JOURNAL OF AEROSOL MEDICINE
Volume 19, Number 1, 2006

Pp. 000–000

Review

Ultrafine Particle–Lung Interactions: Does Size Matter?

WOLFGANG G. KREYLING, Ph.D.,[1,2] MANUELA SEMMLER, M.V.D.,[1] and WINFRIED MÖLLER, Ph.D.[1,3]

ABSTRACT

Epidemiological studies continue to indicate associations between exposure to increased concentrations of ambient fine and ultrafine particles and adverse health effects in susceptible individuals. The ultrafine particle fraction in the ambient atmosphere seems to play a specific role. Yet, the dosimetry (including deposition patterns in the respiratory tract and, particularly, the biokinetic fate of ultrafine particles) is not fully understood. In contrast to fine particles, inhaled ultrafine particles seem to follow different routes in the organism. This paper summarizes the current knowledge. Cardiovascular effects observed in epidemiological studies triggered the discussion on enhanced translocation of ultrafine particles from the respiratory epithelium towards circulation and subsequent target organs, such as heart, liver, and brain, eventually causing adverse effects on cardiac function and blood coagulation, as well as on functions of the central nervous system. Current knowledge on systemic translocation of ultrafine particles in humans and animal models is reviewed. Additionally, an estimate of accumulating particle numbers in secondary target organs during chronic exposure is extrapolated from long-term translocation data obtained from rats. Toxicological studies aim to provide the biological plausibility of health effects of ultrafine particles and to identify cascades of mechanisms that are causal for the gradual transition from the physiological status towards pathophysiologcal alterations and eventually chronic disease. Considering the interaction between insoluble ultrafine particles and biological systems (such as body fluids, proteins, and cells), there still are gaps in the current knowledge on how ultrafine particles may cause adverse reactions. This paper reviews the current concept of interactions between insoluble ultrafine particles and biological systems.

Key words: ultrafine particles, particle dosimetry, deposition, clearance, systemic translocation, uptake in secondary target organs

[1]Institute of Inhalation Biology and [2]Focus Network Aerosols and Health, GSF–National Research Center for Environment and Health, Neuherberg/Munich, Germany.
[3]Clinical Research Group "Inflammatory Lung Diseases," GSF–Institute for Inhalation Biology and the Asklepios Hospital for Respiratory Diseases, Gauting, Germany.

INTRODUCTION

For about a decade, epidemiological studies have indicated associations between exposure to increased concentrations of ambient fine and ultrafine particles and adverse health effects in susceptible individuals.[1–7] Cardiovascular effects observed in epidemiological studies triggered the discussion on enhanced translocation of ultrafine particles from the respiratory epithelium towards circulation and subsequent target organs, such as heart, liver, and brain, eventually causing adverse effects on cardiac function and blood coagulation, as well as on functions of the central nervous system.[8] In addition, because of the high specific surface area, ultrafine particles are attributed to have specific toxicity, which might result in the induction of oxygen radicals and in the catalysis of chemical reactions.[9–11] The dosimetry (including deposition patterns in the different regions of the respiratory tract and the biokinetic fate of ultrafine particles in the human body) is not fully understood.

Toxicological studies on the effects of ultrafine particles aim to provide biological plausibility and to understand particle interactions with proteins and cells and to identify cascades of mechanisms that are causal for the gradual transition from the physiological status towards pathophysiology and eventually chronic disease. This paper reviews the current literature on the interaction between insoluble ultrafine particles and biological systems (such as body fluids, proteins, receptors, and cells), which may be involved in the mechanisms of translocation of ultrafine particles from the site of entrance into the body (respiratory system) towards circulation and towards target organs causing possible adverse health effects.

ULTRAFINE PARTICLES INTERACTIONS WITH THE TRACHEO-BRONCHIAL AIRWAYS EPITHELIUM

There is experimental evidence that not all particles deposited on the epithelium of human tracheo-bronchial airways are cleared immediately via the mucociliary transport to the larynx, but remain for weeks or longer.[12,13] This phenomenon was studied by two experimental approaches: shallow aerosol bolus inhalation[14–16] and extremely slow inhalation.[17–19] In both cases, particles are targeted to deposit as exclusively on tracheo-bronchial airways as possible.

In the first experimental set-up, only monodisperse test particles are inhaled (as an aerosol bolus at the end of a breath of filtered air). Deposition may be enhanced by a breathhold after inhalation. An advantage of this methodology is that any particle size, from ultrafine to super micron, can be administered. To assure exclusive deposition on the tracheo-bronchial airways epithelium, particles should not penetrate in a region that may already be alveolated. So, the more shallowly the aerosol bolus is administered, the higher the probability of exclusive airway deposition.

The second methodology uses only monodisperse particles of 5–10 μm in aerodynamic diameter and makes use of extremely slow inhalation flow rates (≈50 mL/s)—usually at least one order of magnitude less than normal breathing flow—thus minimizing deposition due to impaction in large bronchial airways, but maximizing particle deposition in small bronchial airways due to sedimentation, thereby prohibiting deposition in alveoli.

