Acute Hemodynamic Effects and Blood Pool Kinetics of Polystyrene Microspheres following Intravenous Administration

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Abstract □ The acute hemodynamic effect of intravenous administration of polystyrene microspheres was investigated and correlated with their distribution pattern and kinetics. Microspheres of three diameters (3.4, 7.4, and 11.6 μm) were administered. The 7.4- and 11.6-μm diameter microspheres were filtered by the pulmonary capillary network following intravenous administration, the majority during the first pass. There was no significant hemodynamic effect following administration of the 7.4- and 11.6-μm diameter microspheres in doses as high as 3.0×10^9 and 6.1×10^8, respectively (total cross-sectional area of 1.3×10^{11} and 6.4×10^{10} μm^2, respectively). Intravenous administration of 3.4-μm diameter microspheres produced significant dose-dependent systemic hypotension and depression of myocardial performance at dosages as low as 1.0×10^{10} (cross-sectional area of 9.1×10^{10} μm^2). These differences in acute hemodynamic effect from the 7.4- and 11.6-μm diameter microspheres may be due to the differences in distribution kinetics and fate of the 3.4-μm diameter microspheres, which readily pass through the lungs to the systemic circulation, with late disposition primarily in the liver and spleen. Although elimination of the smaller spheres from the blood during the first 6–8 min was rapid, *i.e.*, $t_{1/2} = 1.62$ and 1.72 min from the venous and arterial blood circulation, respectively, levels of 10^3 spheres/g of blood were present in the circulation for >1 hr. These findings must be considered in the planning of intravenous administration of microspheres as a drug delivery system to target organs.

Keyphrases □ Microspheres, polystyrene—acute hemodynamic effects and blood pool kinetics following intravenous administration to dogs, drug delivery system to target organs □ Drug delivery system—polystyrene microspheres, acute hemodynamic effects and blood pool kinetics following intravenous administration, dogs □ Kinetics—polystyrene microspheres, distribution and clearance in dogs following intravenous administration, drug delivery system □ Radionuclide imaging agents, potential—^{141}Ce-labeled polystyrene microspheres, acute hemodynamic effects and blood pool kinetics evaluated in dogs following intravenous administration

Systemic toxicity of pharmaceutical agents, particularly those used in the chemotherapy of malignancy, has prompted a search for alternative drug delivery methods. Ideally, these methods should be safe, comfortable, and convenient to the patient. To date, attempts at local therapy with surgical implants or chronic catheter placement have achieved only limited success. Intravascular administration of radioactive microspheres has been utilized safely in animals and humans for many years to assess blood flow patterns (1). Detailed investigation regarding their distribution patterns (2, 3), hemodynamic effects (4–6), and induced pathological changes (7, 8) has begun only recently, stimulated by evidence that pharmaceutical agents may be incorporated into subvisible particulates for intravenous administration, with subsequent local release after the particle lodges in the microcirculation (9–11).

While some deaths were recorded in previous studies on microsphere size, distribution time, and distribution pattern in beagle dogs (2, 4, 8), there was little evidence of tissue damage that could be attributed to the microspheres in specific organs. Due to the lack of significant clinical changes, the present study was undertaken to assess the influence of particle size on the hemodynamic changes associated with the intravenous administration of relatively large aggregate-weight bolus injections of microspheres.

EXPERIMENTAL

Administration of Microspheres—Polystyrene divinylbenzene microspheres labeled with cerium 141 were obtained as a suspension in physiological saline from a commercial supplier[1]. The specific activity was ~50 mCi/g; ~500 μCi was administered to each dog. The number of radioactive spheres was calculated from:

$$\text{number of spheres/mg} = \frac{1.55 \times 10^9}{D^3} \qquad \text{(Eq. 1)}$$

where D is the mean sphere diameter in microns. The nonradioactive spheres[2] were obtained as a 10% suspension in saline. The actual size and number of the microspheres were determined microscopically[3] and with an electronic particle counter[4], respectively. The appropriate dose of microspheres was drawn into a syringe, and an accurate radioactivity count was determined by a radioisotope calibrator[5]. The microspheres then were injected intravenously using a three-way stopcock. After thorough flushing of the syringe and stopcock with saline, the syringe was analyzed to exclude significant retention of the microspheres. Immediately prior to dosage administration, the vial of microspheres was placed in an ultrasonic bath for 15 min to ensure dispersion of the spheres. A surfactant, polysorbate 80, was not used in the preparation of the microspheres due to its adverse hemodynamic effect (12).

