

Tracking retention and transport of ultrafine polystyrene in an asthmatic mouse model using positron emission tomography

Heather A. Enright,¹ Jennifer M. Bratt,² Andrew P. Bluhm,³ Nicholas J. Kenyon,² and Angelique Y. Louie³

¹Department of Chemistry, University of California-Davis, Davis, California, USA

²Division of Pulmonary and Critical Care Medicine, University of California-Davis, Davis, California, USA

³Department of Biomedical Engineering, University of California-Davis, Davis, California, USA

ABSTRACT

Upon exposure to particulates, asthmatic individuals are more susceptible to deleterious health effects and increased morbidity and mortality when compared to healthy individuals. These effects are not limited to the respiratory system; increases in acute cardiovascular events have been observed. The development of extra-pulmonary illnesses has led to interest in determining whether particles move out of the lungs and whether transport of particles differs for asthmatic individuals. Differences in particle deposition and retention in asthmatic versus normal subjects have been explored in the literature using the gamma camera, a two-dimensional imaging technique. Herein we report the deposition and fate of ⁶⁴Cu-labeled 100 nm polystyrene particles in a mouse model of asthma using positron emission tomography (PET). All animals were handled humanely under an approved protocol (UC Davis Institutional Animal Care and Use Committee). Particles were administered by intratracheal instillation and animals were imaged over 48 hours using PET. Biodistribution was determined from images using Regions of Interest (ROI) analysis. After 48 hours, for the asthmatic animals, we observed that ~28% of the initial dose is cleared from the lungs; particle accumulation in small amounts is evident in the GI (gastrointestinal) tract, liver, and bladder. This decrease in lung retention is significantly different when compared to the normal mouse (~11%DD), which showed minimal particle transport out of the lung ($P < 0.001$). This study indicates that ultrafine particles (UFP) undergo enhanced transport out of the lungs in an asthma model. This observed transport may facilitate the adverse peripheral effects associated with particulate exposure.

KEYWORDS biodistribution, imaging, lung, particulate matter, ultrafine particles

INTRODUCTION

Asthma, a chronic airway inflammatory disorder, affects approximately 300 million people worldwide and is a major cause of hospitalizations in children [1]. Asthma is associated with airway infiltration of inflammatory cells (mast cells, eosinophils, basophils, macrophages, lymphocytes) [2] and airway hyper-

responsiveness that results in airway narrowing and obstruction [3]. Epidemiological studies have linked particulate air pollution to increased morbidity and mortality in compromised individuals including asthmatics [4]. Other studies have shown increased hospital admissions for asthmatic individuals upon acute exposure to particulates [5–7]. Asthmatic individuals exposed to increased levels of particulate air pollution have shown a variety of physiological responses including decreases in peak expiratory flow (PEF) [8], forced expiratory volume (FEV₁), and forced vital capacity (FVC) [9], an increase in use of medications [8], and an increase in respiratory symptoms, such as coughing and shortness of breath [10].

Furthermore, exposure to particulates has been observed to induce extrapulmonary effects

Received 11 April 2013; accepted 20 June 2013

Imaging work was performed at the Center for Molecular and Genomic Imaging, University of California, Davis. We would like to acknowledge Jennifer Fung for her assistance with the animal imaging.

Address correspondence to Angelique Y. Louie, Department of Biomedical Engineering, One Shields Avenue, University of California, Davis, CA 95616, USA. E-mail: aylouie@ucdavis.edu

particularly in the cardiovascular system, such as decreases in heart rate variability and arterial O₂ saturation in both healthy individuals and asthmatics [11, 12]. But, there is no clear explanation on how particles can elicit these effects. Do particles transport out of the lungs and accumulate in secondary organs, or are nanoparticles retained in the lung where they cause local inflammation that generates a systemic response? To identify the underlying mechanisms for the health effects of particulate matter, many studies in the literature have focused on determining if particles can transport from the lung and, if transport occurs, where particles accumulate.

Studies in the literature have examined the retention and translocation of UFP in healthy humans and animals [13–16]; however, only a few studies to date have investigated whether lung retention and clearance differs for asthmatic individuals. Of particulate matter (PM) size fractions, ultrafine particles (<100 nm) are shown to deposit more efficiently in the respiratory tract in asthmatics compared to healthy subjects [17, 18]. In addition, compared to larger particles, UFP have been shown to have increased toxicity on an equal mass basis [19, 20].

Only a few studies to date have looked at UFP lung retention in asthmatics. In 2006, Wiebert *et al.* investigated if ^{99m}Tc-labeled carbon particles (35 nm, 100 nm) could translocate from the lungs in both healthy and asthmatic individuals using the gamma camera over a 24- and 46-hour time period, respectively. For both of these studies, no significant change in lung retention was observed [21, 22]. In addition, no particle accumulation in secondary organs was observed indicating that the particles used did not enter circulation. The lack of observed translocation may be due to the delayed onset of symptoms after exposure, which will be addressed further in the Discussion, and also due to the relatively low resolution of the gamma camera, which can result in signal disappearing when averaged over large voxel sizes [23]. The trends of transport from the lungs for small populations of particles may be difficult to resolve in humans using the gamma camera. To date, there have been no extensive studies of nanoparticle transport from the lungs in asthmatic animal models, although there have been a number of studies in normal rodents.