Interestingly, the studies applying the aerosol bolus technology clearly show that the long-term retained fraction in the bronchial airways depends on the physical size of the particles (Fig. 1). While this fraction is negligible for particles of 6 μm in physical size and above, it increases

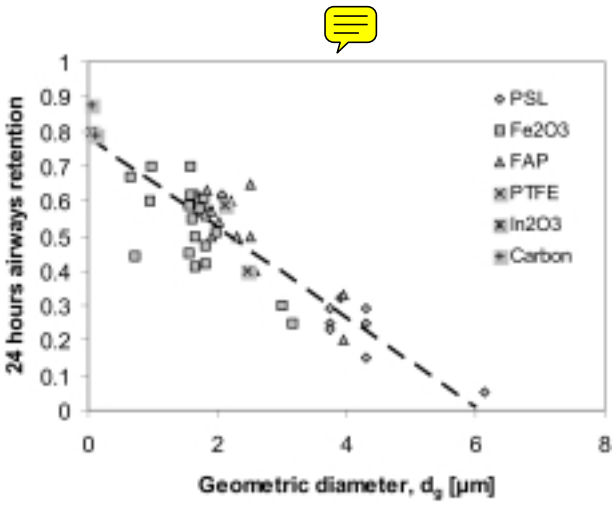

FIG. 1. The 24-h particle retention in the bronchial airways after shallow bolus inhalation.[12] Particles of different types and densities were used: PSL [polystyrene], ρ = 1.05 g/cm^3; Fe2O3 [iron oxide (Fe_2O_3)], ρ = 3.5 g/cm^3; FAP [fused aluminium silicate], ρ = 2.2 g/cm^3; PTFE [Teflon], ρ = 2.2 g/cm^3; In2O3 [indium oxide (In_2O_3)], ρ = 7.2 g/cm^3; carbon, ρ = 2.3 g/cm^3.

steadily with decreasing diameter, such that 80% of the deposited 30-nm ultrafine indium oxide particles are retained long-term.[20] Just recently, we performed a pilot aerosol-bolus-inhalation study during which carbonaceous 50- and 100-nm particles were inhaled to maximize deposition in the bronchial airways.[21] Since there was less than 25% clearance during the first 24 h after inhalation, this study confirmed the long-term retained fraction of carbonaceous ultrafine particles, another particle material, in the airways. These data have significant impact on the dose of toxic or radioactive particles being deposited in the bronchial airways and affecting airway epithelium. In fact, long-term retention in bronchial airways was included into the recent ICRP model of particle deposition and clearance of the inhaled particles.[12]

The reason for the delayed particle clearance and its dependency on particle size is still under debate. It was reported that the airway surface area is not entirely covered with ciliated cells nor with mucus, resulting in discontinuous transport of mucus plugs or streams of plugs to the larynx, as reviewed recently by Geiser et al.[22] Hence, particles may deposit directly onto non-ciliated cells of the bronchial airway epithelium. In addition, because of their size, ultrafine particles are hypothesized to have a higher probability of penetrating between cilia. The direct contact of particles and cell surfaces may lead to adhesion and uptake into the cell. Furthermore, airway macrophages are likely to phagocytize those particles. In fact, the kinetics of this long-term retention mechanism was shown to be similar to that in the lung periphery using 2-μm ferromagnetic tracer particles.[23]

The observed delayed clearance of ultrafine particles leads to the fact that there is only a negligible fast-cleared fraction of ultrafine particles after tidal breathing, although this breathing pattern will result in both bronchiolar and alveolar deposition. Based on model consideration, bronchiolar deposition should account for about 20–30% of total deposition.[12,24] Indeed, we have confirmed that there is negligible fast clearance within 24 and 72 h after inhalation in another study after tidal breathing of 100-nm carbonaceous particles; this was shown not only in healthy adult subjects but also in asthmatic patients and in smokers.[25]

In a dog model, we confirmed the kinetic behavior of the long-term retained fraction as observed in human bronchial airways after aerosol-bolus-inhalation, and we proved at least partially the long-term retention of 2-μm-sized particles in the canine bronchial airways by morphological analysis of particle locations.[26] Morphologically about 50% of the long-term retained particles were found in the bronchial epithelium and the other half in the alveolar epithelium. This may depend on the experimental conditions (intubation in the dog study) and on morphological differences between the human and canine lung structure, leading to different ventilation conditions.

However, the observed long-term UFP retention in human and canine airways does not or only negligibly occurs in rats. After inhalation of 15–20-nm and 80-nm ultrafine iridium particles by endotracheally intubated and ventilated rats, we observed that 30% and 20%, respectively, of the totally deposited particles in the thorax were rapidly cleared from the lungs.[27,28] These rapidly cleared fractions correspond reasonably well to deposition estimates in rats based on earlier data and theoretical calculations.[29,30] Ongoing studies on adult mice demonstrate a similar pattern to that of the rats. Hence, there is a clear-cut different clearance pattern of UFP in the bronchial airways of rats and mice when compared to that of humans and dogs.

Since fewer studies on large animal species, particularly in dogs and monkeys, have been performed in recent times (due to ethical as well as financial reasons) and more studies on rodents will be carried out in the future, these interspecies differences in size-dependent particle clearance need to be taken into account when performing data extrapolation from rodents to humans for human dose estimate and risk assessment.

