A Model for the Radionuclide Measurement of Ascitic Fluid Volumes

William D. Kaplan, Michael A. Davis, Roger F. Uren, Tanya Wisotsky, and Margaret LaTegola

Harvard Medical School and the Sidney Farber Cancer Institute, Boston, Massachusetts

Technetium-99m phytate colloids formed in vitro and in vivo were examined as radioindicators for estimation of the volume of third-space fluid in an ovarian ascites model using C3HeB/FeJ mice. In double-label experiments, the accuracy of the colloids for dilution analysis was found to be equal or superior to that of I-125 HSA. Sampling times 3–5 min after intraperitoneal administration were found to produce the best volume estimates. Four needle-stopcock assemblies inserted sequentially into the quadrants of the peritoneal cavity were used for administration and sampling of the radioindicators. The stopcocks could be closed to prevent leakage of ascitic fluid during the procedure. In contrast to radiolabeled albumin, Tc-99m phytate colloids have clinical use for simultaneous imaging of radiotracer migration to assess potential occlusion of diaphragmatic lymphatics by neoplastic cells, and for dilution analysis to estimate volume of ascitic fluid.

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An increase in the production of peritoneal fluid, with concomitant obstruction in the diaphragmatic lymphatic nodes, can result in ascitic fluid collections (*1*), an occurrence and complication in the natural history of a number of malignancies. It has been suggested that the obstructive component in the formation of ascites may be traced to tumor cells that become lodged within the diaphragmatic lymphatics (*2*).

The existence of malignant ascites may have potential complications in the therapy of the tumor process. In ascitic patients who are receiving high doses of Methotrexate (MTX), it has been shown that the drug can penetrate this third space (*3*), and can persist structurally unchanged when displaced from enzymes or compounds for which it has an in vivo affinity. This phenomenon has been observed up to 26 days after administration (*4*). The bidirectional transport of MTX can result in an increase in serum half-time (*5*) and may lead to toxicity when additional chemotherapeutic agent is administered.

From the concentration of MTX within ascitic fluid (*6*) and the volume of the third space, one can calculate the amount of drug within peritoneal effusions, and by anticipating the transfer of drug from effusion to plasma spaces (*7*), subsequent chemotherapy could be individualized so as to avoid toxicity.

Dilutional analysis studies using concentrations of tumor cells in the peritoneal fluid (*8*), and radiotracer estimation of ascitic fluid volumes using I-131 human serum albumin (HSA) in microcurie amounts, have been performed (*7*). The use of millicurie amounts of Tc-99m sulfur colloid to indicate ascitic-fluid drainage patterns (*1*) suggested the use of this radiolabel as a possible agent for *both* imaging and volume estimations. A scintiphoto depicting uniform distribution of radioactivity would provide an index of confidence in the calculated volume.

Successful lymph-node imaging with Tc-99m antimony sulfide (*9*)—a colloid of less than 20 nm in size (*10*)—suggested that the Tc-99m phytates could have potential for both dilutional analysis and imaging. The phytates in vitro and in vivo formed col-

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For reprints contact: William D. Kaplan, Div. of Nuclear Medicine, Sidney Farber Cancer Institute, 44 Binney St., Boston, MA 02115.

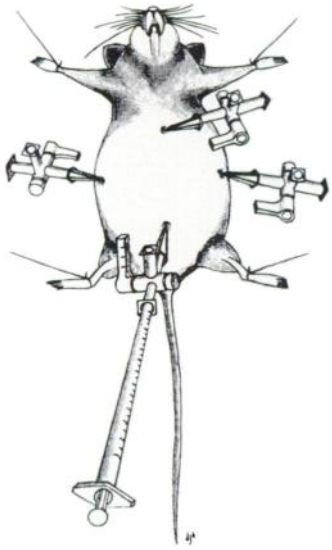

FIG. 1. Needle-stopcock assembly is shown positioned in lower mid-abdomen during administration of radiotracer. Also illustrated is arrangement of stopcocks for subsequent sampling in remaining three quadrants.

loids (*11–13*) smaller in size than sulfur colloid (100–1,000 nm) (*14*) are significantly easier to prepare since neither boiling nor acid-base addition is needed.

MATERIALS AND METHODS

Animals. Five-month-old female C3HeB/FeJ mice weighing 18–23 g were used.

Tumor. A spontaneous ovarian carcinoma that originated in a C3H female mouse at the Jackson Laboratories was maintained through serial transplantation (*15*), and frozen cell samples were carried through 15 intraperitoneal (i.p.) transplantations. Cells from the tenth to twelfth passages were used for these experiments.