In this work, we evaluate if lung retention and biodistribution of ultrafine sized particles in a mouse model of asthma differ from their normal counterparts. In our previous work, polystyrene nanoparticles (~100 nm) were instilled in the normal mouse and the biodistribution and lung retention were evaluated with positron emission tomography (PET) [24]. No significant change in lung retention

of nanoparticles was observed for the normal mice; nanoparticles were found primarily in the gastrointestinal tract with very small amounts in the liver and bladder. The increased inflammatory activity in a mouse model of asthma, however, may facilitate clearance and transport from the lung as endocytosis by immune cells is a common clearance mechanism in both the tracheobronchial and pulmonary regions of the lungs [25]. We have used an ovalbumin sensitization and exposure mouse model of allergic airway inflammation to determine the lung retention and biodistribution of polystyrene nanoparticles (~100 nm) and compared results to a normal mouse control group. The ovalbumin mouse model is well established in the literature and is commonly used to study asthma [17, 26, 27]. This model has proven to exhibit key features of asthma, including airway hyperresponsiveness, eosinophilic bronchitis, acute inflammation, increased airway reactivity, and mild airway obstruction [26]. Polystyrene nanoparticles were used as model particles similar in size to UFP. The nanoparticles were tagged with ⁶⁴Cu (half-life = 12.7 hours, β⁺ 17.8%) for detection by PET.

MATERIALS AND METHODS

Animal Care

All animals were handled humanely under a protocol approved by the UC Davis Institutional Animal Care and Use Committee (approved protocol #13449). Anesthesia was administered for imaging and all other animal procedures. Animals were monitored for discomfort and pain regularly during all animal procedures.

Nanoparticle Labeling

⁶⁴Cu-labeled polystyrene nanoparticles (~100 nm) were prepared as previously reported [24]. Briefly, ⁶⁴CuCl₂ (74 MBq, 0.6 nmol) was buffered with 0.1M triethylammonium acetate (TEAA) buffer (pH 8, 30 μL) and added to *p*-SCN-Bn-DOTA (S-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, Macrocyclics, Dallas, TX, 0.14 μmol). The solution was then incubated at 41°C for 20 minutes. Amine functionalized polystyrene nanoparticles (2% w/v, Molecular Probes, Invitrogen, Eugene, OR, 800 μL) were then added to the reaction mixture and were incubated at 53°C for 2 hours. Nanoparticles were then centrifuged at 14,000 rpm for 15 minutes, the supernatant was removed, and the nanoparticles were resuspended in saline (600 μL). The purification was repeated two additional times and purity evaluated

by radio thin layer chromatography. Purified particles were sized by Transmission Electron Microscopy (TEM, Phillips CM-120) operating at 80 keV after labeling, after decay of radiation to background level. Nanoparticle size was determined with Image J, a Java-based image-processing program. In addition to size, after decay, zeta potential was measured with a NICOMP 380 ZLS (Particle Sizing System, Goleta, CA). Radiolabeling efficiency (RE) was determined with the following expression:

$$\text{RE} = 100 \times \frac{\text{activity before purification (MBq)}}{\text{Activity after purification (MBq)}}$$

The purified nanoparticles were analyzed with radio-thin layer chromatography (TLC) to determine purity. An aliquot of the labeled nanoparticles was first incubated with 10 mM EDTA (ethylenediaminetetraacetic acid) for 20 minutes to chelate any unbound copper and then spotted onto a silica gel plate with ^{64}Cu -EDTA as a negative control. The plate was then run with 50% methanol/50% ammonium acetate (10% w/v) as the eluent. This eluent was chosen because it gave the optimal separation of the labeled particles and ^{64}Cu -EDTA. EDTA was used for this analysis because when chelated with copper, the complex moves with the solvent front, unlike the labeled particles, which remain at the baseline.

Particle stability after labeling was reported in previous work [24]; nanoparticles were incubated in saline, saline at pH 2, and fetal bovine serum for 48 hours. No significant loss of activity was observed for the nanoparticles, indicating no loss of label over time.

Animals, Sensitization and Particle Administration

Male (14 weeks, BALB/c) mice were used in the following experiments. Animals were purchased from Charles River Laboratories (Wilmington, MA) and were maintained in a temperature controlled (22°C) room in ventilated cages on a 12 hour day/night cycle prior to studies. Animal diet and water were provided ad libitum. Allergic asthma sensitization was performed as previously reported in work by Kenyon and colleagues [26, 27]. Briefly, mice (6–8 weeks of age) were sensitized via intraperitoneal injection (i.p.) of ovalbumin (OVA) (10 $\mu\text{g}/0.1$ ml chicken egg albumin, grade V, 98% pure, Sigma, St. Louis, MO) on days 0 and 14. On the 28th day after the first i.p. exposure, mice were exposed to aerosolized OVA (10 mL, 10 mg OVA/ml in PBS) for 30 minutes per day for

six days over a two-week period. Untreated BALB/c mice were housed in parallel as control.

For imaging, mice were anesthetized with inhaled isoflurane (2–3%) and labeled nanoparticles were delivered using a Hamilton (Reno, NV) flat-tipped syringe (40 μL) intratracheally at a concentration of 1.2×10^{14} nanoparticles/mL within 24 hours after the last OVA aerosol challenge ($n = 7$). The instilled dose delivered to each animal was determined by measuring the amount of radioactivity in the delivery syringe before and after the instillation with a dose calibrator (Fluke 34–162 CAL/RAD MARK IV, Cleveland, OH); the difference was used as the initial, or instilled dose (ID). Nanoparticle accumulation data from all later time points were referenced against this ID. Deposited dose (DD) was also determined by referencing the amount deposited in the lungs at time zero with the initial delivered dose. After instillation, anesthesia was maintained with isoflurane (2% maintenance with 100% O_2). Animals were positioned in the PET scanner feet first in supine position for full body PET scanning. Untreated BALB/c mice ($n = 4$) were imaged for comparison as control.

PET Imaging

PET scans were performed on a microPET-Focus 120 (Siemens Medical Solutions, Malvern, PA). The Focus 120 has a transaxial field of view of 10 cm and an axial field of view of 7.6 cm with a spatial resolution of 1.3 mm. The circular detector array consists of 96 detector blocks with a face-to-face diameter of 15 cm [28]. The energy window was 350–650 keV, and arc, decay and dead time corrections were applied. No corrections for signal attenuation were made. Static scans were taken up to 48 hours using ^{64}Cu as the radioisotope (12.7 hour half life). Scan durations were 15 minutes for the zero, 2, 4, and 12 hour time points, 30 minutes for the 24-hour time point and 1 hour for the 48-hour time point. All images were reconstructed using a maximum a posteriori algorithm (MAP).