ULTRAFINE PARTICLES ARE LESS PHAGOCYTIZED BY ALVEOLAR MACROPHAGES AND DISAPPEAR FASTER FROM THE ALVEOLAR EPITHELIUM THAN MICRON-SIZED PARTICLES

When inhaled UFP deposit on the epithelial surface area of the peripheral lungs, they contact the surfactant layer and the underlying epithelial lining fluid (ELF).[22] Hence, they interact immediately with proteins and other biomolecules of ELF, including opsonins.

According to the larger number concentration of UFP in the ambient aerosol (usually exceeding more than one to two orders of magnitude when

compared to the concentration of fine particles), there will be many more deposited UFP spread over the surface of an alveolus. This is likely to lead to a scattered chemoattractant signal, resulting in less recognition and subsequently less phagocytosis by alveolar macrophages (AM), when compared to fewer micron-sized particles coated with relatively more opsonin molecules on their surfaces. In contrast, rapid phagocytosis of micron-sized particles is well documented, based on opsonin-directed migration of AM towards the particle.[31–33]

To study phagocytosis of UFP and micron-sized particles, we have performed exhaustive broncho-alveolar lavage of rat lungs 24 h after a 1-h inhalation of ultrafine iridium particles radiolabeled with ^{192}Ir (^{192}Ir-UFP were either 15–20 nm or about 80 nm in size, geometric standard deviation 1.6), and compared this to the data of Oberdörster et al., who had exposed rats to polystyrene particles of 0.5, 2, and 10 μm in size[8,27]). Only about 20% of the retained UFP were lavageable out of the lungs, and more than 90% of these were associated with alveolar macrophages. In contrast, 24 h after inhalation, the lavageable fraction was about 80% of the retained particles for the 0.5-μm as well as the 2- and 10-μm sized polystyrene particles. The lavaged micron-sized particles were also mostly associated with alveolar macrophages as expected. Since the number of lavaged AM was similar in both cases, these findings suggest that (a) significantly less UFP had been phagocytized in the previous hours and (b) that most of UFP were not accessible for lavage. The fact that we did not find a substantial fraction of free UFP in the lavage fluid may be indicative that these UFP had already disappeared from the luminal side into and beyond the epithelium, as was demonstrated by morphologic studies on ultrafine TiO_2 and silver particles.[34,35] So, these studies support the concept that UFP are less likely to be phagocytized by AM and reside only a short time on the luminal side of the rat alveolar epithelium.

ULTRAFINE PARTICLES INTERACTIONS WITH THE ALVEOLAR EPITHELIUM

Complexes of UFP and proteins

When inhaled particles deposit on the epithelial surface of the peripheral lungs, they will first contact with and, therefore, most likely interact with proteins and biomolecules of the epithelial lining fluid (ELF) such that they may form UFP–protein complexes. There is little known about the behavior and fate of such particle–protein complexes.[36] However, evidence was found that these complexes may be protein specific, depending on the UFP composition and surface structure.[37] An important difference between micron-sized particles and UFP is the fact that, in the former case, these proteins are immobilized on the surface area of the micron-sized particle, which may be phagocytized by alveolar macrophages within the next few hours.[31] However, the UFP–protein complexes may or may not interact with receptors of other cells, like the epithelial cells type I in their direct vicinity, making them less or even more accessible to the phagocytic system. In fact, the complex of the UFP and the protein may not be much larger than the size of the protein itself, such that the fate of the protein may determine the fate of the complexed UFP, resulting in potentially different pathways of metabolism of selected UFP. As a result, some particles may be retained simply on the epithelial surface; others may penetrate the epithelial barrier, providing access to interstitial spaces. Consequently, the specificity of binding allows for specific metabolic fates of such complexes. Depending on the complexing protein, some UFP may find passage into systemic circulation, opening access to various organs of the cardiovascular system, and eventually to other organs, such as the central nervous system; note that we have found a small fraction of ultrafine iridium particles in the brain, similar to that in other target organs after thoracic ventilation.[27] Since the nasal passages were bypassed, transnerval passage from the olfactory epithelium into the brain was excluded; hence, in this case, the iridium UFP had been translocated to the brain via circulation. In any case, in the circulation, such complexes can interact with other proteins or with immuno-competent cells, like T- and B-lymphocytes, or mononuclear as well as polymorphous-nucleated granulocytes, giving rise to immunological signals and responses. In addition, those complexes may simply prevent phagocytosis of the otherwise very efficient defence line of macrophages in liver and spleen, allowing for much longer times of circulation.

We just recently showed that different ultrafine test particles—titanium dioxide, silica, and elemental carbonaceous particles (Printex 90)—bind to a variety of proteins of rat ELF collected by

broncho-alveolar lavage and rat serum, such as albumin.[37] Interestingly, we observed proteins such as albumin binding to each of the test particles used, as well as other proteins binding specifically to a selected particle type, and not or less to the others.

