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Original Article

Acute diesel exhaust particle exposure increases viral titre and inflammation associated with existing influenza infection, but does not exacerbate deficits in lung function

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Background Exposure to diesel exhaust particles (DEP) is thought to exacerbate many pre-existing respiratory diseases, including asthma, bronchitis and chronic obstructive pulmonary disease, however, there is a paucity of data on whether DEP exacerbates illness due to respiratory viral infection.

Objectives To assess the physiological consequences of an acute DEP exposure during the peak of influenza-induced illness.

Methods We exposed adult female BALB/c mice to 100 μg DEP (or control) 3·75 days after infection with $10^{4.5}$ plaque forming units of influenza A/Mem71 (or control). Six hours, 24 hours and 7 days after DEP exposure we measured thoracic gas volume and lung function at functional residual capacity. Bronchoalveolar lavage fluid was taken for analyses of cellular inflammation and cytokines, and whole lungs were taken for measurement of viral titre.

Results Influenza infection resulted in significantly increased inflammation, cytokine influx and impairment to lung function. DEP exposure alone resulted in less inflammation and cytokine influx, and no impairment to lung function. Mice infected with influenza and exposed to DEP had higher viral titres and neutrophilia compared with infected mice, yet they did not have more impaired lung mechanics than mice infected with influenza alone.

Conclusions A single dose of DEP is not sufficient to physiologically exacerbate pre-existing respiratory disease caused by influenza infection in mice.

Keywords BALB/c mice, diesel exhaust particles, inflammation, influenza, lung function.

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Introduction

There are many studies which link exposure to air pollution, and specifically particulate matter (PM), with increased morbidity and mortality due to respiratory illnesses, such as asthma[1–4] bronchiolitis[5,6] and chronic obstructive pulmonary disease.[7–9] These epidemiological studies generally focus on relating mortality and hospital admissions for respiratory or cardiac conditions to a broad range of ''general'' PM, including particles originating from soil and other natural sources, ''road-dust'' from tyre and brake wear, and diesel exhaust particles (DEP). Studies on the respiratory health impacts of DEP specifically are extremely rare due to the difficulty in separating the effects of DEP from other ambient PM.[10,11] Furthermore, as DEP are only part of the complex mixture of particles and gases produced by combusting diesel, many studies investigate the effects of whole diesel exhaust (DE) on health outcomes. However, exhaust gases dissipate rapidly once released to the atmosphere, whereas DEP remain concentrated within the vicinity of the release point for longer such that the respiratory consequences of DEP exposure are of considerable importance.

Accordingly, experimental studies investigating the impacts of DEP on responses to respiratory viral infection are sparse and are typically *in vitro* in nature,[12,13] or involve animal models of long-term exposure to DE.[14–16] Furthermore, such studies usually entail exposing test

animals to DE prior to infection to test the effects of exposure on susceptibility to infection.[14–16] Such studies have shown that exposure to DE results in increased susceptibility to respiratory viral infection,[14–16] higher viral replication,[15] increased pulmonary inflammation,[14,16] lower interferon levels[15,17] and up-regulation of Toll-like receptor 3 (TLR3) receptor expression and signalling[16] in mice.

Data on the effects of acute exposure to DE[18] or carbon black particles[17,19] on existing viral infection are also sparse. Such a scenario is consistent with exposure of an individual with an established respiratory viral infection to high levels of pollution on a bad smog day, while walking next to a busy road, working in a polluted environment or while travelling through a poorly ventilated traffic tunnel.[10,20] It is such a scenario that we are interested in as these acute exposure events may explain, in part, the increase in hospitalization rates due to respiratory illness during periods of high pollution.[21–23] Unfortunately, direct evidence for the impact of DEP specifically on existing respiratory viral-induced disease is lacking. One study modelling this situation.[19] found that instilling mice with ultrafine carbon black particles 3 days after inoculation with respiratory syncytial virus (RSV) caused no change in lung viral titre, but increased neutrophils, lymphocytes, bronchoalveolar lavage (BAL) protein, virus-associated chemokines and responsiveness to methacholine.[19] However, although RSV is a potentially significant human pathogen, it typically produces a mild response in mice. Thus, it may not adequately represent a severe disease such as that observed in mouse models of influenza[24,25] which are more likely to reflect the viral infections resulting in increased hospitalization on high-pollution days. In addition, the use of pure carbon black particles does not reflect the complexity of DEP, which typically consist of a carbon core onto which many toxic substances (e.g. polycyclic aromatic hydrocarbons, nitrates, sulphates and heavy metals) are adsorbed.[26–31]