Analysis of PET Images

Regions of interest (ROI) were obtained by the ASIPro Virtual Machine software to quantify tissue activity. All values (total activity) were decay corrected to the zero hour time point for comparison. The percent of instilled dose (%ID) was determined by dividing the corrected ROI value for the organ by the instilled dose and multiplying by 100. Deposited dose (DD) was calculated by dividing the total activity in the lungs at the initial time point (zero) by the instilled dose (ID). Lung retention is given by

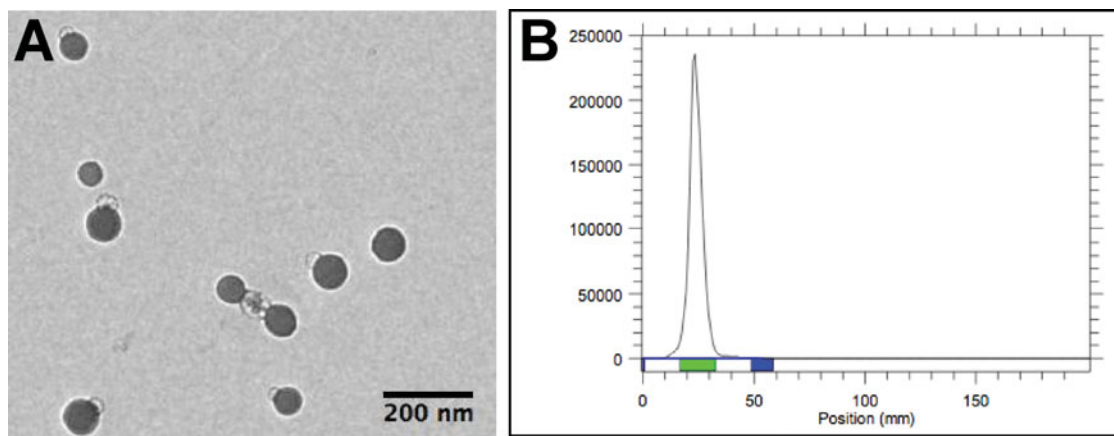


FIGURE 1. Characterization of nanoparticles after labeling with ^{64}Cu . Size determined by TEM is shown in panel A. Radiopurity, verified by radioTLC, is shown in panel B.

the activity remaining at 48 hour relative to DD. Statistical analysis was performed on all lung retention and biodistribution values using a least squares mean t-test. *P*-values are provided in parenthesis when statistical significances were found between data sets.

RESULTS

Nanoparticle Labeling and Characterization

The size of the nanoparticles after labeling was 103 ± 10.9 nm as measured by transmission electron microscopy (TEM); nanoparticles were monodisperse as shown in Figure 1 panel A. Zeta potential was -21.8 ± 1.8 mV; which indicated that the nanoparti-

cles that were stable in solution (nonaggregated) and anionic in charge. After labeling, nanoparticles were 100% pure as determined by radioTLC shown in Figure 1 panel B, only one peak was detected at the baseline. The degree of labeling determined after labeling was 62%.

Biodistribution of Nanoparticles Using PET

Projection images in the ventral-dorsal orientation illustrating the biodistribution of the labeled nanoparticles in the asthmatic mouse model over 48 hours is shown for a representative animal in Figure 2 (0, 4, 12, 24, 48 hours). Note, in the PET images the intensity scale was adjusted with each time point to allow visualization of signal in peripheral organs; thus

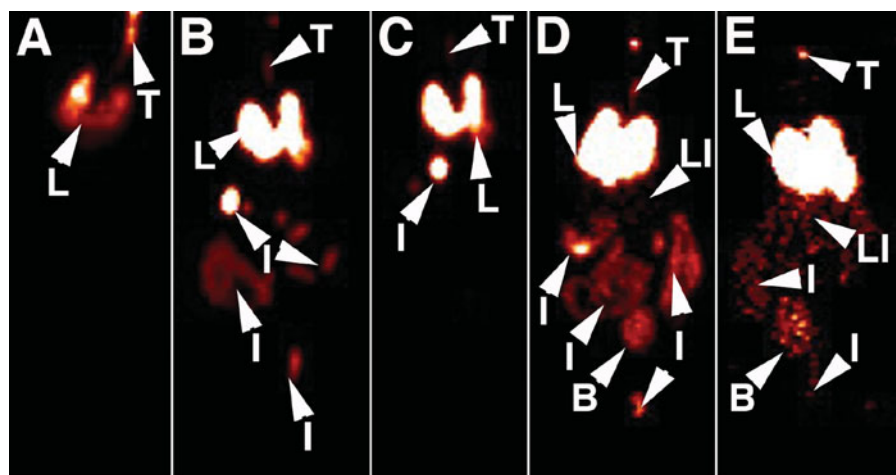


FIGURE 2. Projection images showing the biodistribution of 100 nm labeled polystyrene nanoparticles after instillation in the asthmatic mouse. Biodistribution is shown at (A) 0 hr (B) 4 hr (C) 12 hr (D) 24 hr (E) 48 hr. B = bladder, L = lungs, LI = liver, I = intestines, and T = trachea.

