended to describe the time course of NF-κB initiation, lipid peroxidation and superoxide dismutase expression. This model has provided a means to relate the inhaled silica dose to inflammation and fibrosis.

Keywords: crystalline silica; dosimetry models; exposure–dose–response model; poorly soluble particles; risk assessment; toxicokinetics

INTRODUCTION

Exposure in rats to a high airborne concentration of poorly soluble particles (PSP) of 'low toxicity' is known to cause pulmonary inflammation, severe retardation of particle clearance and increased particle translocation to lung-associated lymph nodes. Particle surface area was shown to be the dose metric of 'low toxicity' PSP (Tran *et al.*, 2000). For 'high toxicity' PSP the health effects mentioned above are likely to occur at much lower particle lung burden (as mass or surface area).

This study aimed to investigate the dosimetry of crystalline silica, a 'high toxicity' PSP.

Specifically, our approach was: (i) to develop a biologically based mathematical model describing the retention and clearance of silica in the lungs and lung-associated lymph nodes of rats; (ii) to determine if there is a critical dose of silica in the lungs that influences the clearance rate; (iii) to extend the model to describe exposure–dose–response at the level of oxidant dose, antioxidant defence and cell damage.

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MATERIALS AND METHODS

Model construction

The starting point for this study was the three-compartment mathematical model derived for coal mine dusts (Kuempel *et al.*, 2001a,b). The inputs to the model were the factors that affect the deposited dose. The model predicts alveolar, interstitial lung and lymph burdens dynamically for any given exposure scenario. To model the rat data, the human model structure was modified to include an alveolar macrophage (AM) compartment. The impairment of

silica particle clearance was attributed to the inability of AMs to remove the inhaled silica once a critical silica dose (m_{crit}) was reached. The model also describes the oxidant dose generated by inhaled silica and products from AM [and also neutrophils (PMN) during inflammation], which unbalance oxidant/antioxidant levels in the lung. This is the 'oxidative stress', which activates the transcription factor NF-κB and pro-inflammatory cytokines, leading to the recruitment of PMNs to the sites of particle deposition.

Data

Data were available from two NIOSH experiments using MIN-U-SIL 5 (Porter *et al.*, 2000, 2001).

In the first experiment (NIOSH I) rats were exposed for 6 h/day, 5 days/week for 28, 54 or 84 days, at 15 mg/m^3, each with a 0 and 36 day recovery group. Lung (lavageable), interstitial (non-lavageable) and lymph node levels of retained silica and AM and PMN cell counts were available.

In the second experiment (NIOSH II) rats were exposed for 6 h/day, 5 days/week for 7, 14, 21, 28, 42, 56, 112 or 161 days at 15 mg/m^3. The data were total lung burden and PMN and AM cell counts, with extra data on the levels of lung hydroxyproline, NF-κB activity of bronchoalveolar lavage (BAL) cells, lung lipid peroxidation and acellular BAL fluid superoxide dismutase (SOD).

In this model, inflammation was represented by the number of PMN cells in the BAL fluid and fibrosis by the level of lung hydroxyproline.

Model calibration

Data from the literature (e.g. value for phagocytosis rate, deposition fraction) and non-linear regression with data from the NIOSH I experiment were used to estimate the model parameters.

A numerical routine (coded in MATLAB) was developed for parameter estimation. This method combines non-linear least squares analysis and the Runge–Kutta algorithm for solving ordinary differential equations.

Model validation

Data from the NIOSH II experiment were subsequently used to validate the model predictions and the model was re-calibrated if necessary. Because of the extra data available only in the NIOSH II experiment (i.e. hydroxyproline, NF-κB, etc.), the model was also extended to describe the time course of these parameters.

RESULTS

A good fit of the model to the data of the NIOSH I experiment was obtained with differences between

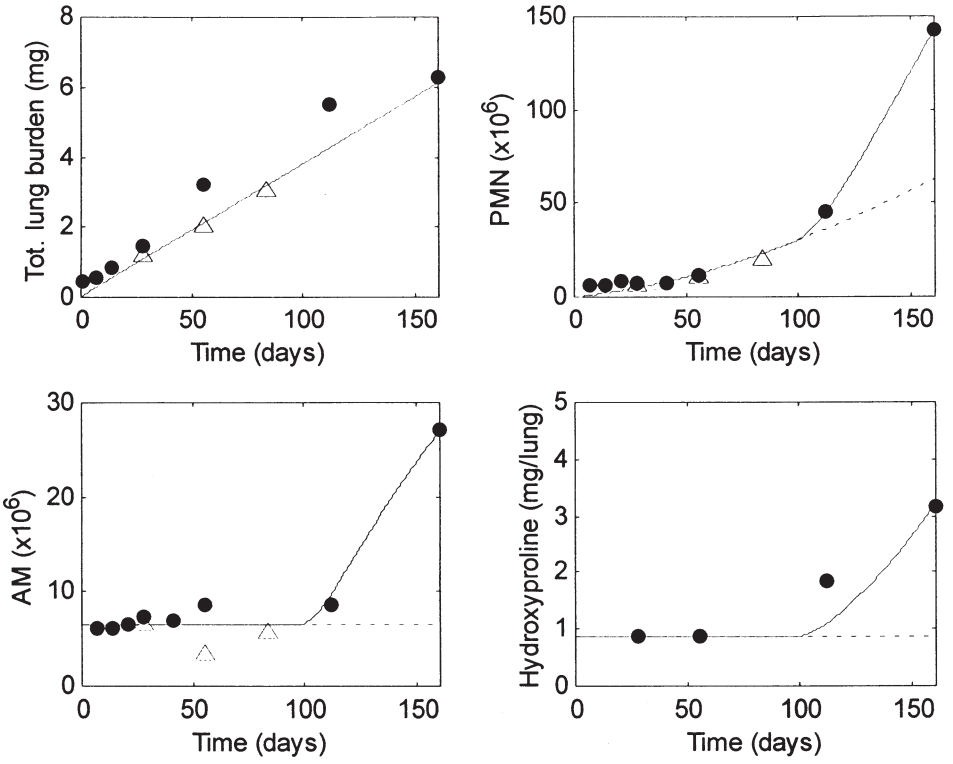

Fig. 1. The re-calibrated model predictions and the experimental data from the NIOSH I experiment with Min-U-Sil 5. The triangles are data from the NIOSH II experiment; the dotted lines are the original model predictions.