One recent study examined the impact of DE exposure on influenza infection in mice by infecting BALB/c mice with 50 pfu of influenza A followed immediately by exposure to DE for 4 hours per day for up to 14 days.[18] Although this protocol is slightly different to what we use in this study (i.e. we waited for 3·75 days post-infection before exposing the mice to a single intranasal dose of DEP), Gowdy *et al.* (2010) showed that beginning DE exposure at the same time as influenza infection and continuing it during infection resulted in increased viral titres, neutrophilia, an interleukin-4-dominated cytokine profile and transiently increased pulmonary responsiveness. As acknowledged by Gowdy *et al.* (2010), whole body plethysmography is ''a rather crude indicator of pulmonary function'', and as they did not use confirmatory methods of lung function assessment based on physical principles, their ''pulmonary responsiveness'' data should be viewed with some caution.[32] In saying this, it is well established that influenza infection results in pulmonary hyper-reactivity in mice[24] and humans,[33] and that diesel exposure causes airway hyper-reactivity in humans.[34] Interestingly, Gowdy *et al.* (2010) also showed that treatment with the antioxidant N-acetylcysteine blocked the DE-induced changes in cytokine profiles and lung inflammation, although it did not alter viral titres. Thus, despite these advances, the physiological impact of an acute exposure to DEP, such as during a high-pollution day, on *established* respiratory viral infection remains unclear.

We aimed to investigate the effects of an acute DEP insult at the peak of influenza-induced respiratory illness by infecting mice with influenza A and exposing them to an acute dose of DEP at the peak of infection induced inflammation. At three time points after DEP exposure, we assessed viral titre, inflammation and lung function. We hypothesized that mice exposed to DEP while already infected with influenza would show greater impairments in lung function, and greater pulmonary inflammation compared with mice subjected to either insult alone.

Methods

Animals

Eight-week-old female BALB/c were purchased from the Animal Resource Centre (Murdoch, Western Australia) and housed in individually ventilated cages (Sealsafe, Tecniplast, Italy) on non-allergic bedding (Shepards Specialty Papers, Chicago, IL, USA). Food (Rat and Mouse Cubes; Specialty Feeds, Glen Forrest, WA, Australia) and water were supplied *ad libitum*. All procedures were approved by the institutional Animal Ethics Committee.

Virus and infection

Influenza A/Memphis/1/71 (H3N1) is a mouse adapted strain of influenza A grown and titrated in Madin-Darby Canine Kidney Cells (MDCK) as previously described.[35] This strain causes lung inflammation and replicates to high titres. Mice were intranasally inoculated with either $10^{4.5}$ plaque forming units (pfu) of influenza A/Mem71 (''influenza mice'') in 50 μl of Virus Production Serum Free Medium (VP-SFM; Gibco, Mulgrave, Vic, Australia) or the supernatant of uninfected MDCK cells diluted in 50 μl VP-SFM (''control mice'') under light methoxyflurane anaesthesia (Medical Development Pty Ltd, Springfield, Vic, Australia). Mice were inoculated by pipetting drops of inoculum onto the nostrils until aspirated.[24] Previous studies have shown that intranasal inoculation of a volume of 50 μl is the most appropriate volume for inoculation in adult mice[36] and ensures that almost exactly 50% of the inoculum reaches the lower respiratory tract.

Diesel exhaust particles

We used US Environmental Protection Agency ''C-DEP'' for all diesel exhaust particle inoculations, which was produced at Research Triangle Park (NC, USA) as previously described.[37] These particles were generated with the use of a 30-kW four-cylinder Deutz diesel engine connected to a 22·3-kW Saylor Bell air compressor. The engine was run at 20% of its full-load rating and emissions were diluted with filtered air and directed to a baghouse. C-DEP contains 18·9% extractable organic matter. The organic carbon to elemental carbon ratio is 0·35.[37] By mass, the majority of C-DEP is carbon, although it also contains small amounts of sulphur (0·37%), chlorine (0·42%), calcium (0·19%), zinc (0·15%) and iron (0·12%).[38] Detailed information about the chemical composition of C-DEP is available.[38]

Diesel exhaust particle exposure

Under light methoxyflurane anaesthesia, mice were intranasally inoculated with either 100 μg DEP suspended in 50 μl of normal saline and 0·05% TWEEN-80 (''DEP mice'') or 50 μl of saline and 0·05% TWEEN-80 (''Saline mice''). A 50 μl instillation volume ensures that ~50% of liquid and particles reach the lower respiratory tract.[36] These mixtures were sonicated using a Vibron USB08CD sonicator (Galsonic Pty Ltd., Valley View, SA, Australia) for 30 minutes prior to exposure to reduce particle aggregation. Drops of the solution were pipetted onto the nostrils until aspirated. The dose of 100 μg of DEP was selected based on our previous work, whereby we identified a dose-dependent inflammatory response in BALB/c mice.[39] Mice were exposed to DEP 3·75 days after inoculation with influenza (at the peak of influenza-induced inflammation). Thus, there were four treatment groups; ''Control-Saline'', ''Control-DEP'', ''Influenza-Saline'' and ''Influenza-DEP''. There were 8–12 mice in each group at each time point.

