

Transport of Particles of Colloidal Gold within and from Rat Lung after Local Deposition by Alveolar Microinjection

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Because inhalation and intratracheal instillation deposit particles throughout the respiratory tract, these methods of administration give little information on the movement of particles within the lung and no direct information on the clearance kinetics from locally defined sites within alveolar tissue. Approximately 0.05 μL of ^{195}Au -labeled gold colloid was administered to 32 rats by microinjection into a small volume of subpleural alveoli. Its fate was studied by whole-body counting and serial sacrifice over 15 months. The kinetics of clearance from the subpleural deposition site showed that there was no rapid removal of particles, and the main clearance process was defined by an exponential term with a half-time averaging 583 days. There was a wide variation between individual animals. The distribution of ^{195}Au at sacrifice showed that the gold colloid was nearly all retained within the respiratory tract. The particles were not appreciably redistributed throughout the lung volume, so most of the material not cleared from the lung remained close to the deposition site. At the later times after microinjection, much of the gold colloid was associated with thickened pleura and adjoining septae.

Introduction

The experimental administration of particles by inhalation results in deposition of particles throughout the respiratory tract. Hence, there is little information on the movement of particles within the lung, except what can be inferred from functionally defined compartments (tracheobronchial and alveolar regions). In principle, alveolar macrophages are capable of transporting particles from one region of alveolar tissue to another, due to the presence of the pores of Kohn, as well as transporting them to the ciliated airways from where they may be cleared from the lung altogether. From autoradiographic studies of asbestos fiber distribution in sections of rat lung, there is evidence that material can be transported toward the pleura (1).

We have investigated aspects of particle movement within the respiratory tract by depositing insoluble gold particles in a small volume of subpleural alveoli in rat lung, using a novel microinjection technique (2). Of special significance is the possibility that a significant fraction of radioactive particles deposited in the alveolar region of the lung can be retained long term at sites close to the epithelium of the conducting airways. Previously we reported the short-term fate of gold colloid particles administered in this way (3). Here we present the first report on the long-term fate of colloidal gold particles, followed for up to 15 months after microinjection.

Another outcome of this long-term study was to determine, for the first time, the clearance kinetics from a locally defined site within alveolar tissue: in this case, close to the pulmonary pleura.

Furthermore, this could be done without the complication of deposition on the conducting airways, where it is known there can be significant retention of particles (4,5).

Materials and Methods

^{195}Au -labeled gold colloid was prepared by a modification of the technique of Watson and Brain (6). We added approximately 3.7 MBq (100 μCi) [^{195}Au]H₂HAuCl₄ to 10 μL HAuCl₄ (20 mg/mL, BDH, Poole, Dorset, UK) in a glass Dreyers tube. This was dried in an oven at 100°C and taken up in 10 μL H₂O. With the temperature maintained at 70°C in a water bath, 4 μL sodium acetate (100 mg/mL) and 3 μL gelatin (40 mg/mL, BDH) were added, then 2 μL ascorbic acid (100 mg/mL) followed by 1 μL sodium citrate (80 mg/mL) and 10 μL H₂O.

Three batches of colloidal particles were used in the study. The particles were sized by transmission electron microscopy and image analysis. The count median diameters ranged from 10.3 to 21.4 nm, and the geometric standard deviations ranged from 1.8 to 1.9.

The gold colloid was injected into the subpleural alveoli of 32 rats by the technique of Patrick and Stirling (2). In brief, glass micropipettes were prepared from 2-mm capillary tubing using a two-stage electrode puller (BioScience, Sheerness, Kent, UK). The tips of the micropipettes were ground back to give a tip diameter of 10–14 μm and a bevel of 30°. A micropipette filled with filtered water was clamped in a holder mounted on a micro-manipulator and connected to a syringe system for loading and ejecting the gold colloid. Shortly before injection, approximately 0.5 μL gold colloid was placed on a wax plate, from which the micropipette was front-loaded under a dissecting microscope.