Short-term translocation towards circulation and uptake into secondary target organs

Conflicting results have been reported about the fractions of translocated UFP from the lung epithelium towards circulation and accumulation into secondary target organs. More recent data demonstrate that systemically translocated fractions are below 5% of the deposited UFP dose in the lungs after a single exposure. This is confirmed in rats[27,28] and humans.[25,38] Particularly in the rat studies that we performed using ultrafine iridium particles, we demonstrated completely balanced metabolic studies with respect to the distribution of the radio-labeled particles in all organs and tissues, as well as in excretion, but also distinguished the metabolic fate of solubilized radio-tracer from that bound firmly to UFP. By that methodology, we were able to determine minute UFP fractions translocated not only to liver and spleen, but also to the heart and even to the brain. Such quantitative studies were not possible in humans, but the fact that there was virtually no clearance, though much care was taken to tightly bind the radio-tracer to the UFP, underlines the limited translocation of these UFP.

Excretion analysis provides an upper estimate of the systemically translocated UFP fraction of <1–5% of the deposit for the carbonaceous and iridium test particles used. However, it needs to be emphasized that other materials (and, particularly, those with other surface composition and structures) may behave differently. Such insight may be taken from a very recent study,[39,40] in which ultrafine titanium dioxide (TiO_2) particles were produced basically by the same method as the iridium particles yielding primary particles of about 5 nm very similar to those of iridium. TiO_2 agglomerates were inhaled by endotracheally intubated and ventilated WKY rats and the lungs were morphometrically studied in great detail immediately after the 1-h inhalation and 24 h later to determine the location of the retained TiO_2 particles within the various cells and lung compartments. Interestingly, a fraction of 10% of the analyzed particles was found at each time point in the vascular compartment, including the endothelium and the vascular lumen indicating a rapid particle translocation towards vasculature which was obviously not the case after the inhalation of iridium particles.

Earlier data suffered from the fact that (1) either the radio-label was not tightly bound to the UFP matrix resulting in severe difficulties ~~to distinguish~~ rapid tracer translocation from rapid UFP translocation (2) or selected sample analysis without a quantitative determination of a balanced kinetics analysis (hamster, Nemmar et al.[41]; human, Nemmar et al.[42]; human, Brown et al.[38]; rat, Takenaka et al.[35]).

To exemplify the importance of stable radio-labelling of the particles we compare two whole body gamma camera scans (Fig. 2): the first (Fig. 2A) was taken from the human study of Nemmar et al.[42] at 1 h after inhalation of ^{99m}Tc-labeled carbonaceous particles generated with a Technegas generator under standard conditions (particle size > 100 nm) used in hospitals presuming a stable labelling. The second (Fig. 2B) was taken one hour after inhalation of soluble ^{99m}Tc-sodium-pertechnetate aerosol particles, the latter were obtained by nebulizing a ^{99m}Tc-pertechnetate-saline solution with a medical nebulizer. In the latter scan, the ^{99m}Tc lung activity had greatly disappeared ~~from the liver~~ and accumulations in the bladder, thyroids, and salivary glands are clearly visible, as expected to be the target organs of soluble ^{99m}Tc-pertechnetate molecules; in addition, the visible contour of the body is indicative of the large fraction of ^{99m}Tc activity circulating in blood.[43] Serial blood samples confirmed high accumulation of ^{99m}Tc in body fluid (blood) and a retarded clearance via urine (half-life of 4–5 days). In contrast, the former whole body scan (Fig. 2A) clearly shows the ^{99m}Tc activity in the lungs indicative of substantial ^{99m}Tc-labeled particle retention. However, it also shows similar accumulations in the same organs as in Figure 2B, indicating the results of the metabolism of soluble ^{99m}Tc-pertechnetate. Because of the predominance of the metabolism of the solute it appears very difficult to distinguish between translocation of the soluble tracer and the ultrafine carbonaceous particles.

While labeling the matrix of carbonaceous UFP with the stable isotope ^{13}C seems ideal,[44] it is limited by the fact that endogenous ^{13}C is abundantly available at a ratio of 1,124 ± 0.006% of ^{12}C in all biological materials[45] (⟨http://deuterium.

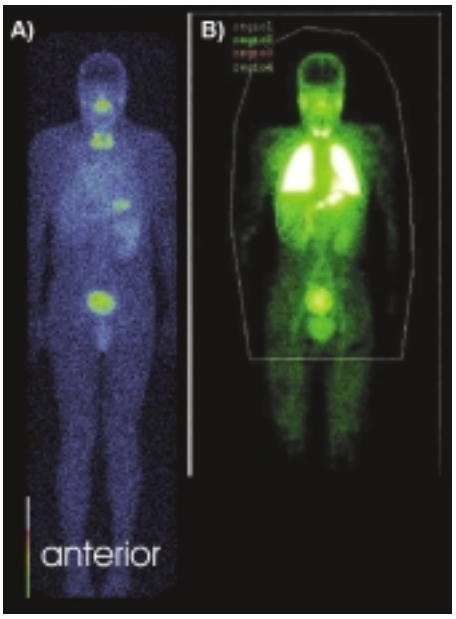

FIG. 2. (**A**) Whole body gamma camera scan (anterior) of inhaled ^{99m}Tc-Technegas (1 h after inhalation) from the study of Nemmar et al.[42] (**B**) Whole body gamma camera scan (anterior) of inhaled ^{99m}Tc-sodium-pertechnetate (1 h after inhalation), and distribution in the body and accumulation in the different organs.

nist.gov/standards.html⟩). Hence, deposition of ^{13}C labeled UFP of a tenth of the endogenously abundant ^{13}C in the lungs of rats requires rather stable ratios of ^{13}C/^{12}C and precise knowledge of the carbon content not only in the lungs but also in all other organs to be analyzed for translocated ^{13}C labeled UFP, and provides a potential source of error. As a result isotope tracer studies provide reasonable results, particularly, when their limitations and potential sources of errors are carefully considered.