TABLE 1. Quantitative Analysis of Images from Asthmatic Group (Average $\pm$ SD)

Asthmatic group	0 h	4 h	12 h	24 h	48 h
Lung	78.4 $\pm$ 7.4	77.8 $\pm$ 8.4	78.7 $\pm$ 10.2	65.8 $\pm$ 6.0	56.4 $\pm$ 6.1
Trachea	6.1 $\pm$ 3.4	2.6 $\pm$ 4.2	1.3 $\pm$ 2.4	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0
Intestines	0.0 $\pm$ 0.0	7.1 $\pm$ 4.3	2.7 $\pm$ 2.4	4.8 $\pm$ 5.9	1.3 $\pm$ 1.3
Bladder	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.3 $\pm$ 0.3	1.2 $\pm$ 1.0	0.5 $\pm$ 0.7
Liver	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2	0.5 $\pm$ 0.1	0.8 $\pm$ 0.4

All values in % Instilled Dose (ID), Average $\pm$ SD.

the normal loss of signal due to decay is not evident in these figures. Initial deposition of the particles to the lungs (L) is verified in Figure 2A with signal localized to the trachea (T) and lungs. Over time, the signal began to disperse to secondary organs, including the gastrointestinal tract (GI), the liver (LI) and bladder (B). At 4 hours postinstillation (Fig 2B) movement of nanoparticles through the GI tract was evident and continued through the 48-hour time point (Fig 2E). At 24 hours (Fig 2D) an increase in organ involvement was observed with signal appearing in the bladder and liver that persisted to the 48-hour time point. In Figure 3, projection images in the ventral-dorsal orientation for a representative control mouse are shown (0, 4 12, 24, 48 hours). Upon instillation, nanoparticles are localized in the lungs and trachea as shown in Figure 3A. Over time, signal is shown to distribute to the intestines (I) as in Figure 3B-3E. Particles are also observed in the liver (LI) at 24- and 48-hour time (Figure 3D and 3E). There appears to be greater flux of particles to the GI for the asthmatic animal, this trend is made clearer in the quantitative analysis of the images.

Quantitative analysis of the image data from all animals in the data sets using region of interest (ROI)

analysis is shown in Figure 4. Figure 4A and 4B present the % instilled dose (ID) of each organ over time in the control group and in Figure 4C and 4D the biodistribution for the asthmatic group. Only organs containing observable signal in the PET images are presented in the plots. Superscripted symbols indicate values that are significantly different than the previous timepoint ($\# = P < 0.05$; $* = P < 0.001$; $** = P < 0.01$). Tables 1 and 2 summarize the values shown in Figure 4. The mean dose deposited into the lungs of the asthmatic group was 78.37% ID $\pm$ 7.38 ($n = 7$) and was 90.0% ID $\pm$ 11.8 for the control animals ($n = 4$). Over time the signal in the lungs of asthmatic animals decreases 28.1% falling to 56.4% ID by the 48-hour timepoint, while decreasing only 10.8% for the control group in the same time period. Signal in the trachea in both groups similarly decreased to zero over the same 48 hours. While both groups showed some evidence of particle transport to the GI system, with signal appearing in the intestines by 4 hours, the signal in liver and bladder persists over time in the asthmatic animals, showing an apparent trend for increasing accumulation in the liver. The enlarged view plots for Figure 4 show statistical comparisons for mean signal

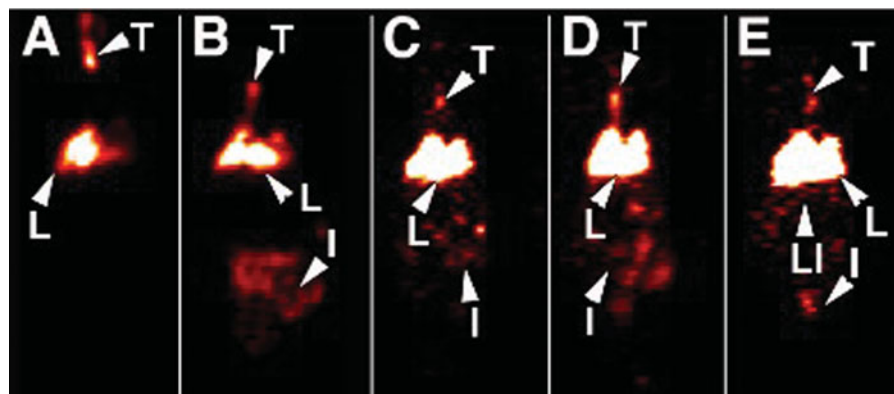


FIGURE 3. Biodistribution of 100 nm polystyrene nanoparticles in control mice (projection images). (A) 0 hr (B) 4 hr (C) 12 hr (D) 24 hr (E) 48 hr. In the images, B = bladder, L = lungs, LI = liver, I = intestines, and T = trachea.

TABLE 2. Quantitative Analysis of Images from Control Group (Average $\pm$ SD)

Control group	0 h	4 h	12 h	24 h	48 h
Lung	90.0 $\pm$ 11.8	89.7 $\pm$ 11.5	87.4 $\pm$ 10.1	86.3 $\pm$ 13.9	80.3 $\pm$ 12.4
Trachea	5.2 $\pm$ 4.9	1.3 $\pm$ 0.7	0.7 $\pm$ 0.6	0.7 $\pm$ 0.3	0.1 $\pm$ 0.1
Intestines	0.0 $\pm$ 0.0	6.6 $\pm$ 6.7	2.0 $\pm$ 0.7	2.2 $\pm$ 1.3	0.7 $\pm$ 0.9
Bladder	0.3 $\pm$ 0.1	0.4 $\pm$ 0.7	0.1 $\pm$ 0.1	0.3 $\pm$ 0.4	0.1 $\pm$ 0.1
Liver	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1	0.2 $\pm$ 0.3

All values in% Instilled Dose (ID), Average $\pm$ SD.

values in the liver and bladder between the asthmatic and control group animals. In the liver, the signal levels in the asthmatic animals are significantly higher than those in the corresponding control animals for the 24 and 48-hour timepoints ($\# = P < 0.05$; $** = P < 0.01$).