the predicted and observed burdens of ~3% on average. The critical dose (m_{crit}) of MIN-U-SIL 5 was calculated for inflammation (0.20 ± 0.19 mg). The model was used to predict the results of the NIOSH II experiment (Fig. 1). The model predicted a linear build-up of total lung burden, parallel with the deposited dose. This appeared to be confirmed by comparing the predicted total lung burden with the burden data of this experiment. Interestingly, for the PMN and AM assays the model significantly under-predicted the last time point (116 days). For hydroxy-proline, the level appeared to increase from 112 days. It was clear that:

- the current model did not predict the formation of fibrosis and therefore required some modifications;
- fibrosis worsened inflammation (i.e. an increase from a relatively steady to an elevated new set point which is noted at the 110 day time point for inflammatory parameters);
- fibrosis is associated with an increase in the AM population.

Thus, the model was subsequently re-calibrated (Fig. 1) to estimate the silica critical burden for fibrosis, m_{crit2} (1.96 ± 0.12 mg).

This model was also extended to describe the time course of NF-κB activation in BAL cells, lipid peroxidation and SOD activity (Fig. 2), although data are not yet available for validation of this part of the model.

DISCUSSION

In the current study we have extended the exposure–dose model of Kuempel *et al.* (2001a,b) to include a macrophage burden compartment. We assume that inflammation begins when AM-mediated clearance becomes impaired. This crucial compartment gave the basis for the threshold doses (m_{crit} and m_{crit2}) of crystalline silica activating inflammation and causing fibrosis.

The model was then used to simulate the long-term retention and clearance of DQ-12 quartz, suggesting similar biological mechanisms for MIN-U-SIL 5 and DQ-12 (Tran *et al.*, 2001).

Finally, the model was extended to cover oxidative stress (lipid peroxidation), antioxidant status (SOD) and transcription factor activation (NF-κB). Although not all sub-models are currently validated, the model offers a unique, quantitative and coherent view of the events leading to oxidant production,

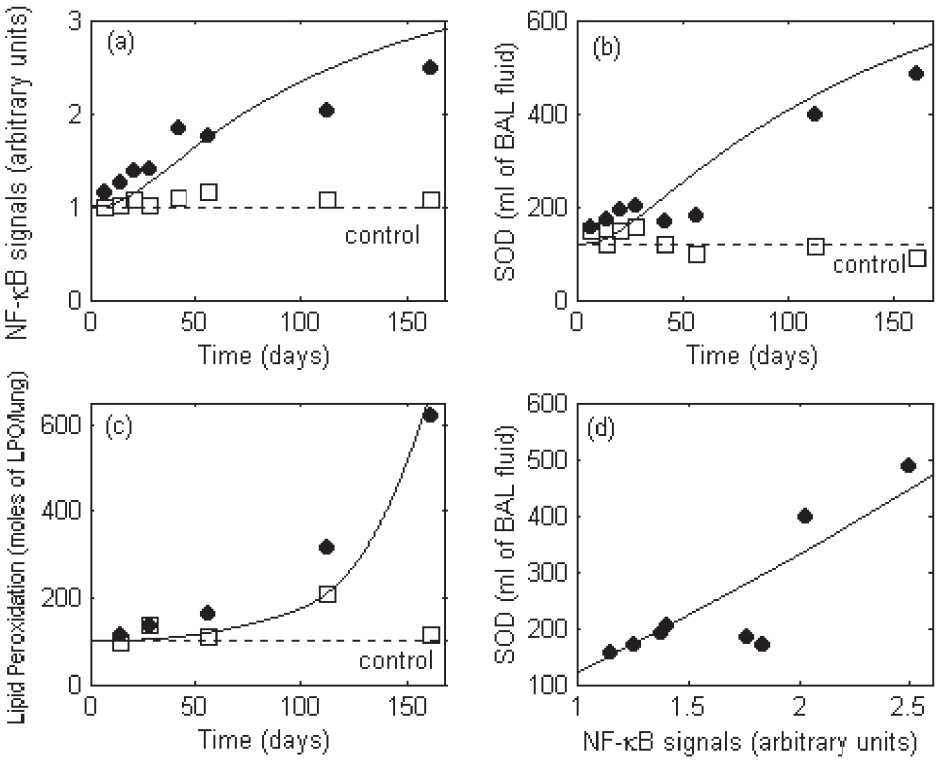

Fig. 2. Time course of (a) NF-kB, (b) SOD and (c) lipid peroxidation and (d) the relationship between inflammation (NF-κB) and the antioxidant response (SOD). Square symbols are data from the control experiment.

inflammation and fibrosis and their inter-dependence.

CONCLUSIONS

In this study we have shown that, for silica (Min-U-Sil 5):

- inflammation and other indicators of pulmonary damage are initiated at relatively low mass lung burdens;
- fibrosis is associated with a further wave of inflammatory reaction which requires higher mass lung burdens.

This model has provided a means to relate the inhaled silica dose to inflammation and fibrosis.

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