Passage of Inhaled Particles Into the Blood Circulation in Humans

A. Nemmar, DVM, PhD; P.H.M. Hoet, PhD; B. Vanquickenborne, MD; D. Dinsdale, PhD; M. Thomeer, MD; M.F. Hoylaerts, PhD; H. Vanbilloen, PhD; L. Mortelmans, MD, PhD; B. Nemery, MD, PhD

Background—Pollution by particulates has been consistently associated with increased cardiovascular morbidity and mortality. However, the mechanisms responsible for these effects are not well-elucidated.

Methods and Results—To assess to what extent and how rapidly inhaled pollutant particles pass into the systemic circulation, we measured, in 5 healthy volunteers, the distribution of radioactivity after the inhalation of "Technegas," an aerosol consisting mainly of ultrafine ^{99m}Technetium-labeled carbon particles (<100 nm). Radioactivity was detected in blood already at 1 minute, reached a maximum between 10 and 20 minutes, and remained at this level up to 60 minutes. Thin layer chromatography of blood showed that in addition to a species corresponding to oxidized ^{99m}Tc, ie, pertechnetate, there was also a species corresponding to particle-bound ^{99m}Tc. Gamma camera images showed substantial radioactivity over the liver and other areas of the body.

Conclusions—We conclude that inhaled ^{99m}Tc-labeled ultrafine carbon particles pass rapidly into the systemic circulation, and this process could account for the well-established, but poorly understood, extrapulmonary effects of air pollution. **(*Circulation*. 2002;105:411-414.)**

Key Words: air pollution ■ particles ■ translocation ■ blood ■ lung

Epidemiological studies have shown that peaks of air pollution by particulate matter with a diameter of <10 μm (PM_{10}) are associated with increased morbidity and mortality, not only from respiratory causes but mainly from cardiovascular diseases.[1–5] Recently, it has been shown that exposure to particulate air pollution for as little as 2 hours increased the occurrence of myocardial.[6] The mechanisms responsible for the cardiovascular effects are not well-elucidated.[7] The main current hypothesis is that the particles produce pulmonary inflammation with a systemic release of cytokines, which may influence cardiovascular endpoints.[8] It has also been proposed that pollutants may cause (reflex) alterations in cardiac autonomic function thus causing changes in heart rate variability and increasing the risk of sudden cardiac death.[9]

An alternative hypothesis, which has not been much investigated so far, is that the smallest particles translocate from the lungs into the circulation and thus influence cardiovascular endpoints more directly. Ultrafine particles, ie, particles with diameter ≤0.1 μm, represent a substantial component, in terms of particle numbers, in PM_{10}, although they represent a relatively small fraction of the total mass.[10] Ultrafine particles have also a much larger surface area, and hence, more toxic potential.[11,12] Recently, we have shown, in hamster, that a substantial fraction of intratracheally instilled ultrafine particles (radiolabeled denatured albumin with diameter <100 nm) rapidly diffuses from the lungs into the systemic circulation.[13] Others have recently described a systemic distribution of inhaled ultrafine silver particles in rats.[14]

Therefore, we wanted to verify whether this also occurs in humans inhaling ultrafine particles. No human data are available on this issue. We utilized a technique, commonly used in diagnostic nuclear medicine for measuring the distribution of ventilation,[15] based on the inhalation of an aerosol of technetium-99m labeled carbon particles (Technegas).

Methods

This study has been approved by our institution's ethical committee for experimentation in human subjects.

Technegas consists on an aerosol suspension of ^{99m}Tc-labeled, ultrafine carbon particles produced in an atmosphere of high-purity argon. It was considered that 100% of the inhaled particles in Technegas were labeled with ^{99m}Tc and that the aerosol did not contain pertechnetate (TcO_4^-).[16] The size of the individualized particles was of the order of 5 to 10 nm, as we confirmed by electron microscopy of particles collected with a thermophoretic precipitator. However, particle aggregates were also seen. Inhalation of these particles enabled static and dynamic images in multiple projections to be acquired.[17]

Received November 14, 2001; revision received December 11, 2001; accepted December 19, 2001.