Preparation of Animals—Female beagle dogs, 13.0–15.0 kg, were randomly assigned to receive 3.4, 7.4, or 11.6-μm diameter microspheres. Following endotrachael intubation, anesthesia was maintained with a mixture of 1–2.5% halothane (depending on the blood pressure response and desired level of anesthesia) and oxygen delivered by a mechanical respirator. A left thoracotomy was performed, and a micromanometer-tipped catheter[6] was inserted into the left ventricle to measure systolic pressure. The rate of change of the left ventricular pressure (dp/dt) was derived electronically. A pair of ultrasonic piezoelectron crystals was implanted in the left ventricular subendocardial wall to measure regional myocardial wall motion and, by inference, left ventricular volume.

Micromanometer-tipped catheters[7] were inserted into the pulmonary artery and descending aorta to measure pulmonary artery pressure. A thermistor-tipped catheter[8] also was placed in the pulmonary artery to enable serial thermodilution cardiac output determinations. Catheters were positioned in the right atrium and descending aorta to allow ease in blood sampling from the venous and arterial circulation, respectively. The surface ECG was monitored continuously. The micromanometer-tipped catheters were referenced to zero and calibrated with a mercury manometer before insertion. Correction for micromanometer transducer drift was performed by superimposing electronically derived mean

[1] Nuclear Products Division, 3M Co., St. Paul, Minn.
[2] Dow Diagnostics, Indianapolis, Ind.
[3] Zetopan universal research microscope, Reichert, Austria.
[4] Coulter Counter model TAII, Coulter Electronics, Hialeah, Fla.
[5] Model CRC-6A, E. R. Squibb & Sons, Princeton, N.J.
[6] Konigsberg Co., Pasadena, Calif.
[7] Millar Co., Houston, Tex.
[8] 4F pediatric thermodilution catheter 702216, Edwards Laboratory, Santa Ana, Calif.

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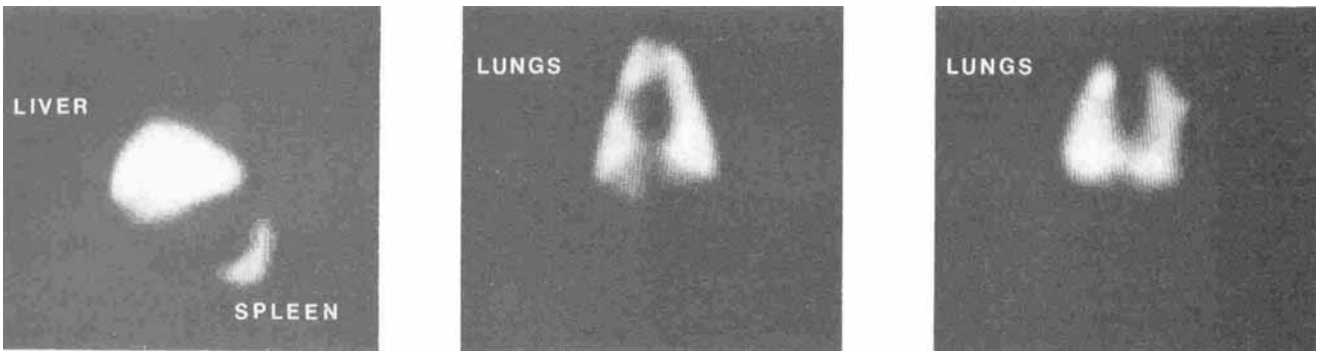

Figure 1—*Distribution of ^{141}Ce-labeled microspheres as determined by external nuclear imaging 3.5 hr following intravenous administration. The larger microspheres (7.4 μm in Dog 6 and 11.6 μm in Dog 4) were localized in the lungs, whereas the smaller (3.4 μm in Dog 1) microspheres distributed to the liver and spleen.*

pressure against that of a fluid-filled catheter system connected to a pressure transducer[9] positioned at midchest level.