Animal preparation

Mice were anaesthetized with an intraperitoneal injection containing 2 mg/ml of xylazine (Troy Laboratories, Glendenning, NSW, Australia) and 40 mg/ml of ketamine (Troy Laboratories) at a dose of 0·01 ml;/g body weight. Three time points were used; 6, 24 hours or 7 days post-DEP exposure. These time points were the equivalent of 4, 5 and 11 days post-influenza inoculation and were chosen based on previous work assessing the peak and resolution of inflammation.[25,39] Mice were injected with ~two thirds of the dose to induce a surgical level of anaesthesia. Anaesthetized mice were tracheostomized with an endotracheal tube (internal diameter = 0·086 cm, length = 1·0 cm) secured with surgical silk, placed inside a plethysmograph and ventilated at 400 breaths per minute with a tidal volume of 8 ml/kg and 2 cm H_2O of positive-end expiratory pressure (HSE Harvard Minivent; Hugo Sachs Harvard Elektronik, March-Hugstetten, Germany). This level of ventilation was sufficient to allow measurement of lung mechanics without paralysis.

Thoracic gas volume

Thoracic gas volume (TGV) was measured using plethysmography as described previously.[40] This technique involves using electrodes to electrically stimulate the intercostal muscles during a pause in ventilation. During this pause, the plethysmograph and tracheal cannula are occluded and plethysmograph and tracheal pressures measured using pressure transducers (Validyne MP45; Validyne Engineering, Northridge, CA, USA and model 8507C-2, Endevco, San Juan Capistrano, CA, USA respectively). Twelve inspiratory efforts were created per mouse by stimulation with individual electrical impulses ~20 V in amplitude, ~1–2 ms in duration and 0·8 s apart (model S44; Grass Instruments, Quincy, MA, USA). Boyle's law was applied to the relationship between tracheal pressure and plethysmograph pressure after correcting for the impedance and thermal characteristics of the chamber to calculate TGV.[40] Measurements were averaged to provide a single TGV measure for each individual.

Lung mechanics

Respiratory system impedance (Z_{rs}) was measured using a modification of the forced oscillation technique as previously described.[41] Briefly, Z_{rs} was measured using a wave-tube system[42] adapted for use in small animals.[41,43] Oscillatory signals consisting of nine non-integer multiple frequency components between 4 and 38 Hz were delivered via a 1 m wave tube (internal diameter = 0·116 cm) to tracheostomized mice during brief apnoeic periods. Lateral pressure at either end of this wave tube was measured using transducers (model 8507C-2; Endevco) and load impedance on the wave tube was used to calculate Z_{rs}. Z_{rs} was partitioned into the airway compartment: airway resistance (R_{aw}) and inertance (I_{aw}) and tissue compartment: tissue damping (G) and tissue elastance (H) using a four parameter model with constant phase tissue impedance.[44] Fitted parameters were corrected for the impedance of the tracheal cannula and wave-tube connectors. After this correction, I_{aw} values were negligible and are not reported.

Cell counts, cytokines and lung protein analysis

Mice were lavaged by slowly washing 0·5 ml of saline in and out of the lungs three times. The cell pellet and supernatant were separated by centrifuging the BAL for 4 min at 250 g. Total cell counts were obtained using a haemocytometer from cells stained with trypan blue. Light microscopy was used to determine differential counts from cytospin cell samples stained with Leishman's stain (BDH

Laboratory Supplies, Poole, England). We used a cytometric bead array (CBA) to assess the levels of IFNγ, IL-6, TNFα and MCP-1 (BD Biosciences, North Ryde, NSW, Australia) and ELISA to assess levels of KC (R&D Systems, Gymea, NSW, Australia) in BAL supernatant as per the manufacturers' instructions. Total protein content was assessed using a colorimetric assay (BIO-RAD, Gladesville, NSW, Australia) measured with a spectrophotometer at 595 nm absorbance [45].