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Male F-344 rats, weighing 268–424 g, were anesthetized with 4% halothane and maintained with pentobarbital sodium, 60 mg/kg at first plus up to 30 mg/kg 25–35 min later. A lateral skin incision was made over the left thorax, and the external and internal intercostal muscle removed under a dissecting microscope until only a small area of the parietal pleural membrane remained. The left lung was clearly visible through this membrane. Breathing was interrupted for up to 30 sec by positive pressure (10 cm H₂O) from a face mask. During this period the micropipette, loaded with gold colloid, was maneuvered over the area of parietal pleura and made to penetrate the lung. The gold colloid was injected promptly and the micropipette withdrawn using the fine control of the micromanipulator, so that its depth at injection could be gauged. The average depth in this study (\pm SD) was $473 \pm 120 \mu\text{m}$. Neither penetration nor withdrawal of the micropipette caused collapse of the lung. Breathing was immediately allowed to resume and the overlying muscle and skin sutured, unless the animal was to be sacrificed immediately (2). The volume of colloid injected was approximately 0.05 μL , containing 0.17 μg gold and 6 kBq (0.17 μCi) ^{195}Au .

All the rats were whole-body counted within 3 hr of injection and also before sacrifice. One group of six rats was counted repeatedly over 462 days. For these measurements the rats were restrained in a plastic tube with a 50-mm diameter \times 50-mm thick NaI detector close to each side of the thorax.

Animals were serially sacrificed after approximately 4 min and 1, 7, 30, 112, 280, and 462 days. They were killed by intratracheal instillation of fluorocarbon FC80 (3M Company, St. Paul, MN) containing 10 mg/mL OsO₄ at 15 cm pressure, which fixed the lungs in inflation suitable for subsequent electron microscopy (7). The trachea, extrapulmonary bronchi, lungs, and thoracic lymph nodes were removed; all these except the lung lobes were replaced in the carcass for a repeat whole-body count. This procedure allowed us to estimate for each time point the fraction of the whole-body count due to the lung content. Interpolated values of this fraction were used to calculate fractional lung contents from the whole body counts of the animals maintained to 462 days. The rest of the carcass was dissected for radioassay of certain organs.

The left lung was cut perpendicular to its major axis into 2-mm slices, which were assayed in a well-type γ counter. The distribution of gold colloid was examined by autoradiography and light microscopy of tissue sections cut from the 2-mm slices. Tissue with sufficient ^{195}Au was further cut into 2-mm cubes from which samples were taken for transmission electron microscopy.

Results

Six rats were studied by whole-body counting over the entire period of the study. The fractional lung content decreased slowly, showing that there was no rapid removal of particles from the subpleural deposition site (Fig. 1). The clearance kinetics were well defined by the sum of two exponential terms: one accounting for 22% of the initial deposit with a half-time of 14 days, and the other with a mean half-time of 583 days:

$$R = 0.218e^{-0.050t} + 0.782e^{-0.00119t}$$

where R = fraction of initial lung content and t = time (days).

Interestingly, there was a wide variation between individual animals in the long-term clearance rate: the half-times for the six rats ranged from 278 to 1246 days, and the fraction remaining after 462 days ranged from 25 to 64%. Regression analysis showed that the half-time was independent of the depth of penetration of the micropipette into the lung.

Animals were killed at seven intervals after microinjection, four during the first phase of overall clearance and three (112, 280, and 462 days) when only the slow phase remained. From the organ distribution of ^{195}Au at sacrifice, it was clear that there was limited transport to the thoracic lymph nodes, with the amount found there exceeding 1% of the body content only by 112 days but reaching 8.4% at 462 days (Table 1). Only small amounts of

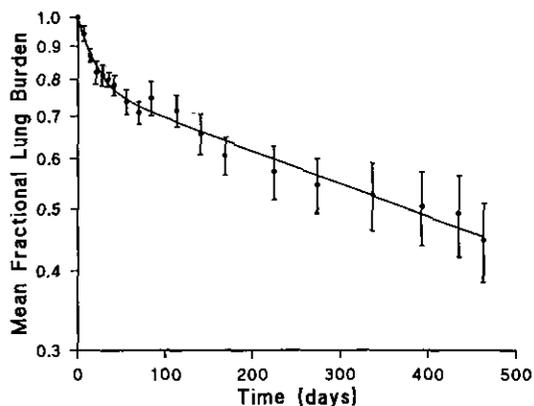


FIGURE 1. Lung retention of ^{195}Au . Mean fractional lung content for six rats studied over 462 days. Error bars represent \pm SEM. See text for fitted curve.