Hemodynamic Protocol—Following the surgical instrumentation, each animal was monitored for 30 min to ensure hemodynamic stability. Continuous direct measurements of the surface ECG, pulmonary artery pressure, systemic artery pressure, left ventricular pressure, rate of change of left ventricular pressure (dp/dt), and left ventricular segmental wall motion were recorded on magnetic tape[10]. Data were printed at predetermined intervals[11]. Serial duplicate cardiac output determinations were recorded at 20-min intervals[12]. Maximum values of $+dp/dt$ and $-dp/dt$ were determined from electronic differentiation of the left ventricular pressure waveform.

The systolic shortening fraction, which is a measure of the left ventricular regional wall motion, was calculated with ultrasonic segment-length measurement *via* the piezoelectron crystals implanted in the left ventricular subendocardium using the following expression: (end diastolic length − end systolic length)/end diastolic length. All animals were stable throughout the 30-min control period. A control animal was monitored for 3 hr following a placebo injection of physiological saline; this animal remained stable.

Following the 30-min control period, a measured number of ^{141}Ce-labeled microspheres of a given size (Table I) was administered as a bolus injection into the right atrium. Two dogs received the 3.4-μm diameter size, two received the 7.4-μm diameter size, and two received the 11.6-μm diameter size microspheres. Hemodynamic measurements and venous and arterial blood samples were taken every 2 min for 40 min, every 5 min for 20 min, and then every 10 min for the next 2 hr.

At the conclusion of the 3-hr monitoring period, a second bolus injection of nonradioactive microspheres of the same diameter (Table I) was administered slowly (~8 ml/min) to assess possible hemodynamic effects of larger doses. Then 3.5 hr after administration of the radioactive microspheres, each animal was sacrificed and sent for nuclear scanning. Two additional dogs were instrumented similarly for additional dosing with nonradioactive 3.4-μm diameter microspheres to complete the dose–response curve.

Scanning of Dogs—With a digital computer[13] attached to a scintillation camera[14], distribution of the radioactive microspheres was assessed by whole body scanning.

Blood Level of Spheres—Blood samples removed during the 3-hr study were analyzed for radioactivity using a well-type γ-counter[15]. Application of the appropriate calibration equation yielded results in microspheres per gram of blood.

RESULTS

Nuclear Scanning—Consistent with previous results, the radioactivity was confined principally to the lungs (>90%) in animals administered ^{141}Ce-labeled 7.4- and 11.6-μm diameter microspheres. The animals given 3.4-μm diameter microspheres had little activity (~12%) remaining in the lungs 3.5 hr following administration, the majority (~83%) of the radioactive particles having accumulated in the liver and spleen (Fig. 1). Table II summarizes the results of external analysis of these organs and demonstrates the clear tendency of the microspheres to distribute in a size-dependent organ-specific pattern.

Table I—Dosing Information

Dog	Microsphere Size, μm	Number	CSA[a], μm²	Weight, mg
Control	—	—	—	—
Control-NR[b]	3.4	1.41×10^{11}	1.28×10^{12}	3525
1	3.4	6.25×10^{8}	5.68×10^{9}	15.6
2	3.4	8.62×10^{8}	7.83×10^{9}	21.6
2NR	3.4	9.45×10^{9}	8.58×10^{10}	236
3	11.6	1.09×10^{7}	1.15×10^{9}	10.9
3NR	11.6	2.02×10^{8}	2.14×10^{10}	202
4	11.6	2.13×10^{7}	2.25×10^{9}	21.3
4NR	11.6	6.06×10^{8}	6.40×10^{10}	606
5	7.4	4.26×10^{7}	1.83×10^{9}	11.2
5NR	7.4	1.50×10^{9}	6.45×10^{10}	394
6	7.4	5.52×10^{7}	2.37×10^{9}	14.5
6NR	7.4	3.00×10^{9}	1.29×10^{11}	789
7NR	3.4	1.00×10^{9}	9.08×10^{9}	25.0
7LNR[c]	3.4	1.00×10^{10}	9.08×10^{10}	250
8NR	3.4	9.55×10^{10}	8.67×10^{11}	2388

[a] CSA = cross-sectional area. [b] NR = dose of nonradioactive spheres. [c] LNR = large dose of nonradioactive spheres.