Lung viral titre

Mice were euthanized and lung tissue was collected under aseptic conditions. Lungs were homogenized in VP-SFM and centrifuged at 250 ***g*** for 5 min. Lung viral titres (normalized to lung weight) were determined by plaque assay as previously described.[35]

Statistics

Analyses were conducted using two-way anova with infection status and exposure status as factors. Data were transformed when required to satisfy the assumptions of the anova. The Holm-Sidak *post hoc* test was used to identify significant differences between groups. Statistical analyses were performed using SigmaStat software (v3·50; SPSS Science, Chicago, IL, USA). Data are shown as mean ± SD for cellular inflammation and cytokines and as maximum/minimum box plots for viral titre and lung function.

Results

Mass

Prior to infection there was no difference in mass between mice randomly allocated to be infected with influenza (17·92 ± 1·04 g) or control solution (17·86 ± 1·45 g; $P = 0{\cdot}809$). Infected mice (16·83 ± 1·34 g) were significantly lighter than non-infected mice 6 hours after DEP exposure (4 days after infection: 17·88 ± 1·25 g: $P = 0{\cdot}018$). There was no difference in mass between infected and non-infected mice either 24 hours ($P = 0{\cdot}120$) or 7 days ($P = 0{\cdot}971$) after DEP exposure. DEP-exposed mice (17·22 ± 1·03 g) were significantly lighter than non-DEP-exposed mice (18·25 ± 1·27 g) 24 hours after exposure to DEP ($P = 0{\cdot}006$), however, this effect was not seen at any other time point. There was no combined effect of DEP exposure and influenza infection on mass.

Viral titre

"Influenza-DEP" mice had significantly higher viral titres ($1{\cdot}91 \times 10^6 \pm 8{\cdot}46 \times 10^5$ pfu/l/g lung) compared with "Influenza-Saline" mice ($1{\cdot}07 \times 10^6 \pm 3{\cdot}08 \times 10^5$ pfu/l/g lung; $P = 0{\cdot}03$) 6 hours after DEP exposure (Figure 1). This difference was not apparent 24 hours after DEP exposure ($P = 0{\cdot}86$). No virus was detected in mice inoculated with control solution, nor was any virus detected in influenza infected mice 7 days after infection.

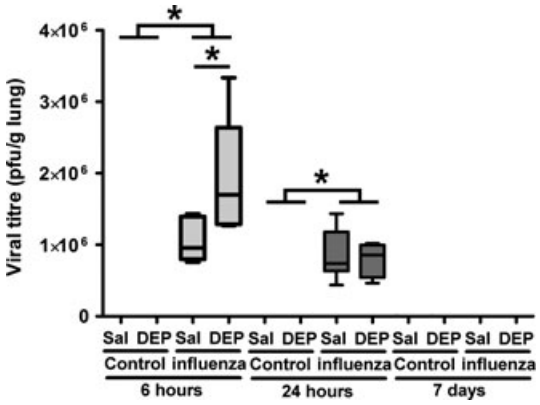

Figure 1. Box and whisker plots of lung viral titre (pfu/g lung) for adult female BALB/c mice infected with influenza (or control) and inoculated with DEP (or saline). *indicates a significant difference between treatments.

Bronchoalveolar inflammation

Influenza infection resulted in significant BAL inflammation at all three time points ($P < 0{\cdot}004$ in all cases; Figure 2). This inflammation was primarily macrophages and neutrophils, with a smaller proportion of lymphocytes. We did not see any eosinophils, which is not unusual for "non-allergic" mice. All three cell types were significantly increased in infected mice (regardless of DEP exposure) at all three time points (macrophages $P < 0{\cdot}002$ in all cases; neutrophils $P < 0{\cdot}005$ in all cases; lymphocytes $P < 0{\cdot}001$ in all cases; Figure 2). DEP exposure had a less severe effect on BAL inflammation, although DEP-exposed mice did have significantly more total BAL cells compared with non-DEP-exposed mice 24 hours and 7 days post-exposure ($P = 0{\cdot}04$ in both cases); there was also some evidence for an effect 6 hours post-exposure ($P = 0{\cdot}06$; Figure 2). The impact of DEP exposure was most evident in the increase in neutrophils seen at all three time points ($P < 0{\cdot}001$ in all cases). The combined effect of DEP exposure and influenza infection was additive in terms of neutrophils, with "Influenza-DEP" mice having significantly more neutrophils than "Influenza-Saline" mice at all three time points ($P < 0{\cdot}01$ in all cases). Importantly, there was a significant interaction between DEP exposure and influenza infection 7 days post-exposure, with "Influenza-DEP" mice having significantly more neutrophils than "Influenza-Saline" mice ($P = 0{\cdot}010$).