The trend for clearance from the lung is shown in greater detail in Figure 5, which gives a comparison of lung retention over time for the asthmatic and control groups. The values are normalized here and presented as percent of deposited dose (each mean value presented against what was de-

posited to the lungs at time zero). Over time, a statistically significant decrease in lung retention was observed at time 24 and 48 hours for the asthmatic group when compared to the normal control group. At 24 hours 84.1%DD remained in the asthmatic lungs compared to 95.7%DD for the control group ($*p < 0.05$). A more significant difference was found for the final time point; at 48 hours, 72.1%DD was retained in the lungs of the asthmatic animals compared to 89.1%DD for the control ($**P < 0.001$). These data are also summarized in Table 3.

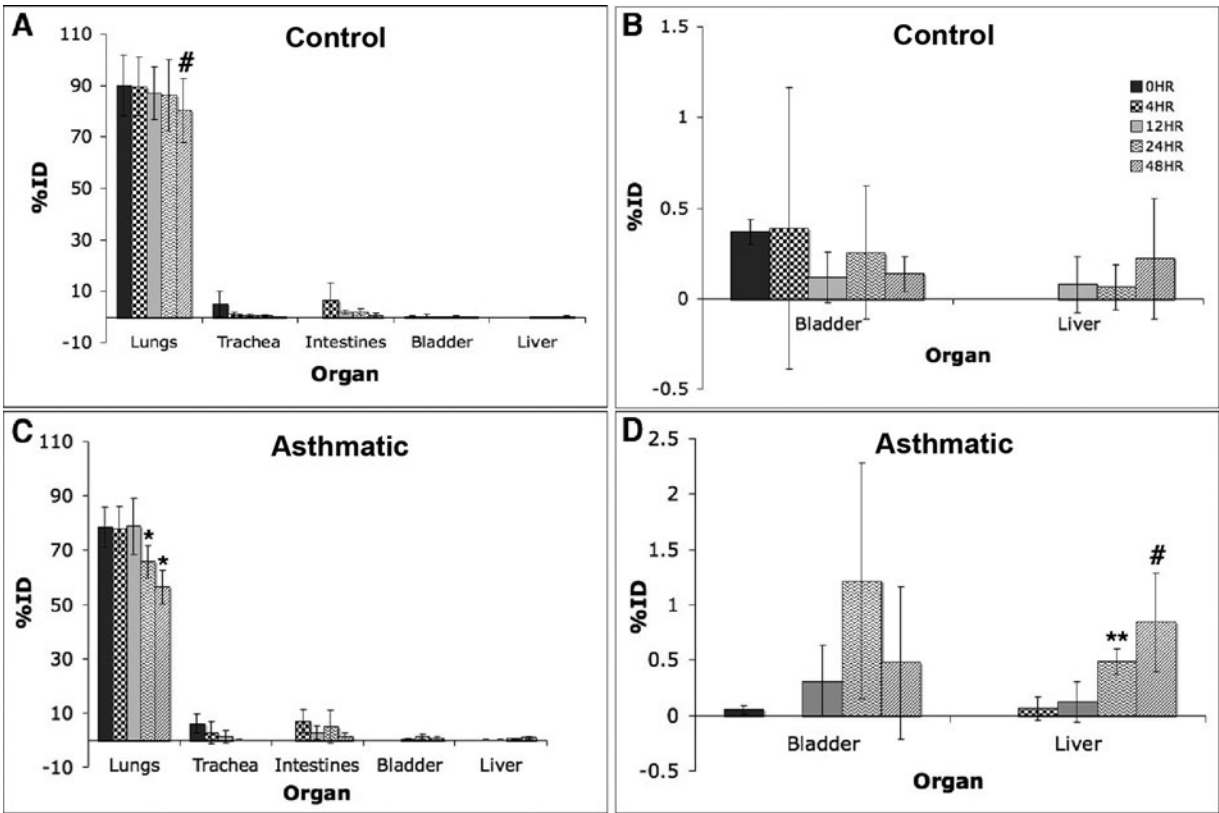


FIGURE 4. Region of Interest (ROI) analysis of labeled nanoparticles over 48 hours in control (A,B) ($n = 4$) and asthmatic (C,D) mice ($n = 7$). Percent instilled dose (%ID) for all organs with signal are shown in A and C. In B and D, bladder and liver are plotted separately to better visualize signal (%ID). $\#P < 0.05$, $*P < 0.001$, $**P < 0.01$.

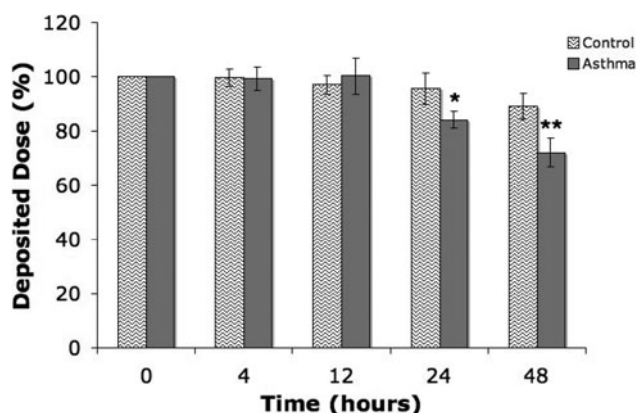


FIGURE 5. Lung retention comparison for control and asthmatic mice over 48 hours. Retention is expressed as percentage of deposited dose (%DD). * $P < 0.05$, ** $P < 0.001$.