However, the picture of systemic particle translocation is more complicated as mentioned above by the morphometric TiO_2 study[39,40] and evidenced by other sets of data of other health relevant outcomes as discussed next. Nemmar et al. introduced a very interesting model on experimentally induced thrombus formation in a peripheral hamster vein.[46,47] They were able to show that particles either injected intravenously into circulation or instilled intratracheally into the lungs will lead to photochemically induced formation of a thrombus in veins and arteries when illuminated with green laser light. For analytical quantification they measured the size of an artificially induced thrombus in hamsters after a preset time. When 500 μg of ultrafine 60-nm polystyrene-test particles (PSL) with positive surface charge (positive zeta potential) were either intratracheally instilled into the lungs or intravenously injected, the size of the thrombus was clearly increased compared to a control group. PSL intratracheally instilled showed slightly less thrombus formation (by about a factor of three to four) than the intravenously injected PSL. In contrast, neither 400-nm large-sized, positively charged PSL, nor negatively charged, carboxylate-coated ultrafine 60-nm sized PSL, nor unmodified ultrafine 60-nm sized PSL led to enhanced thrombus formation. In addition, they demonstrated *in vitro* that hamster platelets showed increased aggregation—the basic mechanism underlying thrombus formation—when co-incubated with as little as 3 μg/mL positively charged PSL.[47] The results of the ultrafine positively charged particles on thrombus formation were confirmed recently by another group which had modified the experimental setup slightly using a rat model.[48] Already in 1977, Berry et al. found 30-nm-sized gold particles in circulating platelets of rats within 30 min after intratracheal instillation into rats (note, however, that no quantitation of the systemically translocated gold particles relative to the instilled particles was given).[49] More evidence on thrombogenic effects of ultrafine carbonaceous particles comes from the study of Khandoga et al.[50] who documented fibrinogenic deposits, increased platelet accumulation and von-Willebrandt factor expression on the endothelium of the liver after intravenous administration of those particles.

Nemmar et al. conclude: the positively charged PSL particles were first translocated into circulation and subsequently the negatively charged platelets were activated by the positively charged PSL particles to aggregate.[46] Questions remain how and to which extend the particles have crossed the air–blood barrier? This elegant set of data clearly provides a number of evidences for translocation of positively charged ultrafine particles across the air-blood-barrier, although the translocated PSL particles are only evidenced by their thrombogenic effect without direct quantitative particle determination in the circulation. The same concern holds for their studies showing enhanced experimentally induced thrombus formation after either intratracheal instillation or intravenous injection of standardized Diesel exhaust particles (DEP).[47] They clearly observed the functional change as an indicator for an impor-

tant adverse health effect, but they only extrapolate the presence of particles at the location of laser-induced thrombus formation.

Therefore, more quantitative research is required to fill in the currently open gaps of knowledge: important questions regarding the translocation of particles into circulation are:

1. Why was platelet activation only increased by a factor of three to four after intravenous injection when compared to intratracheal administration?
2. In humans, classical pathology never has reported such particle loads in secondary target organs under physiological conditions while the increasingly blackening lungs with increasing age of smokers are well documented in many text books since more than a century. Particles in the liver and other organs of the reticuloendothelial system have been reported in coal ~~mine~~ miners and asbestos workers,[51,52] but only in those with long exposure times and high lung burdens of the materials. In these studies it was suggested that the route of translocation was via the lymphatic system and not via the blood circulation.
3. What is the role of the positive surface charge or the amino-coating of PSL particles and is it likely that DEP with an unknown surface charge translocates into circulation similarly rapid? Our measurements of zeta potential of various carbonaceous test particles including NIST standard Diesel Exhaust particles (SRM1650a) obtained from controlled combustion processes always revealed a negative zeta potential.
4. A possible mechanism triggered by upregulation and induction of cytokines or other cellular induced mediators released in the lungs and activating platelets subsequently seems to be unlikely, considering the rapid onset of the observed thrombus formation within minutes after application. This is in contrast to the time constants of upregulation and induction of cellular processes of several hours. Hence, the mechanism of platelet activation, either directly by systemically present UFP or indirectly, remains to be studied in greater details.

Recently, Heckel et al.[53] investigated reverse transport mechanisms of 8 nm sized gold particles coated with autologous albumin from the capillary vasculature to the luminal side of the alveolar epithelium of an endotoxin-triggered rabbit model of inflammation. They observed paracellular particle transport through inter-endothelial junctions, as well as trans-cellular transport via caveolae through endothelial and epithelial type I cells. Interestingly, these particle transport pathways were ~~not~~ only observed in control rabbits without endotoxin-triggered inflammation. At the same time it shows that nanoparticles coated with the appropriate serum protein can rapidly be translocated across a membrane; particularly, when there is a protein gradient between the two sides of the barrier. It remains open whether these albumin-modulated para- and transcellular pathways are important in the human lungs in the reverse direction when an inhaled particle deposited on the alveolar epithelium is translocated into blood circulation.