Cytokines and protein leak

There were distinct effects of influenza infection on levels of all BAL cytokines (Figure 3). Influenza infection alone resulted in significantly increased IFNγ at all three time

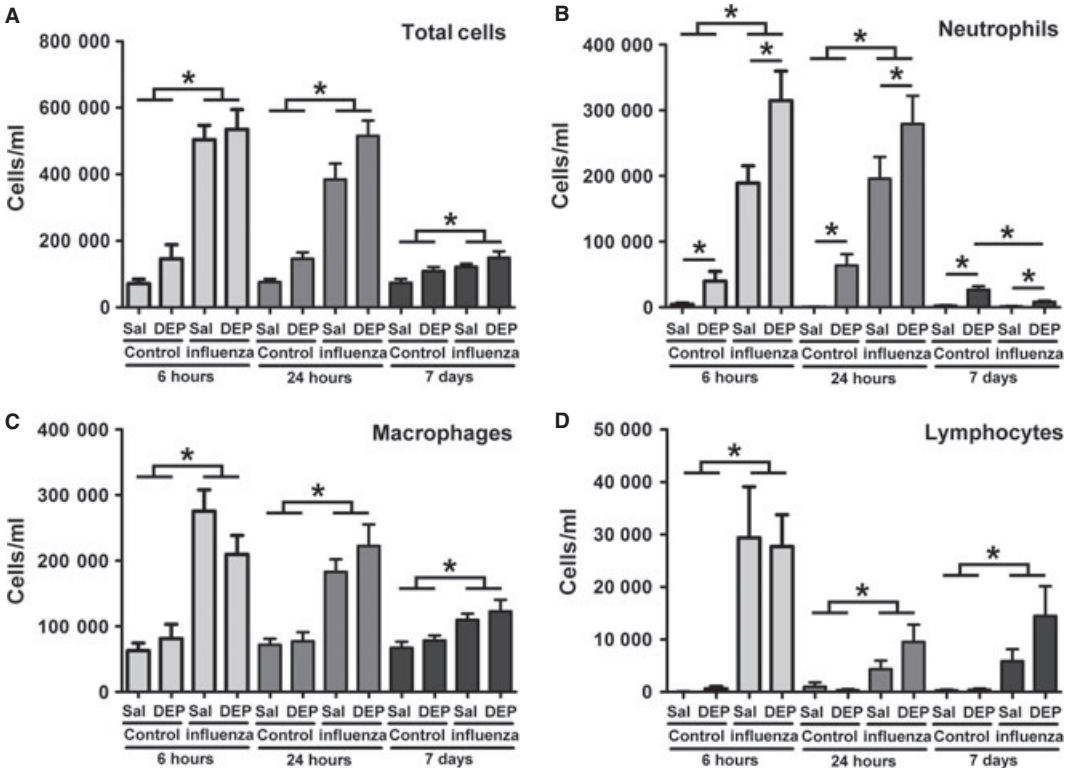

Figure 2. Bronchoalveolar lavage cell counts for total cells (A), neutrophils (B), macrophages (C) and lymphocytes (D) from BALF samples obtained from BALB/c mice infected with influenza (or control) and inoculated with DEP (or saline). Data are mean (SD). *indicates a significant difference between treatments.

points ($P < 0{\cdot}001$ in all cases), however, there was no effect of DEP exposure alone on IFNγ. Influenza infection also resulted in significantly higher levels of TNFα and MCP-1 at the 6-hour time point ($P < 0{\cdot}001$ in both cases), significantly higher IL-6, TNFα, MCP-1 and KC at the 24-hour time point ($P < 0{\cdot}001$ in all cases) and significantly higher KC and protein at 7 days ($P < 0{\cdot}001$ in both cases). DEP exposure alone resulted in increased levels of all cytokines except IFNγ (Figure 3). Six hours post-DEP exposure, ''Control-DEP'' mice had significantly higher levels of IL-6, KC and protein in their BAL compared with ''Control-Saline'' mice ($P < 0{\cdot}001$ in all cases). A similar pattern was seen 24 hours after DEP exposure with ''Control-DEP'' mice having significantly higher levels of IL-6, TNFα, MCP-1 and KC than ''Control-Saline'' mice ($P < 0{\cdot}04$ in all cases). Seven days after exposure, ''Control-DEP'' mice still had higher KC in their BAL compared with ''Control-Saline'' mice ($P < 0{\cdot}001$), but prior DEP exposure did not show any significant effect on the levels of any other cytokine.

