STUDIES OF ACUTE CARDIOPULMONARY TOXICITY OF Sn-MACROAGGREGATED ALBUMIN IN THE DOG

D. R. Allen, W. B. Nelp, F. Cheney, and D. E. Hartnett

University of Washington School of Medicine, Seattle, Washington

A dog model was developed for testing the effect of intravenous injection of stannous macroaggregated albumin particles (Sn-MAA). Throughout the study the most sensitive and conveniently measured indicator of acute particle toxicity was an immediate elevation in mean pulmonary artery pressure. In the studies of normal dogs it was possible to construct a dose-response curve of toxicity relating increase in mean pulmonary artery pressure to the number of 30-micron Sn-MAA particles injected as a single bolus. The dose-response curve indicated the first sign of acute toxicity occurred when approximately 100 times the usual lung scan dose (LSD) was given. A safety factor of 100 in humans for this product was derived by relating the number of particles injected to body weight. In additional studies in dogs with experimental pulmonary hypertension there was no significant effect noted after the single injection of 1×10^6 particles, which is the equivalent of three human lung scan doses of particles.

A number of radiopharmaceutical developments have made lung perfusion imaging with particles labeled with ^{99m}Tc available to many nuclear medicine laboratories (*1–11*). In 1972 Subramanian and coworkers presented a modified preparation of MAA using stannous chloride which could be stored in kit form and instantly and completely labeled with ^{99m}Tc by the addition of pertechnetate (*12,13*). Because of the stability of the kit and the simplicity of labeling, the product was studied in our laboratories for its overall suitability for clinical studies. As part of the product evaluation, acute cardiopulmonary toxicity studies were performed using a comprehensive dog model. This type of evaluation appeared desirable because very few detailed studies of MAA toxicity have been published since those of ^{131}I-MAA by Taplin and associates in 1966 (*14*).

The purpose of this report is to present the dog model used for these studies and report the results of the acute toxicity of the Sn-MAA particles in normal dogs and in those with pulmonary hypertension.

METHODS

Particles of human serum albumin were prepared by adding 25 mg of human serum albumin (25% Hyland salt poor), 100 mg of sodium acetate, and 20 mg of $SnCl_2$ in 1 *N* HCl to a 50-ml serum vial containing a Teflon stir bar. The final pH of the solution was adjusted to 5.5 by adding 1 *N* HCl or 1 *N* NaOH and then brought to a volume of 25 ml by the addition of sterile pyrogen-free water. The reaction mixture was stirred rapidly (magnetic) while heating at 80°C to produce the macroaggregated particles. The sodium acetate buffer was removed by terminal saline wash of the particles after which the particles were resuspended in saline. All procedures were carried out asceptically using sterile and pyrogen-free materials (*13*).

When the particle size reached 30–50 microns, aggregation was stopped by immersing the reaction vessel in cold water. If particle size exceeded the 30–50 micron range, the size could be reduced by forcing the mixture through an aperture. This could be done by placing the aggregate mixture in a 50-cc syringe and attaching it to a Harvard infusion pump which emptied the contents through a 26-gage needle at a rate of 38 ml/min. For these experiments the mean particle size was maintained at 30 microns, a particle size commonly used for human lung perfusion studies.

The numbers of particles injected in these experiments were standardized in terms of lung scan dose equivalents. A lung scan dose (LSD) of particles

Received Nov. 3, 1973; original accepted Jan. 16, 1974.

For reprints contact: D. Allen, Div. of Nuclear Medicine, University of Washington Medical Center, Seattle, Wash. 98105.

was established as 14,000 particles/kg body weight. This number is equal to approximately 1×10^6 particles for a 70-kg man. Each 1 million particles contained approximately 1.0 mg human serum albumin, 0.004 mg of stannous chloride, and 1.0 ml of normal saline solution.

Particles prepared in this manner can be instantly labeled with ^{99m}Tc by adding 1.0–4.0 ml of $^{99m}TcO_4$ pertechnetate in saline to an aliquot of 1 million particles suspended in normal saline solution contained in a multidose vial. In these studies, labeled aliquots showed 97–99% labeling efficiency as determined by ascending paper chromatog aphy (95% methanol:5% water). To ascertain that the particles were trapped in the lung vasculature bed, distribution studies of labeled aliquots were carried out in mice and rabbits. Following intravenous injection of the labeled material, generally greater than 95% of the dose was immediately recovered in the lungs. In the dog studies reported herein, unlabeled particles were used.

