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
We studied 5 healthy, male nonsmoking volunteers (24 to 47 years, mean age 32.8 years). They inhaled, according to a standard procedure, 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 99mTc-pertechnetate (TcO₄⁻) 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 intratracheal (i.t.) administration of 200 μL of 99mTc-pertechnetate 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 percent 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 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 99mTc-label species: one species moved with the solvent front and corresponds to oxidized 99mTc, i.e., pertechnetate (TcO₄⁻), and the other species stayed at the application point and corresponds to particle-bound 99mTc. The framed graphs correspond to the TLC profiles after the direct addition of 99mTc-carbon particles or 99mTcO₄⁻ to blood, showing that the bound radioactivity stays at the origin while the free pertechnetate 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 99mTc-pertechnetate in hamsters showed one peak that moved with the solvent front.

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.1-6 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 to 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 al21 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 hydroxosoluble pertechnetate (TcO₄⁻), 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 ⁹⁹mTc-labeled particles without any appreciable TcO₄⁻. We verified this to be the case by TLC of the material collected on a filter at the mouth. However, after deposition of ⁹⁹mTc-labeled particles in the body, some TcO₄⁻ is produced. Thus, a species behaving as TcO₄⁻ 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 TcO₄⁻ in these organs.24 In the stomach, besides TcO₄⁻ 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 ⁹⁹mTc, ie, pertechnetate (TcO₄⁻), a substantial proportion of radioactivity that stayed at the application point and corresponded to particle-bound ⁹⁹mTc. Such a profile was similar to that obtained after spiking blood with ⁹⁹mTc-carbon particles collected from the Technegas generator. In contrast, there was only one peak at the solvent front after adding ⁹⁹mTc-pertechnetate to blood or in blood collected after the intratracheal administration of ⁹⁹mTc-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 ⁹⁹mTc-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 ⁹⁹mTc-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

Passage of Inhaled Particles Into the Blood Circulation in Humans

Circulation. 2002;105:411-414
doi: 10.1161/hc0402.104118

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/105/4/411

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/