There were complex interactions between DEP exposure and influenza infection for all BAL cytokines, except IFNγ. This was most apparent 24 hours after exposure, whereby levels of IL-6, TNFα, MCP-1 and KC in ''Influenza-DEP'' mice were significantly higher than in ''Influenza-Saline'' mice ($P < 0{\cdot}04$ in all cases). This additive effect was still apparent in KC levels 7 days after DEP exposure ($P < 0{\cdot}001$). Six hours post-exposure to DEP, the effect of DEP exposure on influenza-induced IL-6 and protein was sub-additive ($P < 0{\cdot}015$ in both cases). There was no significant effect of DEP on levels of TNFα or MCP-1 at this time point ($P > 0{\cdot}467$ in both cases), nor was there an effect of influenza on KC at this time point ($P = 0{\cdot}707$).

Thoracic gas volume and lung function

Influenza infection significantly increased TGV in mice studied 6 hours post-DEP exposure ($P = 0{\cdot}02$; Figure 4). This difference had resolved 24 hours post-exposure ($P = 0{\cdot}27$) and was not apparent 7 days ($P = 0{\cdot}88$) after DEP exposure. There was no effect of DEP exposure alone on TGV at any time point ($P > 0{\cdot}31$ in all cases), nor was there any additive effect of DEP exposure and influenza infection on TGV at any time point ($P > 0{\cdot}11$ in all cases).

As influenza infection increased TGV in the 6-hour group, we calculated measures of specific lung function (i.e. corrected for TGV). Influenza infection increased specific airway resistance (sR_{aw}), specific tissue damping (sG) and specific tissue elastance (sH) in the 6-hour group (Figure 4). Influenza infection also resulted in significantly higher sR_{aw} and sG 24 hours after DEP exposure