Table 1. Organ distribution of ^{195}Au .^a

	Time, days						
	0 (4)	1 (4)	7 (5)	30 (5)	112 (4)	280 (4)	462 (6)
Left lung	99.18 \pm 0.39	97.63 \pm 1.46	98.28 \pm 0.37	96.76 \pm 1.75	95.97 \pm 0.66	91.57 \pm 1.76	88.93 \pm 3.27
Trachea and bronchi	0.00 \pm 0.00	0.02 \pm 0.01	0.17 \pm 0.05	0.18 \pm 0.06	0.08 \pm 0.02	0.02 \pm 0.01	0.02 \pm 0.01
Thoracic lymph nodes		0.17 \pm 0.12	0.09 \pm 0.03	0.26 \pm 0.13	1.35 \pm 0.28	2.59 \pm 0.35	8.43 \pm 3.61
Gastrointestinal tract		0.22 \pm 0.10	0.63 \pm 0.15	0.39 \pm 0.08	0.05 \pm 0.05	0.26 \pm 0.26	0.00 \pm 0.00
Liver		0.14 \pm 0.08	0.13 \pm 0.13	0.47 \pm 0.47	0.34 \pm 0.16	2.15 \pm 1.82	0.00 \pm 0.00
Spleen		0.01 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.01	0.01 \pm 0.00	0.02 \pm 0.01	0.02 \pm 0.01
Kidneys		0.05 \pm 0.01	0.07 \pm 0.02	0.13 \pm 0.02	0.38 \pm 0.11	0.50 \pm 0.22	0.50 \pm 0.08
Carcass remainder	0.82 \pm 0.39	1.75 \pm 1.20	0.62 \pm 0.24	1.80 \pm 1.10	1.82 \pm 0.80	2.89 \pm 0.37	2.09 \pm 0.93

^aPercentage of total recovered from the animal at sacrifice, means \pm SEM; number of animals in parentheses.

^{195}Au were detected at any time in the extrapulmonary airways, the gastrointestinal tract, the liver, spleen, kidneys, and the remainder of the carcass. The left lung contained approximately 90% or more of the ^{195}Au remaining in the body throughout the period of the study.

Measurement of the ^{195}Au content of 2-mm lung slices, cut along the long axis of the left lung, showed that 4 min after injection the particles were mostly found within one slice (Fig. 2), confirming previous results (2). The degree of dispersion along the major axis of the lung was expressed as the percentage of the lung content found within the two adjacent slices having the highest amounts. The mean percentage did not fall below 96% (Fig. 3), indicating that the colloidal gold was not appreciably redistributed throughout the lung. Thus there was little relocation to sites up the tracheobronchial tree from where the particles were deposited, inasmuch as there was little or no transfer to the neighboring slice(s) in that direction.

Autoradiographs of lung slices showed that 1 day after microinjection, the gold colloid was all found within about 1000 μm of the deposition site at the pulmonary pleura (Fig. 4). Some ^{195}Au was seen on the epithelium of small bronchioles and near the blood vessel walls, but the amounts there were relatively small.

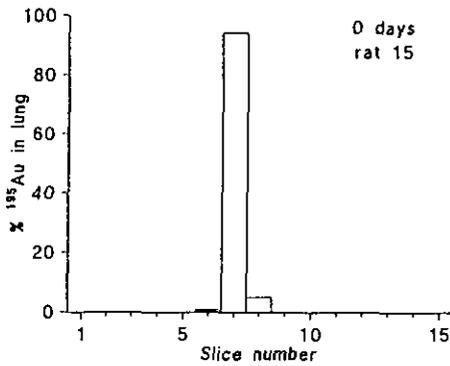


FIGURE 2. ^{195}Au content of 2-mm slices of left lung. Activity expressed as percentage of total ^{195}Au in the lung. Slices numbered from the apical end. Data for one rat sacrificed 4 min after microinjection.