Particularly, the latter study emphasizes that the particle surface and coating may play a crucial role as discussed above regarding UFP–protein complexes (e.g., UFP of any matrix coated with a particular ligand may interact specifically with proteins or cellular receptors of epithelial or endothelial cells), such that enhanced particle translocation may occur across the alveolar air–blood barrier or other membranes.

Long-term translocation towards circulation and uptake into secondary target organs

Even if there is only a small fraction of UFP which translocates within a few days after inhalation into circulation and eventually accumulates in secondary target organs, this process may be continuous, such that even small continuously translocated particle fractions into circulation may result in accumulating loads in secondary target organs. To analyze long-term translocation kinetics we recently extended our previous translocation studies after a single one-hour inhalation of 15–20-nm-sized radio-labeled iridium particles and followed clearance as well as translocation kinetics over a period of 6 months.[28] To quantitatively determine the kinetics we collected total excretion over the entire period to allow for a complete balance of iridium UFP excretion and distribution in the whole body of each individual rat, and killed groups of rats at increasing time intervals. Actually we found a maximum accumulation of iridium UFP in each target organ about one week after inhalation: liver, spleen, kidneys,

heart and brain. In each organ maximum was 0.1–0.5% of the deposited UFP mass. However, thereafter the load in each target organ declined to less than 0.01–0.05% but remained clearly detectable.

Dose estimate of UFP accumulation in secondary target organs during chronic UFP exposure

Taking this long-term kinetics after a single exposure into account we can estimate long-term accumulation in rat secondary target organs during a year-long chronic exposure based on the results obtained from the tested UFP (insoluble iridium) used in our studies. Applying the 6-month rat translocation data to the continuous exposure would result into accumulation of about 0.01–0.05% of each daily lung dose in each of the analyzed target organs, corresponding to about 4–20% of the daily deposited lung dose after one year. Applying this approach to human exposure requires great caution as indicated by other species differences observed between rodents and man. Therefore, this exercise of extrapolating rat translocation data to human may only be considered to represent a rough estimate. The daily dose of insoluble UFP to a human lung can be estimated to be 3×10^9 UFP/day based on the following assumptions: 1×10^3 insoluble UFP per cm^3, daily inhaled gas volume 1×10^4 L/day by an adult human and a deposited fraction of 0.3 of the inhaled UFP concentration in the peripheral lungs. Based on these assumptions $1–6 \times 10^8$ UFP would have accumulated in each secondary target organ during one year of continuous exposure. This compares to a much higher lung dose of 3×10^{11} UFP during one year exposure, assuming about 70% clearance of these insoluble UFP from the lungs.[13] Again we have to point out that the estimate is derived from single exposure rat data under physiological conditions in which translocation biokinetics was very low. Other materials may be translocated differently and translocation in humans may be governed by different biokinetics.

Although the accumulated doses to secondary target organs are two to three orders of magnitude less when compared to the lung dose, the estimated particle numbers are indeed not negligible. Whether these particle numbers and also their total surface area are of biological/toxicological relevance needs further investigations. Considering the insoluble fraction of ambient ultrafine carbonaceous particles being inhaled continuously by each individual, this estimate highlights that not only the elemental carbon load in the lungs increases with age but also the elemental carbon loads in the secondary target organs. Note, in the lungs insoluble particles of all sizes (not being cleared) accumulate while in secondary target organs only the ultrafine particles accumulate. While the number of $1–6 \times 10^8$ of these particles is substantial the mass is rather low in the range of 1–10 ng. Because of this low mass, it is not surprising that particulate uptake has not been documented in secondary target organs in previous literature, except in cases of very high exposures over long time periods, where particle uptake in liver was documented,[51,52] as discussed above.

ACKNOWLEDGMENTS

This work was supported in part by BIODOS–Biokinetics and Dosimetry of Internal Contamination (FIDG-CT-2000-00053) of the European Commission.

REFERENCES

1. Dockery, D.W., C.A. Pope, 3rd, X. Xu, et al. 1993. An association between air pollution and mortality in six U.S. cities. *N. Engl. J. Med.* 329:1753–1759.
2. Wichmann, H.E., and A. Peters. 2000. Epidemiological evidence of the effects of ultrafine particle exposure. *Philos. Trans. R. Soc. A* 358:2751–2769.
3. Wichmann, H.E., C. Spix, T. Tuch, et al. 2000. Daily mortality and fine and ultrafine particles in erfurt, germany part I: role of particle number and particle mass. *Respir. Rep. Health Eff. Inst.* 98:5–94.
4. Ibald-Mulli, A., H.E. Wichmann, W. Kreyling, et al. 2002. Epidemiological evidence on health effects of ultrafine particles. *J. Aerosol Med.* 15:189–201.
5. Peters, A., and C.A. Pope, 3rd. 2002. Cardiopulmonary mortality and air pollution. *Lancet* 360:1184–1185.
6. Pope, C.A., 3rd. 2004. Air pollution and health—good news and bad. *N. Engl. J. Med.* 351:1132–1134.
7. Schulz, H., V. Harder, A. Ibald-Mulli, et al. 2005. Cardiovascular effects of fine and ultrafine particles. *J. Aerosol Med.* 18:1–22.
8. Oberdörster, G. 2004. Kinetics of inhaled ultrafine particles in the organism. *In* U. Heinrich, ed. *Effects of Air Contaminants on the Respiratory Tract—Interpretations from Molecules to Meta Analysis. INIS Monograph.* Fraunhofer IRB Verlag, Stuttgart, 121–143.
9. Donaldson, K., V. Stone, P.S. Gilmour, et al. 2000. Ul-