Blood Pool Kinetics—Serial measurements of circulating microsphere levels from venous and arterial sites drawn following intravenous administration of ^{141}Ce-labeled microspheres are shown graphically by blood level profiles in Fig. 2. The elimination rate of the initial stage following intravenous administration is summarized in Table III. The elimination rate constant was determined by the least-squares method using first-order kinetics. These data, along with the ratios of venous and arterial blood levels presented in Table IV, demonstrate that few of the 7.4- and 11.6-μm spheres reached the arterial circulation; 98% of the 7.4- and 11.6-μm spheres were eliminated from the circulating blood pool in the first 2 min. For the 3.4-μm spheres, 10–12 min was required before 98% was eliminated. Within 10 min, the concentrations of the 11.6, 7.4, and 3.4-μm spheres in venous circulation were <10, ~10, and ~10^3–10^4 spheres/g of blood, respectively. Therefore, a ratio of 0.81 for venous to arterial circulation for the 3.4-μm spheres indicates that the smaller spheres passed readily from the venous to arterial circulation, with high arterial levels present virtually immediately after administration. The

Table II—Microsphere Distribution in Specific Organs 3.5 hr after Intravenous Administration Determined by External Imaging

Size, μm	Lungs, %	Liver, %	Spleen, %	Total in the Three Organs, %
3.4	12	73	10	95
7.4	92	(~2%)[a]		94
11.6	93	—[b]	—[b]	93

[a] Combined level of activity in liver and spleen was ~2%. [b] Negligible level in liver and spleen.

[9] Bentley Trantec mode 800, Irvine, Calif.
[10] Honeywell Co., Denver, Colo.
[11] Brush recorder 200, Gould Co., Cleveland, Ohio.
[12] Model 9520 Cardiac Output Computer, Edwards Laboratories, Santa Ana, Calif.
[13] PDP-11/15, Digital Equipment Corp., Maynard, Mass.
[14] Pho/gamma LFOV, Searle Radiographics, Des Plaines, Ill.
[15] Spectroscaler, Picker Nuclear Corp., Northford, Conn.

Table III—Elimination of Microspheres from Blood at Initial Stage following Intravenous Administration [a]

Sphere Size, μm	Elimination Rate, min^{-1}		$t^{1/2}$, min	
	Venous Blood	Arterial Blood	Venous Blood	Arterial Blood
3.4	−0.426 ± 0.078	−0.403 ± 0.053	1.62	1.72
7.4	−0.810 ± 0.168	−0.776 ± 0.140[b]	0.86	0.89
11.6	−1.33 ± 0.320	—	0.52	—

[a] Average of two dogs in each size. [b] Essentially all 11.6-μm spheres and >95% of the 7.4-μm spheres were removed from the systemic circulation during the first pass. Therefore, this elimination rate is for a relatively small number of spheres that reached the arterial circulation.

blood level profiles show that the venous and arterial levels of 3.4-μm spheres remained essentially the same over ~3 hr, suggesting that these spheres recirculate rather freely. Even after 2 hr, the level in the circulation was 10^2–10^3/g of blood.

Hemodynamic Data—The 7.4- and 11.6-μm diameter microspheres were not associated with significant hemodynamic changes in the dosage ranges administered. The 3.4-μm diameter microspheres, administered at a similar total cross-sectional area dosage, induced significant dose-dependent deterioration in some of the hemodynamic parameters monitored (Fig. 3). These changes are illustrated more clearly in Fig. 4, which depicts a slow continuous recording of the first 7 min following intravenous administration of 1.41×10^{11} 3.4-μm diameter microspheres. A profound drop in the systemic blood pressure, with associated deterioration in $-dp/dt$, $+dp/dt$, and systolic segment shortening, occurred within several minutes of microsphere administration. A later secondary elevation in pulmonary artery pressure also occurred, probably due to a neurohumoral reflex response to the severe systemic hypotension.