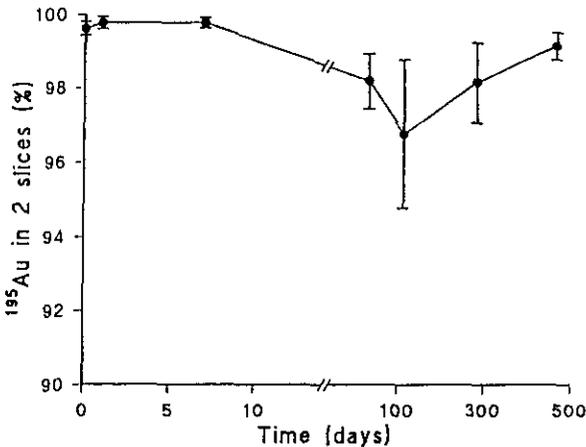


FIGURE 3. Gold colloid distribution as a function of time. Sum of ^{195}Au activity in the two adjacent 2-mm slices having the highest content, expressed as percentage of total ^{195}Au in the lung. Means \pm SEM; see Table I for numbers of animals.

Electron microscopy of lung 1 day after microinjection of colloidal gold showed that the particles were practically all contained within alveolar macrophages (Fig. 5). The same remained true throughout the study. The gold colloid in the macrophages was both in vacuoles and free in the cytoplasm. Autoradiography at later times showed the same general distribution pattern, at least up to 112 days after microinjection, with only small quantities seen further away from the presumed site of deposition (Fig. 6).

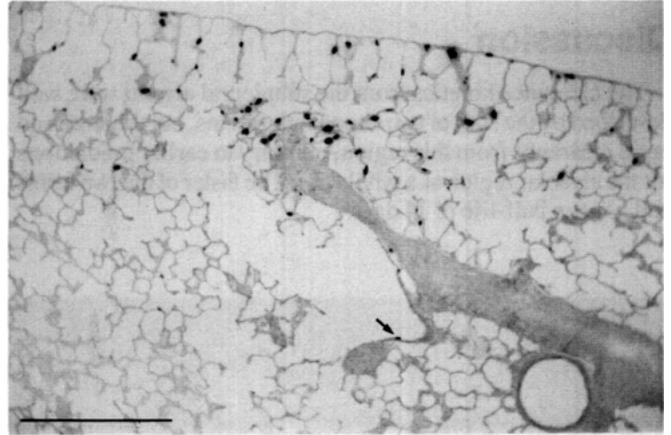


FIGURE 4. Autoradiograph of lung 1 day after microinjection. The dense accumulations of silver grains from ^{195}Au represent all the gold colloid in the section. Note some ^{195}Au near wall of large blood vessel extending to the right, also in association with terminal bronchiole (arrow). Bar = 500 μm .

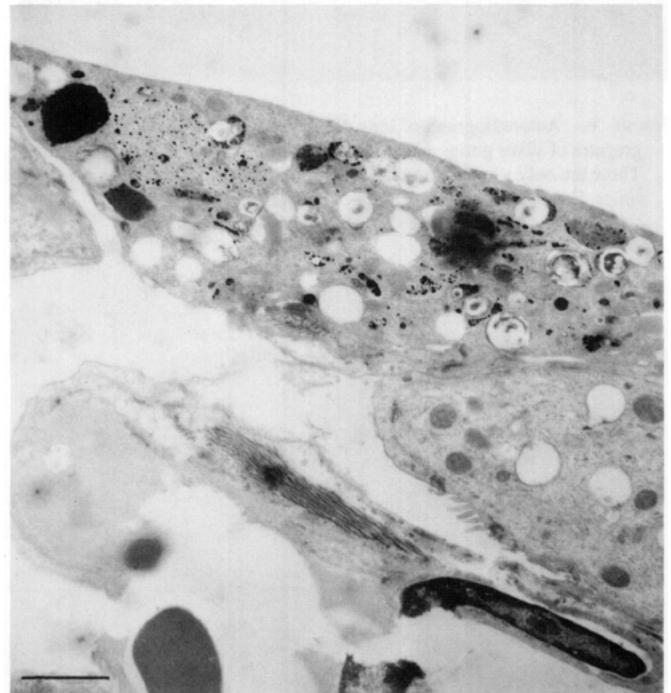


FIGURE 5. Electron micrograph of macrophage after 1 day. The alveolar macrophage is situated close to the pleura and contains many small aggregates of gold colloid particles, both in vacuoles and in the cytoplasm. Note the large aggregate at left. Bar = 3 μm .