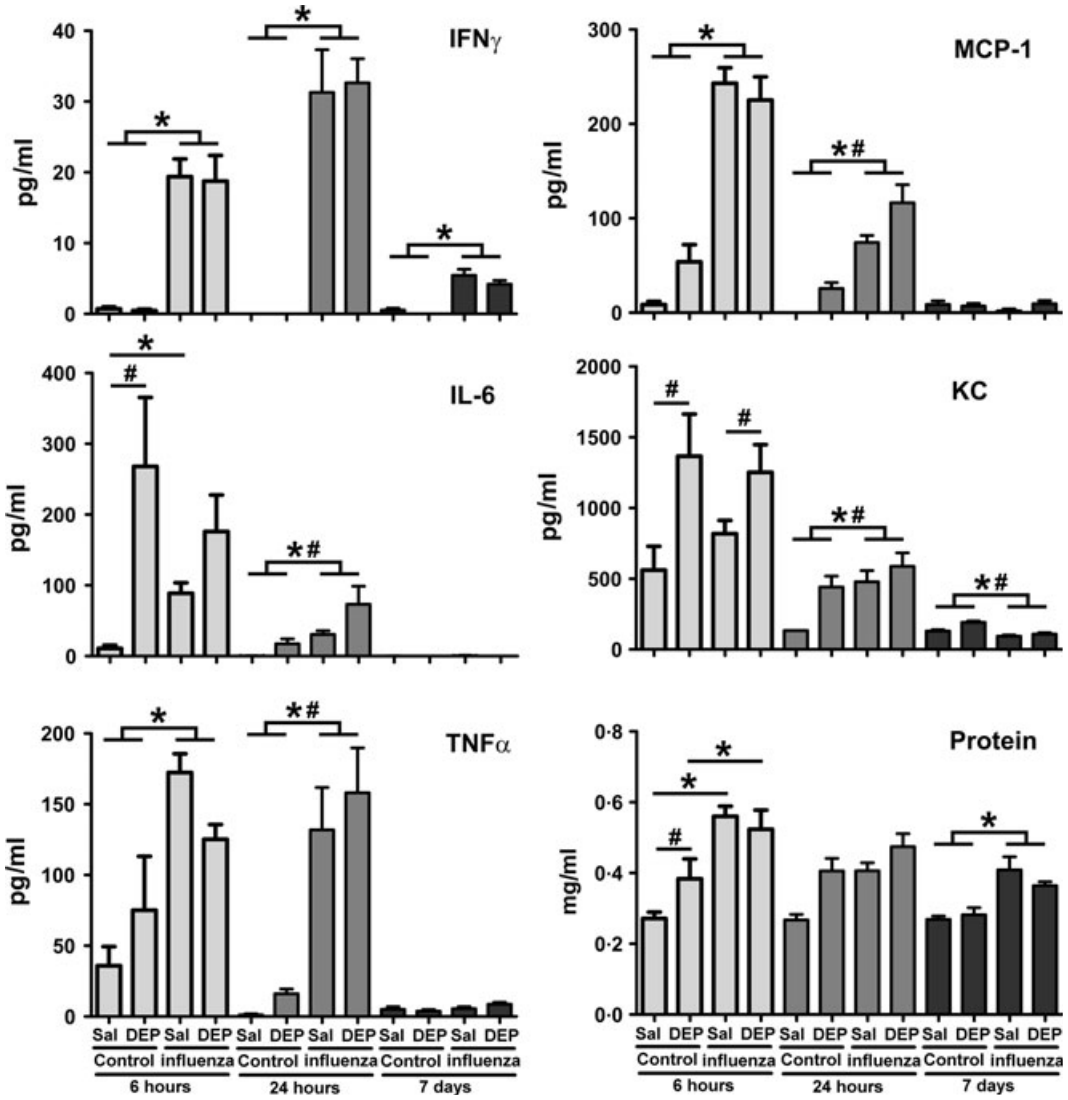

Figure 3. Bronchoalveolar lavage fluid levels of IFNγ, IL-6, TNFα, MCP-1, KC and total protein from adult female BALB/c mice infected with influenza (or control) and inoculated with DEP (or saline). Data are mean (SD). *indicates a significant effect of influenza infection. #indicates a significant effect of DEP.

($P < 0{\cdot}007$ in both cases). There was no effect of influenza infection on any parameter of lung function 7 days post-DEP exposure ($P > 0{\cdot}29$ in all cases).

There was no effect of DEP exposure alone on any parameter of lung function at any time point ($P > 0{\cdot}20$ in all cases), and ''Influenza-DEP'' mice did not have significantly greater impairments in any parameter of lung function compared with ''Influenza-Saline'' mice ($P > 0{\cdot}29$ in all cases; Figure 4).

Discussion

The results of this study show that an acute exposure to DEP during influenza infection can have significant impacts on disease severity including increasing viral load, cellular inflammation and levels of certain inflammatory mediators. Despite this, we found that DEP exposure during infection did not exacerbate influenza-induced changes in lung volume or lung function measured at functional residual capacity.

Importantly, we measured higher viral titres in ''Influenza-DEP'' mice, compared with ''Influenza-Saline'' mice 6 hours after DEP exposure. This is contrary to the only other study in which mice with an existing respiratory viral infection were exposed to PM[19] which showed that exposure to carbon black particles did not alter viral replication in mice previously infected with RSV. As previously mentioned, the inert chemical composition of carbon black particles may explain this discrepancy.[46] Furthermore, RSV may respond differently to co-exposure with carbon black