For the particle toxicity studies 11 dogs (15–25 kg) were used. The animals were maintained on light pentobarbital anesthesia and mechanically ventilated with room air by a Harvard animal respirator. Polyethylene catheters were inserted in the femoral artery and vein and a balloon-tipped Swan Gantz catheter passed through an external jugular vein into the pulmonary artery. These catheters were used for pressure measurements, particle injections (femoral vein), cardiac output measurements, and for arterial and mixed venous blood sampling.

The pulmonary and femoral arterial catheters were interfaced to a Beckmann Type R Dynograph with Strathem pressure transducers for continuous pressure recording. Expired air and blood gas values were determined periodically with a Laboratory Instrument, Inc. Model 113 gas analyzer and a Model 182 Co-Oximeter. Cardiac output was measured within 2 min after particle injections using indocynine green dye with a Gilford Model 103 Cuvette Densitometer recorded with a Varian Instruments Model G-2000 strip chart recorder.

Measurements that were used directly or calculated are included in Table 1.

TABLE 1. MEASUREMENTS READ OR CALCULATED

1. $\overline{PaP}$—mean pulmonary artery pressure; cm H_2O
2. PaW—pulmonary artery wedge pressure; cm H_2O
3. $\overline{Ap}$—mean arterial pressure; mmHg
4. PaO_2—partial arterial oxygen tension; mmHg
5. $PaCO_2$—partial arterial carbon dioxide tension; mmHg
6. $\dot{Q}_t$—cardiac output; liters/min
7. EKG—electrocardiogram
8. P_AO_2—alveolar oxygen tension; mmHg
9. P_ACO_2—alveolar carbon dioxide tension; mmHg
10. Vd/Vt—deadspace-to-tidal volume ratio
11. S_VR—systemic vascular resistance; dyn sec cm^{-5}
12. P_VR—pulmonary vascular resistance; dyn sec cm^{-5}
13. AP—airway pressure; cm H_2O
14. HR—heart rate
15. $Q_s/\dot{Q}_t$—right-to-left pulmonary shunt; %

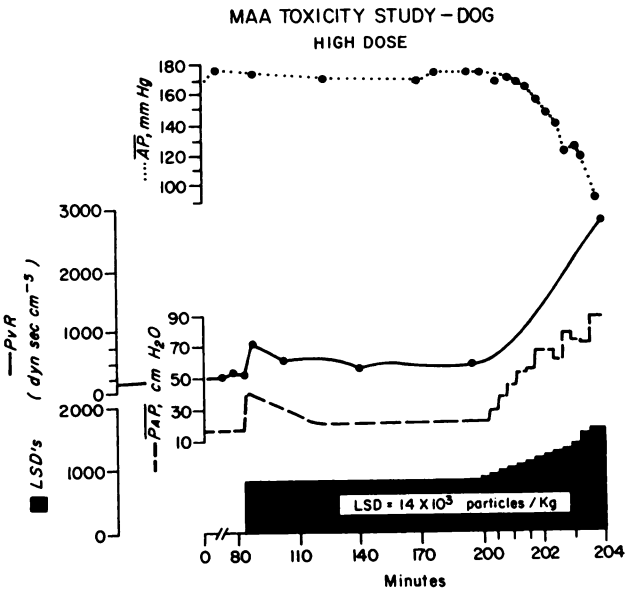

FIG. 1. An initial injection of 750 LSDs produced marked elevation in $\overline{PaP}$ and P_VR. Arterial pressure remained unchanged. $\overline{PaP}$ and P_VR returned to baseline values within 45 min. Multiple injections of particles over 4 min (starting at 200 min) produced dramatic and stepwise increases in $\overline{PaP}$ and P_VR. Rapid fall in $\overline{AP}$ was noted. This ordinarily signaled impending death of animal.

RESULTS

The effect of injecting large numbers of particles in the normal dog is illustrated in Fig. 1. In this graph changes are shown in $\overline{PaP}$, $\overline{Ap}$, and P_VR. The number of particles injected is recorded in terms of LSDs as standardized on a weight basis (horizontal axis).