A somewhat different retention pattern became evident at 112 days, and by 462 days the pattern was clearly established. Some of the material was seen as more dense deposits close to or within the pleura, and by 462 days this accounted for most of the lung content (Fig. 7). At these sites there was ultrastructural evidence of fibrotic change, with the macrophages packed within the thickened pleura or adjoining interalveolar septae. The macrophages containing the colloidal gold had characteristic vacuoles, which did not stain (Fig. 8). Otherwise, the histological appearance of the lungs was entirely normal throughout the study.

Discussion

The clearance kinetics from the subpleural alveoli were well described by the sum of two exponential terms, but there was no rapid clearance from this region, contrary to earlier predictions for the alveolar region as a whole (8). The faster of the two terms had a mean half-life of 14 days.

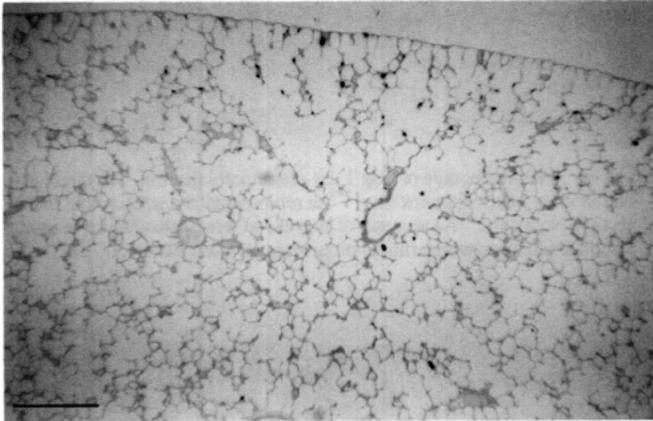


FIGURE 6. Autoradiograph of lung 112 days after microinjection. The aggregates of silver grains again represent all the gold colloid in the section. There are only a few accumulations farther from the pleura than at earlier times. Bar = 500 μm .

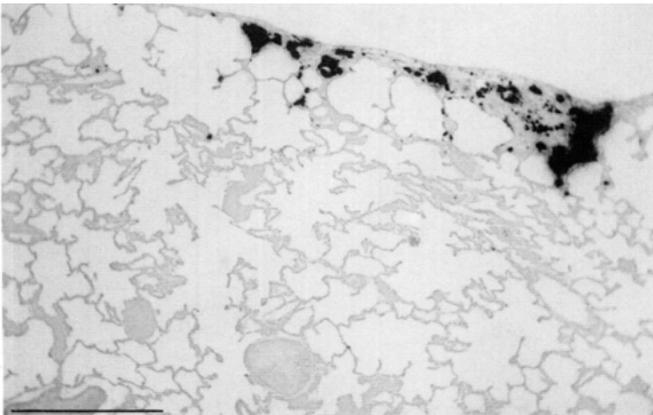


FIGURE 7. Autoradiograph of lung 462 days after microinjection. Most of the gold colloid is as dense aggregates associated with thickened pulmonary pleura. Some ^{199}Au remains as separate foci in normal alveoli, as at left. Bar = 500 μm .

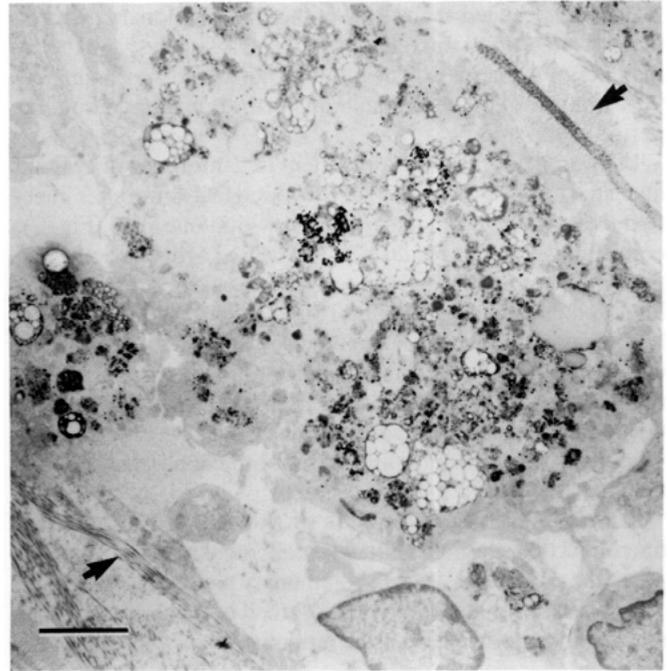


FIGURE 8. Electron micrograph of macrophage after 462 days. The macrophage shown contains many aggregates of colloidal gold particles, mostly in the cytoplasm, and has characteristic electron-lucent vacuoles. It is part of a collection of macrophages within the thickened pleura: note collagen fibers (arrows) around the cell. Bar = 2 μm .