From the Laboratory of Pneumology (Lung Toxicology) (A.N., P.H.M.H., M.T., B.N.), Nuclear Medicine (B.V., H.V., L.M.), and Center for Molecular and Vascular Biology (M.F.H.), Katholieke Universiteit Leuven, Leuven, Belgium; and the MRC Toxicology Unit (D.D.), Leicester, UK.

Correspondence to Prof B. Nemery, K.U.Leuven, Laboratorium voor Pneumologie (Longtoxicologie), Herestraat 49, B-3000 Leuven, Belgium. E-mail ben.nemery@med.kuleuven.ac.be

***Circulation* is available at http://www.circulationaha.org**

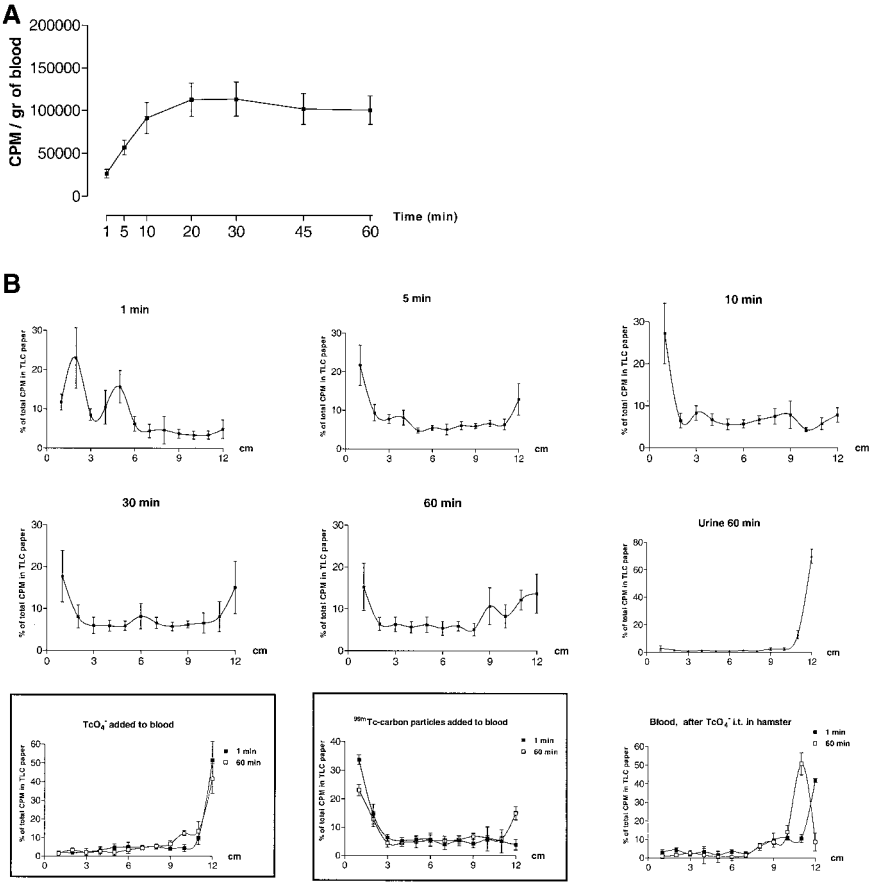

Figure 1. A, Radioactivity in blood at intervals after Technegas inhalation (mean±SEM, n=5). B, distribution of radioactivity after thin layer chromatography (TLC). The y-axis represents percent of total CPM (counts per minute) measured on the TLC paper; x-axis, distance (in cm) on the chromatogram. In blood (unframed graphs), TLC showed the presence of 2 ^{99m}Tc-label species: one species moved with the solvent front and corresponds to oxidized ^{99m}Tc, ie, pertechnetate (TcO_4^-), and the other species stayed at the application point and corresponds to particle-bound ^{99m}Tc. The framed graphs correspond to the TLC profiles after the direct addition of ^{99m}Tc-carbon particles or $^{99m}TcO_4^-$ to blood, showing that the bound radioactivity stays at the origin while the free technetate moves with the solvent front. The TLC in urine at 60 minutes and in blood at 1 and 60 minutes after the intratracheal (i.t.) administration of 200 μL of ^{99m}Tc-pertechnetate in hamsters showed one peak that moved with the solvent front.