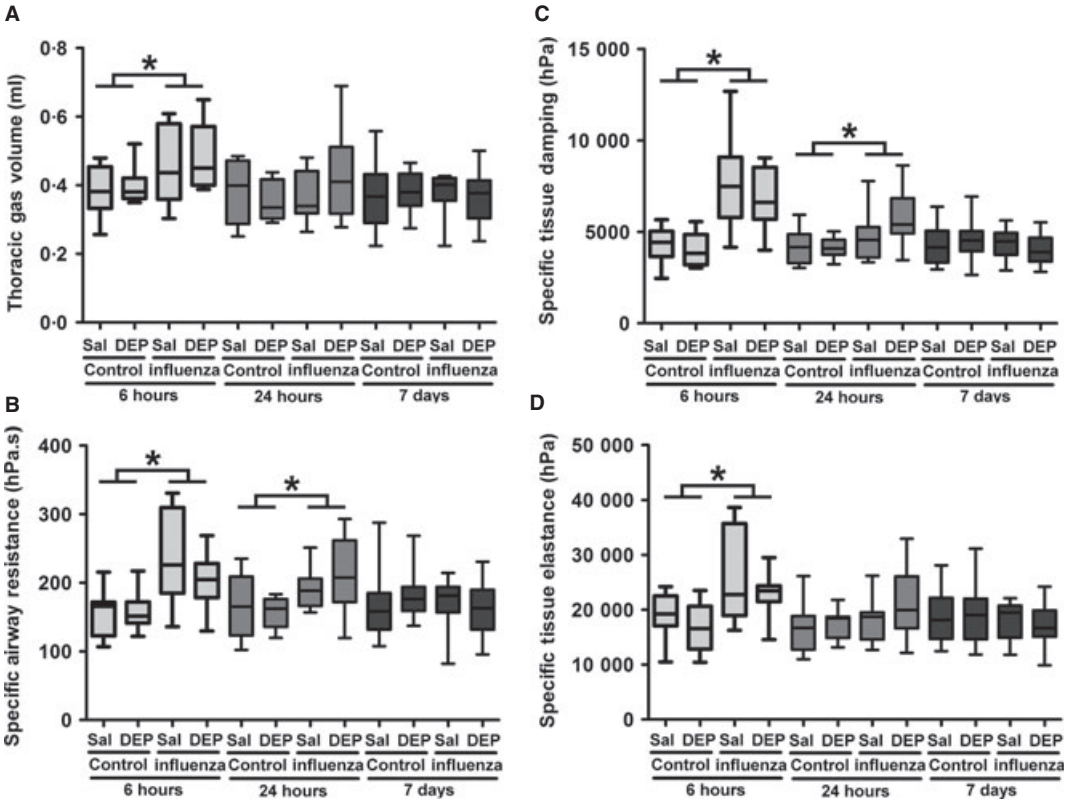

Figure 4. Box and whisker plots of thoracic gas volume (A), specific airway resistance (B), specific tissue damping (C) and specific tissue elastance (D) measured at functional residual capacity of adult female BALB/c mice infected with influenza (or control) and inoculated with DEP (or saline). *indicates a significant difference between treatments.

compared with how influenza interacts with DEP. Without a larger body of literature to draw upon, this is difficult to assess. Similar studies where mice received DE and virus simultaneously[18] or chronic DE before viral infection[14,15] show that DE can enhance influenza replication. Due to the complexity of interacting host responses to respiratory viruses and DE, a myriad of potential mechanisms for this have been postulated.[47] It is well known that long-term exposure to DE/DEP can significantly alter host immune responses to pathogens, however, we were interested in identifying how an acute exposure to DEP could exacerbate existing respiratory viral-induced illness. Potential mechanisms for this include DE exposure reducing mucociliary clearance of pathogens,[48,49] DEP decreasing alveolar macrophage function[50–52] and/or DE reducing production of antiviral defences.[14] Several studies have also shown that exposure to DE increases markers of oxidative stress *in vitro*.[53–55] For example, Jaspers *et al.* (2005)[13] showed that oxidative stress generated by pre-treatment with DE increased susceptibility to influenza infection and viral attachment to respiratory epithelial cells. In mice, blocking DE-induced oxidative stress during influenza infection does not affect viral titres, but does decrease pulmonary inflammation.[18] These results indicate that the role of oxidative stress in DE enhanced respiratory viral infection is still unclear.

In terms of inflammation, our findings partially agree with those of the one previous study which has considered the effects of PM exposure on existing respiratory viral-induced disease.[19] Lambert *et al.* (2003a) found that instilling BALB/c mice with carbon black on day 3 of RSV induced illness resulted in limited exacerbation of cellular inflammation but increased chemokine (RANTES, MIP-1α and MCP-1) expression. They reported a complex pattern in BAL cellular inflammation, whereby 4 days post-infection, mice exposed to carbon black and RSV had significantly *less* neutrophils and macrophages in their BAL, compared with mice exposed to carbon black alone. Mice infected with RSV alone had virtually no BAL neutrophils at this time point. This was despite increased levels of neutrophil chemoattractants in RSV and carbon black exposed mice. We found that influenza infection significantly increased macrophage, neutrophil and lymhocyte numbers in BAL, whereas DEP alone only increased neutrophilia (Figure 2). We also found that DEP significantly increased influenza-induced neutrophilia above that caused by

influenza infection alone. It has previously been shown that excessive neutrophilia is detrimental to mice during influenza infection,[56] although neutrophils are also necessary for viral clearance.[57,58] It was not surprising that DEP-induced exacerbation of neutrophilia was accompanied by similar increases in levels of keratinocyte chemoattractant (KC; a mouse analogue of IL-8) which is involved in chemotaxis and cell activation of neutrophils. In addition, we again showed that a single dose of 100 μg of DEP alone is sufficient to elicit long-term cellular inflammation in BALB/c mice.[39] The significant neutrophilia measured 7 days after instillation of DEP alone is likely due to the relatively gradual processing and removal of elemental carbon by pulmonary inflammatory cells. We have previously shown that DEP remains detectable in the lungs of mice for at least 4 weeks post a single exposure.[39]

Despite enhanced viral load and cellular inflammation, an acute exposure to DEP during influenza infection did not exacerbate physiological impairments in lung function above those already caused by influenza. This may be due to the strain of influenza we used (Influenza A/Memphis/1/71) which causes significant impairments in lung function in mice.[24,25] In this study, DEP exposure did not alter lung function on its own, so any potential further impairments caused by it in conjunction with influenza infection would likely have been eclipsed by the significant physiological impairments due to influenza infection. This finding is supported by the work of Gowdy *et al.* (2010). Our results suggest it would be beneficial for people with an established respiratory viral infection to remain indoors on high-pollution days. This is similar to daily ''air pollution alerts'' announced by various governmental bodies,[59] which are targeted at reducing the exposure of people with chronic lung diseases to air pollution.

Acknowledgements

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