trafine particles: mechanisms of lung injury. *Philos. Trans. R. Soc. A* 358:2741–2748.
10. Donaldson, K., C. Lang Tran, and W. MacNee. 2002. Deposition and effects of fine and ultrafine particles in the respiratory tract. *Eur. Respir. Monogr.* 7:77–92.
11. Kreyling, W. G., M. Semmler, and W. Möller. 2004. Dosimetry and toxicology of ultrafine particles. *J. Aerosol Med.* 17:140–152.
12. ICRP. 1994. Human respiratory tract model for radiological protection. A report of a Task Group of the International Commission on Radiological Protection. *Ann. ICRP* 24:1–482.
13. Kreyling, W. G., and G. Scheuch. 2000. Clearance of particles deposited in the lungs. *In* P. Gehr and J. Heyder, eds. *Particle–Lung Interactions.* Marcel Dekker, New York, 323–376.
14. Stahlhofen, W., J. Gebhart, G. Rudolf, et al. 1986. Measurement of lung clearance with pulses of radioactively-labelled aerosols. *J. Aerosol Sci.* 17:333–336.
15. Stahlhofen, W., G. Scheuch, and M.R. Bailey. 1995. Investigations of retention of inhaled particles in the human bronchial tree. *Rad. Protec. Dosimetry* 60:311–319.
16. Scheuch, G., W. Stahlhofen, and J. Heyder. 1996. An approach to deposition and clearance measurements in human airways. *J. Aerosol Med.* 9:35–41.
17. Anderson, M., K. Philipson, M. Svartengren, et al. 1995. Human deposition and clearance of 6-micron particles inhaled with an extremely low flow rate. *Exp. Lung Res.* 21:187–195.
18. Camner, P., M. Anderson, K. Philipson, et al. 1997. Human bronchiolar deposition and retention of 6-, 8- and 10-micrograms particles. *Exp. Lung Res.* 23: 517–535.
19. Svartengren, M., K. Svartengren, F. Aghaie, et al. 1999. Lung deposition and extremely slow inhalations of particles. Limited effect of induced airway obstuction. *Exp. Lung Res.* 25:353–366.
20. Roth, C., G. Scheuch, and W. Stahlhofen. 1993. Clearance of the human lungs for ultrafine particles. *J. Aerosol Sci.* 24:S95–S96.
21. Möller, W., J. Seitz, K. Sommerer, et al. 2003. Deposition and clearance of radiolabelled ultrafine carbon particles in the human airways after bolus inhalation. *J. Aerosol Med.* 16:201.
22. Geiser, M., V. Im Hof, S. Schürch, et al. 2000. Structural and interfacial aspects of particle retention. *In* P. Gehr and J. Heyder, eds. *Particle–Lung Interactions.* Marcel Dekker, New York, 291–322.
23. Möller, W., K. Haeussinger, R. Winkler-Heil, et al. 2004. Mucociliary and long-term particle clearance in the airways of healthy non-smokers. *J. Appl. Physiol.* 97:2200–2206.
24. Asgharian, B., W. Hofmann, and R. Bergmann. 2001. Particle deposition in a multiple-path model of the human lung. *Aerosol Sci. Technol.* 34:332–339.
25. Wiebert, P., A. Sanchez-Crespo, J. Seitz, et al. 2005. High retention of 100 nm 99mTc-labeled carbonaceous particles in healthy and affected human lungs. *Fiber Particle Toxicol.* (submitted).
26. Kreyling, W.G., J.D. Blanchard, J.J. Godleski, et al. 1999. Anatomic localization of 24- and 96-h particle retention in canine airways. *J. Appl. Physiol.* 87: 269–284.
27. Kreyling, W.G., M. Semmler, F. Erbe, et al. 2002. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J. Toxicol. Environ. Health A* 65:1513–1530.
28. Semmler, M., J. Seitz, F. Erbe, et al. 2004. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhal. Toxicol.* 16:453–459.
29. Anjilvel, S., and B. Asgharian. 1995. A multiple-path model of particle deposition in the rat lung. *Fundam. Appl. Toxicol.* 28:41–50.
30. Hofmann, W., and B. Asgharian. 2002. Comparison of mucociliary clearance velocities in human and rat lungs for extrapolating modeling. *Ann. Occup. Hyg.* 46:S323–S325.
31. Brain, J.D. 1985. Macrophages in the respiratory tract. *In* A.P. Fishman and A.B. Fisher, eds. *Handbook of Physiology.* American Physiology Society, Bethesda, MD, 447–471.
32. Warheit, D.B., L.H. Overby, G. George, et al. 1988. Pulmonary macrophages are attracted to inhaled particles through complement activation. *Exp. Lung Res.* 14:51–66.
33. Sim, R.B., and S.A. Tsiftsoglou. 2004. Proteases of the complement system. *Biochem. Soc. Trans.* 32:21–27.
34. Ferin, J., G. Oberdorster, and D.P. Penney. 1992. Pulmonary retention of ultrafine and fine particles in rats. *Am. J. Respir. Cell Mol. Biol.* 6:535–542.
35. Takenaka, S., E. Karg, C. Roth, et al. 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ. Health Perspect.* 109:547–551.
36. Barrett, E.G., C. Johnston, G. Oberdorster, et al. 1999. Silica binds serum proteins resulting in a shift of the dose-response for silica-induced chemokine expression in an alveolar type II cell line. *Toxicol. Appl. Pharmacol.* 161:111–122.
37. Semmler, M., J. Regula, G. Oberdörster, et al. 2004. Lung-lining fluid proteins bind to ultrafine insoluble particles: a potential way for particles to pass airblood barrier of the lung? *Eur. Respir. J.* 24:100s.
38. Brown, J.S., K.L. Zeman, and W.D. Bennett. 2002. Ultrafine particle deposition and clearance in the healthy and obstructed lung. *Am. J. Respir. Crit. Care Med.* 166:1240–1247.
39. Kapp, N., W. Kreyling, H. Schulz, et al. 2004. Electron energy loss spectroscopy for analysis of inhaled ultrafine particles in rat lungs. *Microsc. Res. Tech.* 63:298–305.
40. Geiser, M.R.-R.B., N. Kapp, S. Schürch, et al. 2005. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ. Health Perspect.* (in press).
41. Nemmar, A., H. Vanbilloen, M.F. Hoylaerts, et al. 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am. J. Respir. Crit. Care Med.* 164:1665–1668.