These acute changes are most consistent with peripheral vascular collapse, not unlike those seen in anaphylactic shock. That direct myocardial depression occurs, but is not the predominant cause of shock, is evidenced by the lack of elevation in left ventricular end diastolic pressure and the reduction in end diastolic segment length. Only after a period of profound hypotension was there evidence of depressed myocardial function, *i.e.*, decreased $+dp/dt$ and decreased systolic segment shortening. Sustained ventricular tachycardia developed late following administration of 3.4-μm diameter microspheres in one animal. Cardiac output also was well maintained initially, dropping only after sustained hypotension, with its deleterious effect on the myocardium, had been present. Progressive increases in dosage of these 3.4-μm diameter microspheres resulted in progressive worsening of the severity (Fig. 5) and duration of the systemic hypotension.

DISCUSSION

Intravascular injection of particulate matter has been performed to image organs and assess blood flow characteristics. Early investigators utilized distribution kinetics of different size microspheres to assess the physical dimensions and biological characteristics of the microcirculation of various organs (1). As early as 1961, it was reported that the majority of microspheres less than 4.0 μm in diameter injected into the femoral vein passed through the pulmonary capillary network, whereas only a relatively small portion of similarly injected microspheres of >8.0 μm diameter passed into the systemic circulation (13). Previous work in our laboratories amplifies these points and, along with the present results, demonstrates that localization of the 7.4- and 11.6-μm diameter microspheres in the lungs, presumably by mechanical filtration, occurs in the first 2 min. This rapid localization suggests passive filtration and is consistent with ultrastructure studies of the pulmonary capillary network (14, 15) and the results of other investigators working with similarly sized microspheres (13, 16). The localization in the lungs appears to be complete for the 11.6-μm spheres, but there is evidence of gradual clearance and relocation in the liver and spleen of the 7.6-μm spheres.

Because of the recirculation characteristics of the microspheres of <4.0-μm diameter, their use was generally abandoned since they were of little value in organ imaging or assessing blood flow patterns. Of considerable interest is the recent documentation (2, 8) that, following intravenous administration, the 3.4-μm diameter microspheres pass through the pulmonary circulation and distribute in a pattern consistent with reticuloendothelial system uptake. This localization of the 3.4-μm diameter microspheres in the liver and spleen requires considerably more time than localization of the larger (≥7.4-μm diameter) microspheres in the lungs, suggesting a biologically active process, *e.g.*, phagocytosis (17) rather than passive filtration. The prolonged time course required for the 3.4-μm diameter microsphere clearance from the circulating blood pool correlates well with the duration of their acute hemodynamic effects (*vide supra*) and suggests some association with blood cells, a gradual release from the lungs, or a combination of the two.

Allen *et al.* (18) suggested that pulmonary hypertension is the most sensitive sign of hemodynamic deterioration following intravenous injection of microspheres larger than 13.5-μm in diameter. These authors also demonstrated a dose–response curve that correlated the increase in pulmonary artery pressure with increasing dosage and increasing sizes of administered microspheres (5). As expected from their dose–response curve, no significant pulmonary artery pressure rise was observed following intravenous administration of 7.4- and 11.6-μm diameter microspheres. While these particles are smaller than those that Allen utilized, they are nearly completely filtered by the pulmonary capillary network during the first pass through the pulmonary circulation.

The number of 3.4-μm diameter microspheres required to produce serious hemodynamic compromise is much smaller than would be predicted using the formula suggested by Allen *et al.* (5). This result appears due to the demonstrated ability of these small microspheres to pass through the pulmonary vascular network. Indeed, the period of hemodynamic compromise coincides with the time during which the 3.4-μm

Table IV—Ratio of Microsphere Levels in Venous and Arterial Blood (VBC/AVC) Samples following Intravenous Administration

Minutes	VBC/AVC		
	3.4 μm	7.4 μm	11.6 μm
2	0.81	6.4	85
4	0.69	3.1[a]	18[a]
10	0.81[b]	—[c]	—[c]
20	1.0	—	—
30	1.1	—	—

[a] Approximate venous concentration is <50 spheres/g of blood. [b] Venous concentration is 10^3–10^4 spheres/g of blood. [c] Venous concentration is <10 spheres/g of blood.