We studied 5 healthy, male nonsmoking volunteers (24 to 47 years, mean age 32.8 years). They inhaled, according to a standard procedure,[18] approximately 100 MBq of Technegas in 3 to 5 breaths via a mouthpiece. Immediately after the Technegas inhalation, body images were acquired as follows: static acquisition (1 to 3 minutes) of lungs and thyroid followed by dynamic acquisition (5 to 45 minutes) of the abdomen, including liver, stomach, and bladder, and then successive images of the whole body (50 to 60 minutes). Blood samples were collected (via a venous catheter) at 1, 5, 10, 20, 30, 45, and 60 minutes after Technegas inhalation, and their radioactivity was measured in a gamma counter. At each time point, thin layer chromatography (TLC) was done on a droplet of blood using silica impregnated glass fiber ITLC-SG strips (Gelman Sciences) with NaCl 0.9% as the mobile phase. The chromatograms were cut into 1-cm lengths and their radioactivity measured with a gamma counter (1480W12ARD, Wallac) with a correction for background radiation. TLC was also done on a urine sample at 60 minutes.

Results

Figure 1a illustrates the time course of the radioactivity in blood expressed as counts per minute (CPM) per gram of blood. The radioactivity was detected in blood already after 1 minute, reached a maximum between 10 and 20 minutes, and remained at this level up to 60 minutes. At all time points, TLC of blood (Figure 1b) showed a peak of radioactivity at the application point and another peak that moved with the solvent front. In urine, there was mainly the latter peak. For comparison, we also present the results of TLC after the direct addition of Technegas particles (collected on a filter, at the mouth) or ^{99m}Tc-pertechnetate (TcO_4^-) to blood, showing that the bound radioactivity stays at the origin while the free pertechnetate moves with the solvent front. We also show a TLC of blood at 1 and 60 minutes after the instillation of 200 μL of free ^{99m}Tc-pertechnetate (3.7 MBq) in hamsters (n=3), showing a single peak of radioactivity at the solvent front.

The radioactivity recorded over the liver and bladder was expressed as a percentage of the initial lung radioactivity. In liver, the radioactivity remained stable at around 8%, while in the bladder it increased with time (Figure 2).

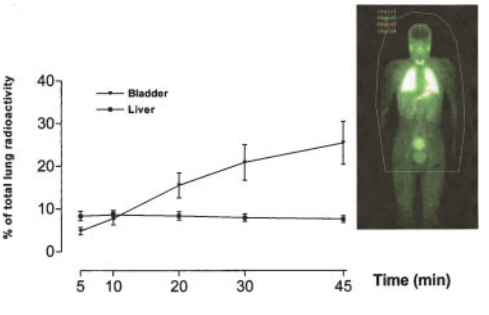

Figure 2. Time-activity curve over liver and bladder expressed as percent of initial lung radioactivity. Insert, Whole body gamma camera image of 1 representative volunteer recorded at 60 minutes. The radioactivity over the organs is expressed as counts per minute (CPM) per pixel within each region of interest (ROI). The values recorded over the stomach were not included because this radioactivity may also come partly from swallowing of particles deposited in the mouth.

Discussion

This study was designed to investigate a plausible mechanistic explanation for the consistent but puzzling epidemiological observations that particulate air pollution is associated with cardiovascular effects.[3–6] Our working hypothesis, which is different but not opposed to more traditional ones,[8,9] is that ultrafine particles may pass into the circulation and thus exert direct effects on the heart and vessels.

The type of aerosol used in our study is probably relevant urban air pollutant aerosols. Particles with diameters ranging from 0.02 μm to more than 100 μm are measurable in the air of cities.[11,19] Ultrafine particles (smaller than 100 nm) are often emitted from combustion and other high-temperature processes in the form of fractal-like aggregates composed of solid nanoparticles. The primary particle size of atmospheric aggregates ranges from 6 to 100 nm. This broad polydispersity has been related to the fact that atmospheric aggregates come from a variety of sources with different primary particle sizes.[20] In addition, Shi et al[21] found that the primary particles in diesel aggregates range from 10 to 40 nm. The size of the individualized particles used in our study (5 to 10 nm), as well as the aggregates that were also seen by electron microscopy is therefore relevant to atmospheric ultrafine particles.