The slower phase described here had a half-time of 583 days, which was long compared with the overall long-term clearance rate in the same rat strain after inhalation. In that case the half-time has been found to be 57–173 days for highly insoluble fused aluminosilicate particles (9) and for the mechanical clearance component of the clearance of cobalt oxide particles (10). Similarly, the clearance half-time in the F-344 rat was 64 days for particles of the mineral tourmaline (11) and 247 days for uranium dioxide (12). Thus, the mean half-time for long-term clearance of particles administered by microinjection was 2–10 times larger than the values for a variety of inhaled particles.

There is more than one possible explanation for this difference. First, the site of deposition after microinjection is purely within alveoli and, moreover, alveoli that are close to the lung periphery, whereas after inhalation, particles are deposited in alveoli throughout the lung, as well as in alveolar ducts. The rate of clearance from more proximal alveoli and alveolar ducts would be expected to be greater than that from subpleural alveoli because of the difference in the distance that macrophages have to migrate to reach the ciliated airways.

A second possibility is that the slower clearance seen after microinjection might be some artifact of the method of administration. This cannot be ruled out, but is perhaps less likely because the fate of the particles soon after administration was the same as seen after inhalation: most of the particles were quickly phagocytized by alveolar macrophages, whose ultrastructural appearance was quite normal (Fig. 5).

The marked variation in the long-term clearance rate between rats was not expected given the uniform experimental conditions.

Again, this could be a function of the relationship of the deposition site to the local microarchitecture of the lung, e.g., the distance to the proximal end of the alveolar duct. However, the individual half-times of the slow phase of clearance did not correlate at all with the depth of the micropipette tip at injection.

The distribution of gold colloid within the left lung changed remarkably little throughout the study. We had observed earlier that by 5 hr particles of colloidal gold were dispersed up to a few hundred micrometers from the subpleural deposition site (3). Here it was found that the range of dispersion was about 1000 μm after 24 hr (Fig. 4), changing very little up to 112 days (Fig. 6), with most of the material that was not cleared from the lung remaining close to the deposition site. Yet, by 112 days, an average of approximately 30% of the initial deposit had been cleared from the lung (Fig. 1). Thus it appears that the fate of the great majority of the particles was either to be cleared completely from the respiratory tract or to remain quite close to the original site of deposition.

Nearly all the colloidal gold particles were found within macrophages at all times from 1 to 462 days (Figs. 5 and 8). Previously we had observed this to be the case by 5 hr after microinjection (3).

The concentration of particle-containing macrophages in thickened pleural tissue from 112 days, and especially at 462 days (Figs. 7 and 8), is of interest. The fibrotic thickening may be due to the presence of the ^{195}Au -labeled gold particles over an extended period of time; by 462 days the mean gamma- and X-ray dose within a radius of 1000 μm of the deposition site was estimated to be 16 Gy. Otherwise it may be an age-related phenomenon: 462 days after injection the rats were 20 months old. In certain respects the finding resembled the subpleural accumulation of asbestos fibers observed by Morgan et al. (1), where the fibers were retained in foamy macrophages in areas of nodular fibrosis.

Regarding the mechanism of the long-term clearance of colloidal gold particles, it should be noted that the fractional lung content was still decreasing at 462 days (Fig. 1), although by this time most of the colloidal gold was within macrophages enclosed within connective tissue at the pleura. Therefore, removal by mucociliary clearance could presumably relate only to those macrophages still free in alveoli. Some clearance could be due to dissolution, which can be gauged from metabolic data to be

reported elsewhere. Otherwise, macrophages could be moving to lymph nodes via the pleural lymphatics; however, this could not account for all of the lung clearance rate because the total body content was also still decreasing at the end of the study.

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