Ascites. Animals were administered 1 million tumor cells i.p. and observed for 14 days before use*.

Radiolabeled particulates. Five radiotracers were employed in the study: Tc-99m phytate, both as a preformed colloid (*12*) and as a colloid formed in vivo; human serum albumin labeled with either Tc-99m or I-125; and Tc-99m sulfur colloid.

Injection and sampling. Animals were lightly anesthetized with ether. Volumes of radiotracers of about 0.1 cc were administered, with total activities of 30–50 μCi (Tc-99m-labeled agents) and 1–5 μCi (I-125 HSA). In a subset of 11 animals, I-125 HSA and either Tc-99m phytate or Tc-99m HSA were administered simultaneously. Syringes containing radiotracers were counted in a dose calibrator before administration, and the empty syringe-and-stopcock assembly was recounted following administration, to determine the actual injected dose.

Injection and sampling were performed with a 26-gauge sterile disposable needle, ½-in. in length, which was part of a needle-stopcock assembly (Fig. 1). The initial introduction of the tracer was through a stopcock located in the midline at the lower portion of the peritoneal cavity. After introduction of tracer, the remaining three quadrants were sampled at 3–5 min by means of additional needle-stopcock assemblies. Selected additional animals were sampled again 30 min following radiotracer administration. Following either radionuclide administration or sample withdrawal, the stopcock was closed, the syringe removed, and the needle-stopcock assembly was left in place.

At the termination of each study, the animal was killed by ether overdose, the peritoneal cavity opened and drained, and the total quantity of ascitic fluid collected and measured.

Counting. Single-label samples of ascitic fluid were counted in a NaI(Tl) well counter. Double-labeled samples (I-125 and Tc-99m) were assayed by counting the Tc-99m activity immediately following death, reserving the sample for 72 hr, and then counting on an I-125 channel, correcting the values for physical decay. A calibration factor was used to convert counts recorded on the well counter into microcurie values as obtained from the dose calibrator.

TABLE 1. CORRELATION BETWEEN AGENTS AND MEAN PERCENTAGE ABSOLUTE ERROR IN ESTIMATE OF ASCITIC-FLUID VOLUME

	No. of animals	Mean fluid volume measured	Mean fluid volume estimated	Mean percentage absolute error
Tc-99m HSA	8	16.27	16.53	6.60
Tc-99m Phytate	11	13.88	14.49	9.04
Tc-99m Phytate with Ca^{++}	11	15.72	14.58	9.66
I-125 HSA	14	14.12	15.01	13.87
Tc-99m sulfur colloid	15	18.50	15.99	15.77

RESULTS

Ascitic-fluid volumes measured at the time of sacrifice ranged from 8.7 to 27.6 ml, with a mean of 15.7 ml. The individual ascitic-fluid measurement for each animal served as the "actual" volume in the comparison with the tracer-estimated volume.

TABLE 2. DOUBLE-LABEL EXPERIMENTS COMPARING Tc-99m-LABELED AGENTS FOR VOLUME ESTIMATION IN 11 ASCITIC MICE

Agent	Animal No.	Actual volume (ml)	$\bar{x}$ estimated volume		% error	
			Tc	I	Tc	I
Tc-99m HSA and I-125 HSA	1	15.16	15.02	18.31	−0.90	+20.80
	2	15.27	14.11	16.97	−7.60	+11.10
	3	15.80	14.60	17.11	−7.60	+8.30
	Avg	15.41	14.58	17.46	−5.37	+13.40
Tc-99m phytate + calcium and I-125 HSA	4	18.20	15.70	14.50	−13.74	−20.30
	5	11.53	12.31	10.71	+6.81	−7.10
	6	9.25	8.44	6.66	−8.83	−28.00
	7	10.18	9.05	11.73	−11.10	+15.20
	8	15.68	14.70	15.55	−6.25	−0.83
	9	13.48	14.97	15.12	+11.05	+12.17
	Avg	13.05	12.53	12.38	±9.63	±13.93
I-125 HSA, and Tc-99m phytate without calcium	10	14.30	15.64	16.27	+9.37	+13.78
	11	14.75	15.84	16.64	+7.39	+12.81
	Avg	14.53	15.74	16.46	+8.38	+13.30

Technetium-99m HSA showed the lowest mean percentage error in volume estimates. Technetium-99m phytate, as either in vitro or in vivo formed colloid, produced similar results with a slightly higher error (Table 1).

Double-label experiments (Table 2) indicated that volume determinations tended to be slightly overestimated when Tc-99m phytate was used as an in vivo formed colloid, along with I-125 HSA. Also the I-125 HSA showed greater variability in both directions.