As seen in Fig. 1, an initial injection of 750 LSDs (20 sec) caused a prompt and sustained elevation in $\overline{PaP}$ and P_VR. The change in P_VR is determined according to the relationship

$$P_VR = \frac{\overline{PaP}\text{-PaW} \times 80}{\dot{Q}_t}.$$

In this instance no change in $\dot{Q}_t$ (cardiac output) or PaW occurred. There were also no changes in any of the other variables measured. After 200 min the dog in Fig. 1 was subjected to a separate series of rapid incremental injections of particles. One can see with each injection the stepwise increase in $\overline{PaP}$ and P_VR. After six increments of particles were injected, a progressive decrease in the mean arterial pressure occurred. At this stage of the experiments, the animal was severely insulted and there was a pro-

TABLE 2. CHANGES IN VARIOUS PARAMETERS MEASURED IN CONJUNCTION WITH EXPERIMENT IN FIG. 1

Cumulative LSDs*	0	3.5	750	750	750	754	1,450
Time†	0	80	85	140	170	200	204
$\dot{Q}_t$	4.05	3.87	3.58	3.33	3.38	2.99	3.08
$Q_s/\dot{Q}_t$	7.0	9.0	9.0	4.0	6.0	4.0	23.0
PaO_2	88.5	83.0	74.0	80.0	63.6	80.0	50.0
$PaCO_2$	35.0	35.5	35.0	33.5	33.0	29.0	32.7
$\overline{Bp}$	166.0	178.0	175.0	169.8	168.2	174.8	91.5
HR	145.0	150.0	140.0	150.0	140.0	140.0	140.0
$\overline{PaP}$	15.6	17.0	38.0	27.6	23.3	20.3	95.9
PaW	3.0	3.5	2.0	3.0	3.0	4.0	0
P_VR	250.1	279.0	804.4	583.0	473.0	435.0	2,490.0
Vd/Vt	0.43	0.51	0.43	0.47	0.46	0.36	0.36

* (1 LSD = 14 × 10^3 particles/kg).
† Time course measured in minutes after first injection of Sn-MAA.

gressive drop in systemic blood pressure ($\overline{AP}$). At this stage there was also a decrease in PaO_2 and an increase in $Q_s/\dot{Q}_t$. It was of interest that significant changes in other measured variables were not observed until the animal was near death at the end of the experiment (Table 2).

The effects of injecting smaller numbers of particles in normal dogs are shown in Fig. 2. In this instance effects were limited to changes in $\overline{PaP}$ and P_VR. An initial injection of 125 LSDs produced a small elevation in $\overline{PaP}$ which returned to nearly baseline readings after 30 min but remained slightly elevated for the remainder of the experiment. A slight elevation in P_VR was also noted that followed closely the changes in $\overline{PaP}$. After 30 min repeated bolus injections of 125 LSDs produced similar results. Throughout this type of experiment it was clear that an elevation in the $\overline{PaP}$ proved to be the most easily measured and consistently most sensitive indicator of toxic effect of particle injection.

From the variety of studies performed in the 11 normal animals it was possible to derive a dose-response curve using a definite elevation in $\overline{PaP}$ as the most sensitive indicator of toxicity. The results of this analysis are seen in Fig. 3. As seen in Fig. 3, no changes were observed when up to 60 LSDs were given as a single injection (20 sec). The first evidence of MAA toxicity occurred when single injections of 100–125 LSDs were given. Beyond this dose larger numbers of particles tended to produce consistent and progressively larger elevations in $\overline{PaP}$, up to 300% greater than the baseline normal value. From this type of dose-response curve one could conclude that the toxic response would be expected when 100 or more LSDs were injected as a single bolus. In man this could be equated to approximately 100 million 30-micron-sized particles.

An additional consideration and perhaps more important one was to evaluate the effect of single

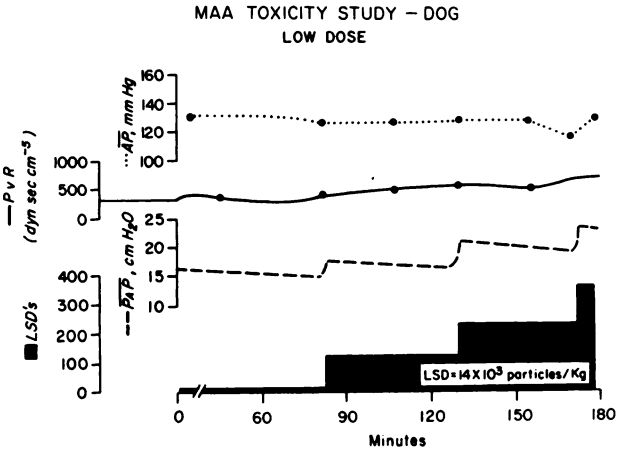

FIG. 2. Effect of repeated injections of 125 LSDs in same animal. Each injection produced immediate elevation in $\overline{PaP}$. In minutes following injection, the $\overline{PaP}$ returned toward baseline value but never returned to normal. A slight increase in P_VR is also noted during course of experiment. $\overline{AP}$ remained normal during course of experiment.