DISCUSSION

Comparison of Asthmatic Versus Healthy Transport

In the present study, we evaluated the lung retention and biodistribution of 100 nm polystyrene nanoparticles *in vivo* using PET imaging in an asthmatic mouse model over a 48-hour period and compared these results to a normal control group. Our results suggest that in the asthmatic model, there was a greater loss of nanoparticles from the lungs and concomitant accumulation was observed in peripheral organs. After 48 hours we observed ~28% DD ($P < 0.001$) cleared out of the lungs for asthmatic mice, compared to ~11%DD for controls, with statistically significant particle accumulation found in the liver of asthmatic mice. This accumulation was indicative of blood transport, as there is not another direct pathway from the lung to the liver; the liver [29] could be accumulating signal by filtering circulating nanoparticles from the bloodstream. The trends in the data show particle loss from the lung first observed at 24 hours.

The observed change in lung retention could be due to the physiological and cellular changes present in the asthmatic animal model. The asthmatic animal model has been shown to contain increased levels of inflammatory cells including macrophages, lymphocytes, mast cells, eosinophils, and neutrophils [30].

Macrophages are known to be key players in clearing particles and pathogens from the lungs [25, 31]. Amplified macrophage activity may lead to increased clearance of nanoparticles from the lungs by mucociliary clearance [32] and/or transport to the bloodstream via the lymphatic system [33]. Macrophages have been shown to ingest and transport particles out of the bronchial tree for subsequent swallowing and clearance through the GI tract [32, 34]. While there are conflicting reports on whether macrophages transport into lymphatic vessels [31], some reports have shown that macrophages appear in the lymph nodes as soon as 1–3 days post instillation [35]. Therefore, particle clearance by macrophages into the bloodstream via the lymphatic system may be possible within our observation period of 48 hours. In addition to variable levels of inflammatory cells, the initial deposition of nanoparticles for the asthmatic animals could have been different from the control group. As discussed earlier, asthma is associated with airway narrowing [3]; therefore, deposition into the alveoli could have occurred to a lesser extent for the asthmatic animals. Airway secretions are also elevated in the lungs for the asthmatic model, which may have influenced clearance [36].

Although, to date, there have been no other literature reports studying ultrafine particle transport in an asthmatic mouse model, a few human studies have been reported and have observed minimal transport of ultrafine particles out of the lungs. As mentioned earlier, in 2006 Wiebert et al. studied ^{99m}Tc -labeled carbon nanoparticles in humans (100 nm) using a gamma camera and concluded that there was minimal translocation (99% remaining at 46 hours) in both healthy and asthmatic individuals [22]. Wiebert and colleagues also conducted a similar study with 35 nm carbon particles and observed no significant change in lung retention; at 24 hours 102% $\pm$ 4.7 of the initial dose remained in the lungs [21]. Beside species, differences between these studies conducted in humans and the work presented here could be due to variations in study parameters including particle characteristics (composition, surface coating), study model and the chosen detection method. Asthmatic individuals enrolled in the studies by Wiebert et al. had milder cases of asthma, at least in regards to their lung function [22] or their lack of evident

TABLE 3. Normalized Data for Signal in Lungs Over Time in Asthmatic and Control Groups (Average $\pm$ SD)

	0 h	4 h	12 h	24 h	48 h
Lung Asthmatic	100	99.2 $\pm$ 4.4	100.2 $\pm$ 6.7	84.1 $\pm$ 3.2	72.1 $\pm$ 5.3
Lung Control	100	99.7 $\pm$ 3.4	97.0 $\pm$ 3.4	95.7 $\pm$ 5.9	89.1 $\pm$ 4.7

All values in % Deposited Dose (DD), Average $\pm$ SD.

asthma symptoms or airway hyperreactivity [21]. The asthmatic mouse model used for the work presented here exhibited key features representative of moderate asthma including both hyperresponsiveness and eosinophilic bronchitis. Increased inflammatory activity was present in this model and may have contributed to the change in lung retention and distribution observed. However, it is important to note that there are several anatomical differences between human and mouse lungs. Mouse lungs exhibit a relatively larger airway diameter, an increased airway to lung parenchyma ratio and a single lobe for the left lung [37]. These anatomical differences appear to result in decreased resistance to airflow in mice as a percentage of total lung resistance when compared to humans. Based on these differences, it is often discussed that the OVA mouse model is not a true model of asthma. This is correct; the OVA mouse model is a model of allergic airway inflammation and airway hyperresponsiveness. Therefore, as with any study that has utilized an animal model to understand disease, interpretation of the underlying mechanisms is preliminary until confirmed in humans. In addition, the gamma camera was used in the studies by Wiebert *et al.*; this imaging modality has lower sensitivity compared to PET (<1% vs. 2–10%, respectively) [24], limiting the level of detection possible in the human studies [38, 39].

For this work, nanoparticles were administered via intratracheal instillation, a liquid bolus that is introduced into the respiratory system. This technique provides rapid and accurate delivery of a known amount of test material to the respiratory tract without contamination to the fur and/or nasal passages that is commonly a problem in whole body or nose-only inhalation systems [40, 41]. Instillation is also well suited for delivering radioactive or highly toxic materials; it allows the delivery in a very small volume and does not require decontamination of large pieces of equipment [40]. Although instillation is a rapid and accurate delivery method, it also has some disadvantages. Instillation does not distribute as evenly as an inhalation technique and can often result in an inhomogeneous lung distribution [42, 43]. Despite these drawbacks, instillation is an accepted delivery method for PM studies in the literature and has been widely used in the field to study the effects of PM in the lung and to study particle transport [40, 44] [45–48].

Nanoparticle Stability

A common concern when utilizing radiolabeled particles is whether or not the label dissociates from the particle *in vivo*. DOTA, the chelator used for these studies, exhibits a high stability constant (22.25, pH

7.4) for capture of ^{64}Cu [49]. The stability of metal complexes is measured by log K values, where K is the equilibrium constant between the ligand and the complexed metal [50]. In addition, we have shown in previous work for particles prepared by the same synthesis that over 48 hours of observation the nanoparticles remain stable in environmental conditions similar to those that can be encountered *in vivo*. These incubation conditions included acidic pH such as found in environments of the stomach and lysosomal compartments in cells, and exposure to plasma, which is encountered through blood transport [24]. Therefore, we are confident that the loss of signal from the lungs observed here is not due to loss of label. Furthermore, if the observed change in lung signal were due to a simple loss of radiolabel, the results should have been kinetically similar for asthmatic and control groups, which was not observed here.