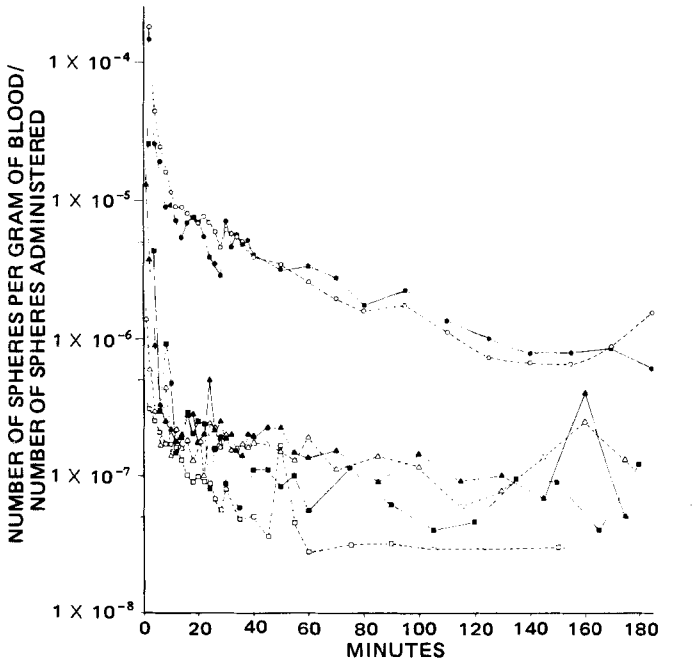

Figure 2—*Level of ^{141}Ce-labeled microspheres in venous and arterial samples following intravenous administration in beagle dogs. Key: ●, 3.4 μm, venous; ○, 3.4 μm, arterial; ▲, 7.4 μm, venous; △, 7.4 μm, arterial; ■, 11.6 μm, venous; and □, 11.6 μm, arterial.*

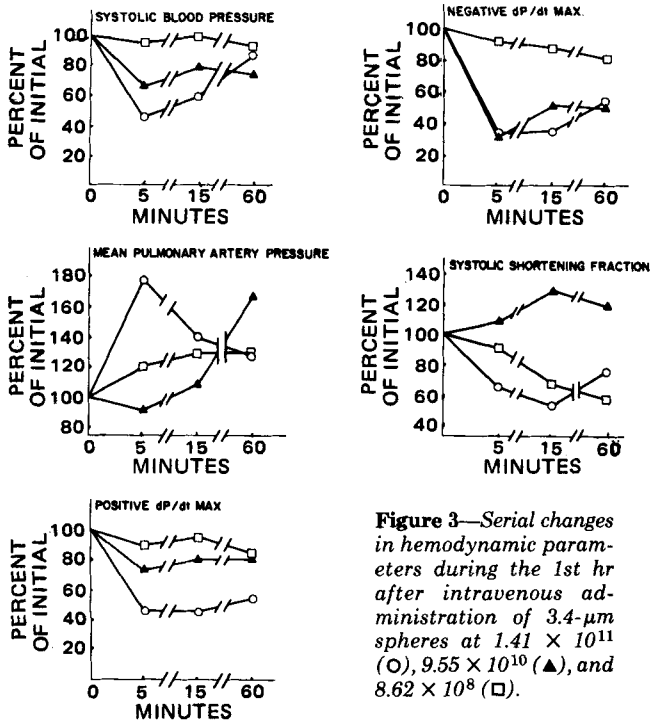

Figure 3—*Serial changes in hemodynamic parameters during the 1st hr after intravenous administration of 3.4-μm spheres at 1.41 × 10^{11} (○), 9.55 × 10^{10} (▲), and 8.62 × 10^{8} (□).*

diameter microspheres are numerous in the circulating blood pool (Figs. 2–4).