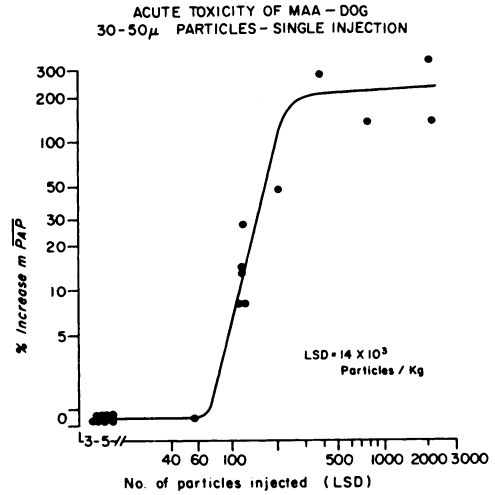

FIG. 3. Curve indicates that single injections of 30–50μ MAA particles would not be expected to increase PaP until more than 100 LSDs are injected. Injections of 300 or more LSDs routinely produced sustained elevations in $\overline{PaP}$.

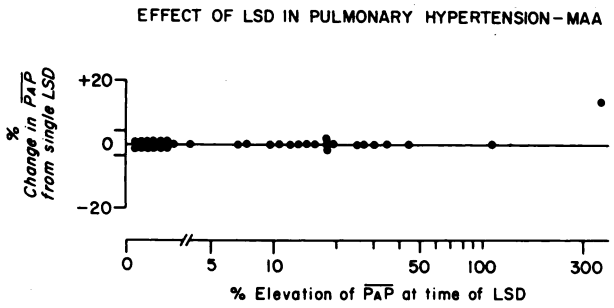

FIG. 4. When single injection of scanning dose of particles was injected into animal with various degrees of pulmonary hypertension (horizontal axis), no further increase in $\overline{PaP}$ was noted except in one animal. In this instance $\overline{PaP}$ was three times normal and additional injection of LSD caused further 14% increase in $\overline{PaP}$.

human lung scan doses of MAA in the abnormal state. This effect was evaluated in animals who had sustained pulmonary hypertension by virtue of previous injection of macroaggregates. After pulmonary hypertension was produced, an additional single dose of 1 million particles was given. On the basis of body weight this was equivalent to giving approximately three human diagnostic lung scan doses of particles to the dog. As seen in Fig. 4 this maneuver generally produced no further increase in $\overline{PaP}$ even in those animals with severe pulmonary hypertension. One exception was an animal near death with severe pulmonary hypertension (last point on right of Fig. 4) when the injection of a LSD resulted in an additional 10% elevation in $\overline{PaP}$.

DISCUSSION

Throughout these experiments it was evident that the most sensitive and easily measured toxic response to the bolus injection of MAA in the normal dog was an immediate elevation in the $\overline{PaP}$. Changes in pulmonary vascular resistance were also observed as might be expected; however, continuous monitoring of cardiac output would be required to establish the immediate change in the resistance. Once pulmonary artery pressure and vascular resistance were elevated, they tended to return to the baseline level but for the periods of observations in these studies the previous baseline levels were generally not reached. With the larger doses of MAA, the elevation in the $\overline{PaP}$ was more sustained. This model also demonstrated that changes in the other variables such as cardiac output, arterial pressure, or dead-space-to-tidal volume ratio (Vd/Vt) were not seen until a severe particle insult occurred such as is seen in Fig. 1.

By combining the series of observations it was possible to derive a dose-response curve in the normal animal relating an elevation in $\overline{PaP}$ to the number of MAA particles given as a single 20-sec bolus injection. This relationship, seen in Fig. 3, indicates that the first toxic effect was experienced when 100–125 LSDs were given as a single injection. With larger doses there was a progressive accentuation of the effect. Using this data it may be possible to translate this into the concept of a safety margin, i.e., the ratio of the minimum toxic dose of particles to the usual diagnostic dose. Using the dog model and adjusting the relative LSD to a weight basis (14×10^3 particles/kg body weight) would indicate a "safety" factor of 100–125; that is, 100 lung scan doses of MAA would need to be given as a single bolus to produce the first measurable abnormal effect on the cardiopulmonary circulation.

This level of toxicity is considerably more than that reported by Taplin and associates where a safety factor of approximately 1,000 was suggested for MAA (*14*). The reasons for this difference could be due to a basic difference in the particle. In his studies MAA particles were of similar size, appearance, and protein content but were prepared without $SnCl_2$ as in this case. Alternately, the dog model used here may be more sensitive to detecting the effects of particle insult.