CONCLUSIONS

In summary, the observed particle biodistribution for the asthmatic model differed significantly when compared to the normal mouse, indicating that UFP transport out of the lungs may occur more readily in a diseased model. This enhanced particle transport is consistent with the hypothesis that the increased morbidity and mortality due to cardiovascular effects that are observed after acute and chronic particle exposure is related to particle transport out of the lungs, and that asthmatic individuals may be more susceptible. Compromised individuals, including asthmatics have been shown to be at higher risk of death and illness, which include health end points due to both cardiovascular and respiratory causes. The observed changes in lung retention and particle accumulation in the present work help support this risk assessment, that asthmatic individuals are more susceptible to these deleterious effects after particle exposure, potentially due to enhanced particle transport out of the lungs and subsequent accumulation in peripheral organs.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- [1] Braman SS: The global burden of asthma. *Chest*. 2006;130(1):4S–12S.
- [2] Hamid Q, Tulic M, Liu M, Moqbel R: Inflammatory cells in asthma: mechanisms and implications for therapy. *J Allergy Clin Immunol*. 2003;111:S5–S17.

- [3] (WHO) WHO: Prevention of allergy and allergic asthma. World Health Organization. 2002;1–14.
- [4] Pope C. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk? *Environ Health Perspect*. 2000;108:713–723.
- [5] Atkinson RW, Anderson HR, Sunyer J, Ayres J, Baccini M, Vonk JM, Boumghar A, Forastiere F, Forsberg B, Touloumi G, Schwartz J, Katsouyanni K: Acute effects of particulate air pollution on respiratory admissions - results from APHEA 2 project. *Am J Respir Crit Care Med*. 2001;164(10):1860–1866.
- [6] Burnett RT, Dales R, Krewski D, Vincent R, Dann T, Brook JR: Associations between ambient particulate sulfate and admissions to Ontario hospitals for cardiac and respiratory diseases. *Am J Epidemiol*. 1995;142(1):15–22.
- [7] Lin M, Chen Y, Burnett RT, Villeneuve PJ, Krewski D: The influence of ambient coarse particulate matter on asthma hospitalization in children: Case-crossover and time-series analyses. *Environ Health Perspect* 2002;110(6):575–581.
- [8] Peters A, Dockery D, Heinrich J, Wichmann H: Short-term effects of particulate air pollution on respiratory morbidity in asthmatic children. *Eur Respir J*. 1997;10(4):872–879.
- [9] Koenig JQ, Larson TV, Hanley QS, Rebolledo V, Dumler K, Checkoway H, Wang SZ, Lin DY, Pierson WE: Pulmonary-function changes in children associated with fine particulate matter. *Environ Res*. 1993;63(1):26–38.
- [10] Pope CA, III, Bates DV, Raizenne ME: Health Effects of Particulate Air Pollution: Time for Reassessment? *Environ Health Perspect*. 1995;103(5):472–480.
- [11] Gong H, Linn WS, Clark KW, Anderson KR, Sioutas C, Alexis NE, Cascio WE, Devlin RB: Exposures of healthy and asthmatic volunteers to concentrated ambient ultrafine particles in Los Angeles. *Inhal Toxicol*. 2008;20(6):533–545.
- [12] Gong H, Linn WS, Terrell SL, Clark KW, Geller MD, Anderson KR, Cascio WE, Sioutas C: Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles. *Inhal Toxicol*. 2004;16(6–7):335–343.
- [13] Brown JS, Zeman KL, Bennett WD: Ultrafine particle deposition and clearance in the healthy and obstructed lung. *Am J Respir Crit Care Med*. 2002;166(9):1240–1247.
- [14] Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdorster G, Ziesenis A: Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health A*. 2002;65(20):1513–1530.
- [15] Nemmar A, Hoet PHM, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B: Passage of inhaled particles into the blood circulation in humans. *Circulation*. 2002;105(4):411–414.
- [16] Semmler M, Seitz J, Erbe F, Mayer P, Heyder J, Oberdorster G, Kreyling WG: Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhal Toxicol* 2004 Jun;16(6–7):453–459.
- [17] Alessandrini F, Semmler-Behnke M, Jakob T, Schulz H, Behrendt H, Kreyling W: Total and regional deposition of ultrafine particles in a mouse model of allergic inflammation of the lung. *Inhal Toxicol*. 2008;20(6):585–593.
- [18] Chalupa D, Morrow P, Oberdorster G, Utell M, Frampton M: Ultrafine particle deposition in subjects with asthma. *Environ Health Perspect*. 2004;112:879–882.
- [19] Oberdorster G, Ferin J, Finkelstein G, Wade P, Corson N: Increased Pulmonary Toxicity of Ultrafine Particles .2. Lung Lavage Studies. *J Aerosol Sci*. 1990;21(3):384–387.
- [20] Oberdorster G, Ferin J, Soderholm S, Gelein R, Cox C, Baggs R, Morrow PE: Increased pulmonary toxicity of inhaled ultrafine particles: due to lung overload alone? *Ann Occup Hyg*. 1994;38(inhaled_particles_VII):295–302.
- [21] Wiebert P, Sanchez-Crespo A, Falk R, Philipson K, Lundin A, Larsson S, Moller W, Kreyling WG, Svartengren M: No significant translocation of inhaled 35-nm carbon particles to the circulation in humans. *Inhal Toxicol* 2006;18(10):741–747.
- [22] Wiebert P, Sanchez-Crespo A, Seitz J, Falk R, Philipson K, Kreyling WG, Moller W, Sommerer K, Larsson S, Svartengren M: Negligible clearance of ultrafine particles retained in healthy and affected human lungs. *Eur Respir J*. 2006;28(2):286–290.
- [23] Petersson J, Sanchez-Crespo A, Larsson SA, Mure M: Physiological imaging of the lung: single-photon-emission computed tomography (SPECT). *J Appl Physiol*. 2007;102(1):468–476.
- [24] Palko HA, Fung JY, Louie AY: Positron emission tomography: a novel technique for investigating the biodistribution and transport of nanoparticles. *Inhal Toxicol*. 2010;22(8):657–688.
- [25] Oberdorster G: Lung clearance of inhaled insoluble and soluble particles. *J Aerosol Med*. 1988;1(4):289–330.
- [26] Kenyon NJ, Last JA: Reversible and irreversible airway inflammation and fibrosis in mice exposed to inhaled ovalbumin. *Inflamm Res*. 2005;54(2):57–65.
- [27] Lim Y, Vasu VT, Valacchi G, Leonard S, Aung HH, Schock BC, Kenyon NJ, Li C-S, Traber MG, Cross CE: Severe Vitamin E deficiency modulates airway allergic inflammatory responses in the murine asthma model. *Free Radic Res*. 2008;42(4):387–396.
- [28] Kim JS, Lee JS, Im KC, Kim SJ, Kim SY, Lee DS, Moon DH: Performance measurement of the microPET focus 120 scanner. *J Nucl Med*. 2007;48(9):1527–1535.
- [29] Illum L, Davis SS: Targeting of colloidal particles to the bone marrow. *Life Sci*. 1987;40(16):1553–1560.
- [30] David E, Bice JS, Francis H. Y., Green: Animal models of asthma: potential usefulness for studying health effects of inhaled particles. *Inhal Toxicol*. 2000;12(9):829–862.
- [31] Lehnert BE: Pulmonary and thoracic macrophage subpopulations and clearance of particles from the lung. *Environ Health Perspect* 1992;97:17–46.
- [32] Ferin J: Pulmonary retention and clearance of particles. *Toxicol Lett*. 1994;72(1–3):121–125.
- [33] Harmsen AG, Muggenburg BA, Snipes MB, Bice DE: The role of macrophages in particle translocation from lungs to lymph nodes. *Science*. 1985;230(4731):1277–1280.
- [34] Lippmann M, Yeates DB, Albert RE: Deposition, retention, and clearance of inhaled particles. *Br J Ind Med*. [Review]. 1980;37(4):337–362.
- [35] Corry D, Kulkarni P, Lipscomb MF: The migration of bronchoalveolar macrophages into hilar lymph-nodes. *Am J Pathol*. 1984;115(3):321–328.
- [36] Kenyon NJ, Ward RW, Last JA: Airway fibrosis in a mouse model of airway inflammation. *Toxicol Appl Pharmacol* 2003;186(2):90–100.
- [37] Bates JHT, Irvin CG: Measuring lung function in mice: the phenotyping uncertainty principle. *J Appl Physiol* 2003;94(4):1297–1306.
- [38] Bushberg JT, Seibert JA, Leidholdt EM, Boone JM: *The Essential Physics of Medical Imaging*. 2nd ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2002.
- [39] Cherry SR: *Physics in Nuclear Medicine*. 3rd ed. Philadelphia, PA: Saunders; 2003.
- [40] Osier M, Oberdorster G: Intratracheal inhalation vs intratracheal instillation: differences in particle effects. *Fundam Appl Toxicol*. 1997;40(2):220–227.