At present, the cause of the observed acute hemodynamic deterioration is unknown. Because of its similarity to anaphylaxis, a possible explanation would be the liberation of vasoactive amines as the microspheres pass through the pulmonary and peripheral vascular networks (19, 20). Alternatively, the hypotension could reflect a neurogenic reflex caused by high levels of the microspheres entering the central nervous system (21, 22). The former appears more likely based on the sequence of hemodynamic changes and the platelet clumping that occurred only around the 3.4-μm diameter microspheres upon histological examination (8, 23). The possibility that toxic monomers leaching from the smaller microspheres could promote vasomotor instability and initiate anaphylaxis was considered and discarded when injection of 20 ml of supernate from the sphere suspension into a dog caused no change in blood pressure over 0.5 hr. Furthermore, *in vitro* analyses of the supernate included UV spectra devoid of any peaks characteristic of monomer and a clean TLC analysis of a chloroform extract. The amount of residue left after drying the chloroform extract was too small to quantitate or to obtain an IR spectrum.

Incorporation of pharmacotherapeutic agents into microspheres for intravascular administration appears feasible from a technological standpoint and may have the advantages of achieving locally high drug concentrations in the target organ. Larger size (7.4- and 11.6-μm diameter) microspheres lodge in the pulmonary capillary network whereas 3.4-μm diameter microspheres appear to be taken up by the reticuloendothelial system. These differences in distribution fate following intravenous administration may be of significance in planning future chemotherapy of disease of the lungs and reticuloendothelial system.

CONCLUSION

This study demonstrates that intravenous administration of particulate matter may be associated with significant acute hemodynamic deterioration in the experimental animal and reveals that very small microspheres (3.4-μm diameter) have a different distribution fate and time course than larger microspheres (≥7.4-μm diameter). Since the difference in distribution is associated with a more severe, acute hemodynamic effect than would be expected based on size and number alone, the limitation of particulate material for target organ drug administration may be the ability to incorporate a therapeutic dose in a finite number of particles