AU2

AU3

42. Nemmar, A., P.H. Hoet, B. Vanquickenborne, et al. 2002. Passage of inhaled particles into the blood circulation in humans. *Circulation* 105:411–414.
43. Beasley, T.M., H.E. Palmer, and W.B. Nelp. 1966. Distribution and excretion of technetium in humans. *Health Phys.* 12:1425–1435.
44. Oberdörster, G., Z. Sharp, V. Atudorei, et al. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J. Toxicol. Environ. Health A* 65:1531–1543.
45. Craig, H. 1957. *Geochim. Cosmochim. Acta* 12:133–149.
46. Nemmar, A., M.F. Hoylaerts, P.H. Hoet, et al. 2002. Ultrafine particles affect experimental thrombosis in an *in vivo* hamster model. *Am. J. Respir. Crit. Care Med.* 166:998–1004.
47. Nemmar, A., P.H.M. Hoet, D. Dinsdale, et al. 2003. Diesel exhaust particles in lung acutely enhance experimental peripheral thrombosis. *Circulation* 107: 1202–1208.
48. Silva, V.M., N. Corson, A. Elder, et al. 2005. The rat ear vein model for investigating *in vivo* thrombogenicity of ultrafine particles (UFP). *Toxicol. Sci.* (in press).
49. Berry, J.P., B. Arnoux, G. Stanislas, et al. 1977. A microanalytic study of particles transport across the alveoli: role of blood platelets. *Biomedicine* 27:354–357.
50. Khandoga, A., A. Stampfl, S. Takenaka, et al. 2004. Ultrafine particles exert prothrombotic but not inflammatory efffects on the hepatic microcirculation in healthy mice *in vivo*. *Circulation* 109:1320–1325.
51. Auerbach, O., A.S. Conston, L. Garfinkel, et al. 1980. Presence of asbestos bodies in organs other than the lung. *Chest* 77:133–137.
52. LeFevre, M.E., F.H. Green, D.D. Joel, et al. 1982. Frequency of black pigment in livers and spleens of coal workers: correlation with pulmonary pathology and occupational information. *Human Pathol.* 13:1121–1126.
53. Heckel, K., R. Kiefmann, M. Dorger, et al. 2004. Colloidal gold particles as a new *in vivo* marker of early acute lung injury. *Am. J. Physiol. Lung Cell Mol. Physiol.* 287:L867–L878.

Received on May 16, 2005
in final form, August 25, 2005

Reviewed by:
~~Gunter~~ Oberdörster, D.V.M., Ph.D.

Address reprint requests to:
Wolfgang G. Kreyling, Ph.D.
GSF–National Research Center for Environment and Health
GSF-Focus: Aerosols and Health
Institute for Inhalation Biology
Ingolstaedter Landstr. 1
D-85764 Neuherberg/Munich, Germany

E-mail: Kreyling@gsf.de

KREYLING

AU1
Is “half-life of 4–5 days” as meant?

AU2
Please update ref. 25.

AU3
Please update ref. 40.

AU4
For reference 45, please provide article title.

AU5
Please update reference 48.