Technegas is different from Pertechnegas, which is also used in nuclear medicine (for measuring lung permeability).[22] Pertechnegas is produced in an atmosphere of argon and oxygen, thus allowing the technetium to become oxidized to the hydrosoluble pertechnetate (TcO_4^-), the kinetics of which have been studied.[23] In contrast, Technegas is generated in a pure argon atmosphere, and therefore, the aerosol only consists of ^{99m}Tc-labeled particles without any appreciable TcO_4^-. We verified this to be the case by TLC of the material collected on a filter at the mouth. However, after deposition of ^{99m}Tc-labeled particles in the body, some TcO_4^- is produced. Thus, a species behaving as TcO_4^- was found by TLC in blood and in urine (Figure 1b), as well as in saliva (not shown), and the intense radioactivity detected over the thyroid, salivary glands, and stomach (Figure 2) is essentially due to the well-known accumulation of TcO_4^- in these organs.[24] In the stomach, besides TcO_4^- from saliva and gastric secretion, some radioactivity also came from swallowed particles that had deposited in the mouth or been cleared from the trachea via the mucociliary escalator.

However, 3 lines of evidence indicate that the radioactivity that we measured in blood consists, at least partly, of particle-bound radioactivity, ie, radioactivity associated with carbon particles having passed the air-blood barrier, rather than free radioactivity. Firstly, TLC of all blood samples showed, in addition to radioactivity having moved with the solvent front and corresponding to oxidized ^{99m}Tc, ie, pertechnetate (TcO_4^-), a substantial proportion of radioactivity that stayed at the application point and corresponded to particle-bound ^{99m}Tc. Such a profile was similar to that obtained after spiking blood with ^{99m}Tc-carbon particles collected from the Technegas generator. In contrast, there was only one peak at the solvent front after adding ^{99m}Tc-pertechnetate to blood or in blood collected after the intratracheal administration of ^{99m}Tc-pertechnetate to hamsters. This excludes the possibility that the noneluting radioactivity was due to free technetium having become bound to plasma proteins. Secondly, the TLC of urine showed only one peak at the solvent front, and this is in agreement with an elimination of free ^{99m}Tc-pertechnetate via the urine.[24] Finally, the presence of radioactivity in the liver is compatible with an accumulation of particles by Kupffer cells, as is known to occur with colloidal particles.[25,26] Because of the rapidity of the accumulation of radioactivity in liver, we think it is unlikely that the liver radioactivity came from the stomach. Admittedly, the above reasoning only provides indirect arguments that radioactivity outside the lungs corresponded to particles, and we would have liked to have more direct evidence. However, we were unable to detect the carbon particles in ultrathin sections of blood by electron microscopy, most probably because of their low electron density. Nevertheless, despite this limitation, we are confident that our findings provide plausible evidence for particle translocation from the lung into the blood and then its distribution to the organs. This conclusion is supported by recent studies in animals.[13,14] The exact mechanism for this translocation remains to be established, but its rapidity makes it unlikely that phagocytosis by macrophages and/or endocytosis by epithelial and endothelial cells are (solely) responsible for particle-translocation to the blood. There are experimental data suggesting the existence of (functional) pores in the alveolar-blood barrier,[27] and this is supported by the fact that "pneumoproteins" may be found in the blood.[28]

We conclude that inhaled ultrafine ^{99m}Tc-carbon particles, which are very similar to (the ultrafine fraction of) actual pollutant particles, diffuse rapidly into the systemic circulation, and this should be considered relevant for the cardiovascular morbidity and mortality related to ambient particle pollution.

Acknowledgments

This work was supported by the funds of K.U.Leuven (F/00/058). We are very grateful to K. Stessel (Nuclear Medicine, K.U.Leuven) for his excellent technical assistance.