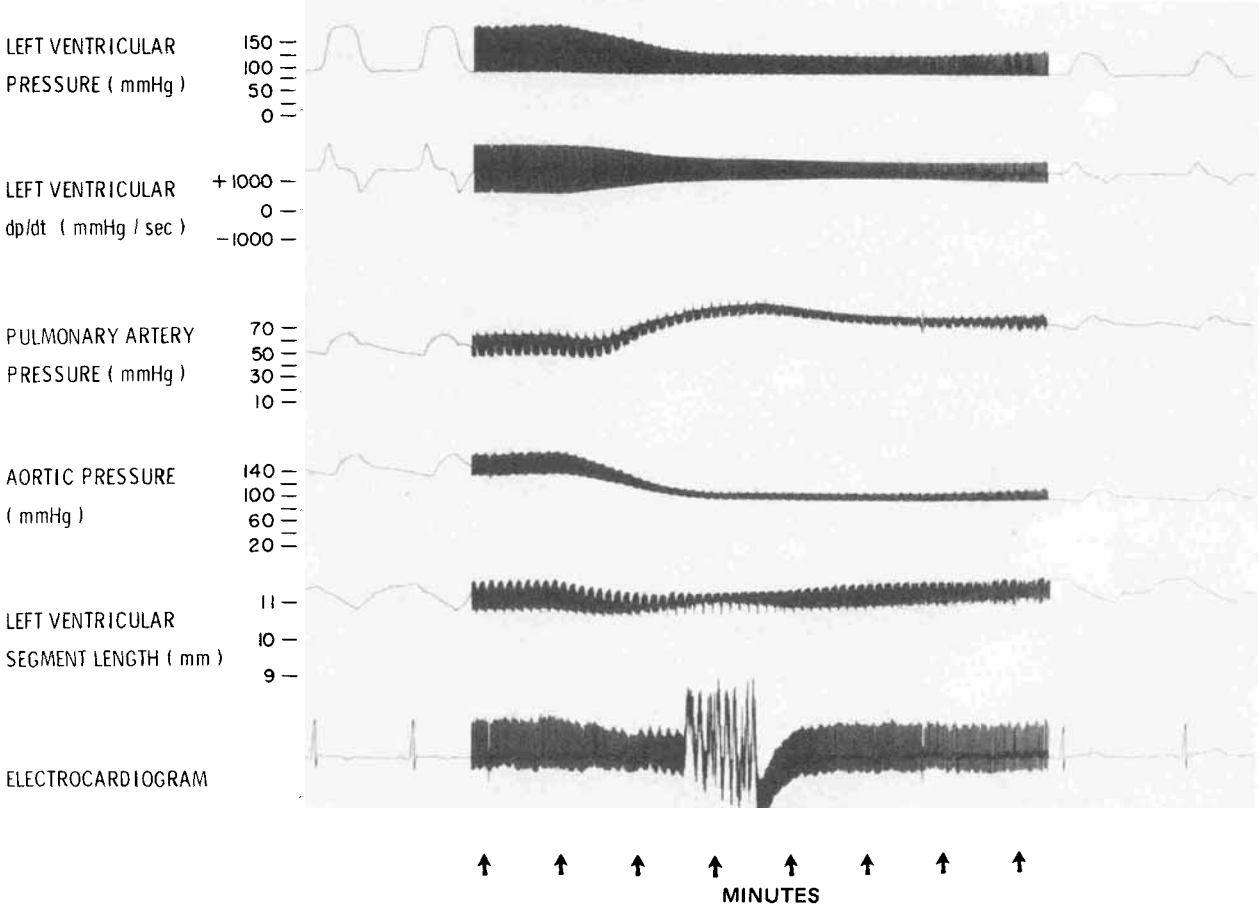

Figure 4—*Continuous display of the hemodynamic changes in the first 7 min following intravenous administration of 1.41 × 10^{11} microspheres of 3.4-μm size (area = 1.28 × 10^{12} μm² and weight = 3525 mg). A short lead and exit strip at chart speed of 25 mm/sec are provided to allow appreciation of individual waveforms.*

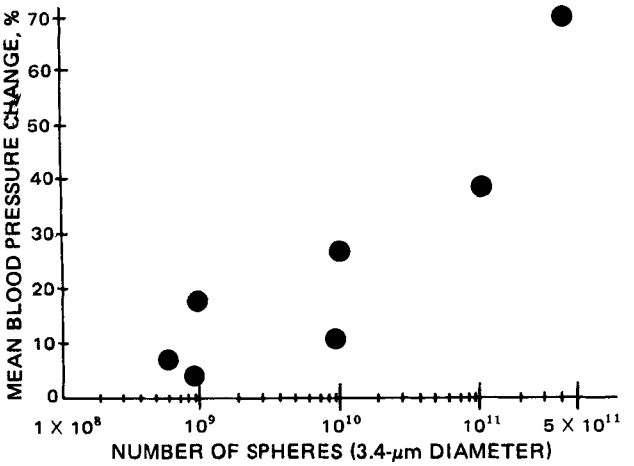

Figure 5—*Acute effect of intravenously administered 3.4-μm spheres on mean blood pressure as a function of concentration (r = 0.82).*

determined by their hemodynamic effect. Nevertheless, bolus intravenous injections of ~1 g of microspheres were administered to beagle dogs (13–15 kg) without serious consequences.

Therefore, the potential exists to incorporate sufficient amounts of drug into the microsphere during manufacture to provide therapeutic dosages. The influences of particle composition, surface charge, dissolution rate, and antigenicity will need to be determined. Furthermore, the acute hemodynamic effect evidenced following administration of the 3.4-μm diameter microspheres was consistently brief and reversible in the smaller dosage range. This finding suggests that a different dosage pattern, *i.e.*, smaller, more frequent administrations rather than a large bolus injection, or slower administration could avoid the risks of hemodynamic effects, thereby delivering a selected chemotherapeutic agent in relatively high local tissue concentrations to the target organ with safety.

Finally, both histological and hemodynamic studies of the long-term effects of repeated intravascular injections of microspheres must be performed to exclude the possibility of irreversible tissue damage with chronic administration.

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