- [41] Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, Schlesinger RB: Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicol Sci.* 2000;55(1):24–35.
- [42] Brain JD, Knudson DE, Sorokin SP, Davis MA: Pulmonary distribution of particles given by intratracheal instillation or by aerosol Inhalation. *Environ Res.* 1976;11(1):13–33.
- [43] Tronde A, Baran G, Eirefelt S, Lennernas H, Bengtsson UH: Miniaturized nebulization catheters: a new approach for delivery of defined aerosol doses to the rat lung. *J Aerosol Med-Depos Clear Eff Lung.* 2002 Fal;15(3):283–296.
- [44] Oberdorster G, Cox C, Gelein R: Intratracheal instillation versus intratracheal inhalation of tracer particles for measuring lung clearance function. *Exp Lung Res* 1997;23(1):17–34.
- [45] Ghio AJ, Devlin RB: Inflammatory lung injury after bronchial instillation of air pollution particles. *Am J Respir Crit Care Med.* 2001;164(4):704–8.
- [46] Bagate K, Meiring JJ, Gerlofs-Nijland ME, Vincent R, Cassee FR, Borm PJA: Vascular effects of ambient particulate matter instillation in spontaneous hypertensive rats. *Toxicol Appl Pharmacol.* [doi: DOI: 10.1016/j.taap.2004.02.005]. 2004;197(1):29–39.
- [47] Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PHM, Verbruggen A, Nemery B: Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am J Respir Crit Care Med.* 2001;164(9):1665–1668.
- [48] Furuyama A, Kanno S, Kobayashi T, Hirano S: Extrapulmonary translocation of intratracheally instilled fine and ultrafine particles via direct and alveolar macrophage-associated routes. *Arch Toxicol.* 2009;83(5):429–437.
- [49] Delgado R, Felix V, Lima LMP, Price DW: Metal complexes of cyclen and cyclam derivatives useful for medical applications: a discussion based on thermodynamic stability constants and structural data. *Dalton Trans.* 2007(26):2734–2745.
- [50] Raos N, Milifçevifä A: Prediction of stability constants of coordination compounds from their molecular graphs. *J Phys: Conf Ser.* 2013;410(1):1–4.