References

1. Dockery DW, Pope CA III, Xu X, et al. An association between air pollution and mortality in six U.S. cities. *N Engl J Med*. 1993;329: 1753–1759.
2. Schwartz J. What are people dying of on high air pollution days? *Environ Res*. 1994;64:26–35.
3. Samet JM, Dominici F, Curriero FC, et al. Fine particulate air pollution and mortality in 20 U.S. cities, 1987–1994. *N Engl J Med*. 2000;343: 1742–1749.
4. Peters A, Perz S, Doring A, et al. Increases in heart rate during an air pollution episode. *Am J Epidemiol*. 1999;150:1094–1098.
5. Peters A, Doring A, Wichmann HE, et al. Increased plasma viscosity during an air pollution episode: a link to mortality? *Lancet*. 1997; 349: 1582–1587.
6. Peters A, Dockery DW, Muller JE, et al. Increased particulate air pollution and the triggering of myocardial infarction. *Circulation*. 2001;103: 2810–2815.
7. Ware JH. Particulate air pollution and mortality: clearing the air. *N Engl J Med*. 2000;343:1798–1799.
8. Seaton A, MacNee W, Donaldson K, et al. Particulate air pollution and acute health effects. *Lancet*. 1995;345:176–178.
9. Pope CA III, Verrier RL, Lovett EG, et al. Heart rate variability associated with particulate air pollution. *Am Heart J*. 1999;138:890–899.

10. Peters A, Wichmann HE, Tuch T, et al. Respiratory effects are associated with the number of ultrafine particles. *Am J Respir Crit Care Med*. 1997;155:1376–1383.
11. Oberdörster G. Pulmonary effects of inhaled ultrafine particles. *Int Arch Occup Environ Health*. 2001;74:1–8.
12. Donaldson K, Stone V, Seaton A, et al. Ambient particle inhalation and the cardiovascular system: potential mechanisms. *Environ Health Perspect*. 2001;109(suppl 4):523–527.
13. Nemmar A, Vanbilloen H, Hoylaerts MF, et al. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am J Respir Crit Care Med*. 2001;164:1665–1668.
14. Takenaka S, Karg E, Roth, et al. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ Health Perspect*. 2001; 109(suppl 4):547–551.
15. Rizzo-Padoin N, Farina A, Le Pen C, et al. A comparison of radiopharmaceutical agents used for the diagnosis of pulmonary embolism. *Nucl Med Commun*. 2001;22:375–381.
16. Senden TJ, Moock KH, Gerald JF, et al. The physical and chemical nature of technegas. *J Nucl Med*. 1997;38:1327–1333.
17. James JM, Testa HJ. The use of ^{99}Tcm-Technegas in the investigation of patients with pulmonary thromboembolism. *Nucl Med Commun*. 1995; 16:802–810.
18. Cook G, Clarke SE. An evaluation of Technegas as a ventilation agent compared with krypton-81m in the scintigraphic diagnosis of pulmonary embolism. *Eur J Nucl Med*. 1992;19:770–774.
19. Brändli O. [Are inhaled dust particles harmful for our lungs?]. *Schweiz Med Wochenschr*. 1996;126:2165–2174.
20. Xiong C, Friedlander SK. Morphological properties of atmospheric aerosol aggregates. *Proc Natl Acad Sci U S A*. 2001;98:11851–11856.
21. Shi JP, Mark D, Harrison RM. Characterization of particles from a current technology heavy-duty diesel engine. *Environ Sci Technol*. 2000;34: 748–755.
22. Mouratidis B, Lising J, Nogrady S, et al. Increased pertechnegas lung clearance in interstitial lung disease. *Clin Nucl Med*. 1999;24:105–108.
23. Kotzerke J, van den HJ, Burchert W, et al. A compartmental model for alveolar clearance of pertechnegas. *J Nucl Med*. 1996;37:2066–2071.
24. Ercan MT, Tuncel SA, Caner BE, et al. Evaluation of ^{99m}Tc-labelled monodisperse polystyrene/polyacrylate latex particles for the study of colon transit and morphology. *Int J Rad Appl Instrum B*. 1991;18: 253–258.
25. McEntee MF, Ficken MD. Blood clearance of radiolabeled gold colloid by the turkey mononuclear phagocytic system. *Avian Dis*. 1990;34: 393–397.
26. Simon BH, Ando HY, Gupta PK. Circulation time and body distribution of 14C-labeled amino-modified polystyrene nanoparticles in mice. *J Pharm Sci*. 1995;84:1249–1253.
27. Conhaim RL, Eaton A, Staub NC, et al. Equivalent pore estimate for the alveolar-airway barrier in isolated dog lung. *J Appl Physiol*. 1988;64: 1134–1142.
28. Hermans C, Bernard A. Lung epithelium-specific proteins: characteristics and potential applications as markers. *Am J Respir Crit Care Med*. 1999;159:646–678.