The dose-response curve in Fig. 3 is based on administration of the particles as a single bolus (20 sec injection). It is clear that a very slow rate of injection or an intermittent injection of a large number of particles as seen in Fig. 2 will greatly reduce the resulting elevation in $\overline{PaP}$. For example, in the experiment diagrammed in Fig. 2, 370 LSDs given over 180 min eventually elevated the $\overline{PaP}$ to 40–50% above the normal baseline. On the other hand, a similar amount given as a single bolus (Fig. 3) produced an immediate 300% increase in $\overline{PaP}$. This phenomenon is similar to that reported by Rhodes and associates in studies of albumin microspheres (*15*).

Another aspect and perhaps more meaningful one regarding the toxicity of lung scanning agents is what is the effect of the single lung scan dose in abnormal patients such as one with pulmonary emboli? In these patients cardiopulmonary reserve is reduced and pulmonary hypertension may be present. This cannot be determined easily in a clinical setting but was examined in these studies by observing the effect of small doses of MAA in dogs with pulmonary hypertension (Fig. 4). In this case the equivalent of three additional LSDs (single bolus) showed little or no elevation of $\overline{PaP}$ or other parameters. Although the experimental situation is not an ideal comparison, one would infer that the single dose of MAA injected in the presence of significant pulmonary hypertension would not be expected to produce further abnormal change.

ACKNOWLEDGMENTS

The technical assistance of Richard Tuck is hereby gratefully acknowledged. This work was supported in part by USPHS grant GM 15991-05 from the National Institute of General Medical Sciences, National Institutes of Health.

REFERENCES

1. HARPER PV, LATHROP KA, GOTTSCHALK A: Pharmacodynamics of some technetium-99m preparations. In *Radioactive Pharmaceuticals*, Andrews GA, Kniseley RM, Wagner HN, eds, Symposium Series 6, CONF-651111, Springfield, Va, National Bureau of Standards, 1966

2. HONDA T, KAZEM I, CROLL MN, et al: Instant labeling of macro- and microaggregated albumin with ^{99m}Tc. *J Nucl Med* 11: 580–585, 1969

3. GWYTHER MM, FIELD EO: Aggregated ^{99m}Tc-labeled albumin for lung scintiscanning. *Int J Appl Radiat Isot* 17: 485–486, 1966

4. BENJAMIN PP, VOELKER WH, FRIEDELL HL: Semiautomated laboratory production of ^{99m}Tc-albumin. *J Nucl Med* 12: 325–326, 1972

5. ROBBINS PJ, FORTMAN DL, LEWIS JT: A kit for the rapid preparation of ^{99m}Tc-macroaggregated albumin. *J Nucl Med* 13: 463–464, 1972

6. SURPRENANT EL, WEBBER MM, BENNETT LR: Technetium lung scanning. *Int J Appl Radiat Isot* 20: 77–80, 1969

7. FICKEN V, HALPERN S, SMITH C, et al: Evaluation of $^{99m}Tc_2S_3$ macroaggregates for lung scanning. *J Nucl Med* 10: 402, 1969

8. FICKEN V, HALPERN S, SMITH S, et al: ^{99m}Tc-sulfur colloid macroaggregates for lung scanning. *J Nucl Med* 12: 432–433, 1971

9. BURDINE JA, RYDER LA, SONNEMAKER RE, et al: ^{99m}Tc-human albumin microspheres (HAM) for lung imaging. *J Nucl Med* 12: 127–130, 1971

10. LIN MS, WINCHELL HS: Macroaggregation of an albumin-stabilized technetium-tin (11) colloid. *J Nucl Med* 13: 928–931, 1972

11. FICKEN V, HALPERN S, SMITH C, et al: ^{99m}Tc-sulfur colloid macroaggregates. *Radiology* 97: 289–295, 1970

12. SUBRAMANIAN G, ARNOLD RW, THOMAS FD, et al: Evaluation of an instant ^{99m}Tc-labeled lung scanning agent. *J Nucl Med* 13: 790, 1972

13. SUBRAMANIAN G: Personal communication, 1972

14. TAPLIN GV, JOHNSON DE, KENNADY JC, et al: Aggregated albumin labeled with various radioisotopes. In *Radioactive Pharmaceuticals*, Andrews G, Kniseley RM, Wagner HN, eds, Symposium Series 6, CONF-651111, Springfield, Va, National Bureau of Standards, 1966, p 525

15. RHODES BA: Personal communication, 1973