Volume estimations made on samples obtained at 30 min produced larger percentage errors.

DISCUSSION

The results of our study indicate that particulates labeled with Tc-99m can be used to quantify the volume of ascitic fluid, as well as simultaneously to assess radiotracer migration into intrathoracic lymph nodes. In no instance did mice with ovarian tumor and ascites show uptake in intrathoracic lymph nodes, whereas in normal, nonascitic mice, Tc-99m-labeled particulates readily migrated to the mediastinal nodes (Fig. 2).

Data indicating the continuous transfer of radiolabeled albumin from the intraperitoneal to the vascular compartment (*7*) suggest that volume determinations using particulates should be made soon after intraperitoneal tracer instillation. In this study, in addition to the initial 3–5 min sampling, some animals were sampled again 30 min following tracer administration. When compared with values obtained at 3–5 min, later sampling resulted in a greater percentage error and tended to overestimate the volumes.

These findings correlated with our data on recovery of the percentage injected dose. When animals were killed within 5 min of the administration of radiotracer, recovery of the injected dose in ascitic fluid approximated 96%; 90% of the counts were associated with the supernatant and the remaining 10% were in the pellet. Beyond 30 min, approximately 90% of the injected dose could still be recovered in the ascitic fluid. Within this fluid, however, the distribution of radiotracer had changed such that the pellet composed of 90% tumor cells, with macrophages and cellular debris accounting for 8% and 2%, respectively, now accounted for about a fifth of the counts. This finding is the most likely explanation for the overestimated volume determinations seen at later sampling times.

Although particles as large as 22.5 μ have been shown capable of passing from the peritoneal cavity into the diaphragmatic lymphatics (*16*), different transfer rates have been recorded when monitoring the kinetics of I-131 HSA as compared with Cr-51-labeled erythrocytes (*7*). In our study however, the

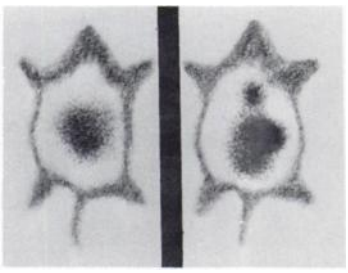

FIG. 2. Left: mouse with ascites secondary to ovarian tumor shows radiotracer confined to abdominal cavity. Right: a normal mouse shows good visualization of mediastinal lymph nodes.

similar results obtained with phytate colloids or radiolabeled human serum albumin, suggest that particulate as contrasted with soluble material is not a critical consideration in the selection of an agent for volume determinations.

The validity and success of this model relate to two factors: first, that it mimics the pattern of malignant ascitic fluid formation seen in humans, with obliteration of diaphragmatic lymphatic channels (*4*); and, second, that an arrangement of stopcocks remains in place throughout the experiment, thereby preventing leakage of ascitic fluid from the distended and tense abdomen. Failure to maintain a seal in the sampling puncture sites results in continuous loss of both peritoneal fluid and radiotracer, producing considerable errors in volume determinations.

With I-125 HSA as the standard for a comparative analysis of agents, our results suggest that either of two substances, each tagged with Tc-99m, can be used routinely to estimate ascitic volumes: phytate —either as a preformed colloid or as one formed in vivo—and HSA. These agents give results equal or superior to those obtained with I-125 HSA. When patients harboring ascitic fluid collections require concomitant imaging of radiotracer migration to assess potential alteration of peritoneal drainage, we suggest that Tc-99m-phytate can be substituted for radioiodinated compounds. Although a particle size of about 8 nm has recently been found for Tc-99m stannous phytate (G. N. Ege, A. Warbick, unpublished data), upon injection this material reacts with calcium to form in vivo particles whose size is still unknown.

FOOTNOTE

* Animals were kindly supplied by Robert C. Knapp, M.D., Chief, Gynecologic Oncology, Beth Israel Hospital, Boston Hospital for Women, and Sidney Farber Cancer Institute, Boston, Mass.

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REFERENCES

1. COATES G, BUSH RS, ASPIN N: A study of ascites using lymphoscintigraphy with ^{99m}Tc-sulfur colloid. *Radiology* 107: 577–583, 1973

2. FELDMAN GB, KNAPP RC, ORDER SE, et al: The role of lymphatic obstruction in the formation of ascites in a murine ovarian carcinoma. *Cancer Res* 32: 1663–1666, 1972

3. TATTERSALL MHN, PARKER LM, PITMAN SW, et al: Clinical pharmacology of high-dose methotrexate (NSC-740). *Cancer Chemother Rep Part 3* 6: 25–29, 1975

4. JOHNS DG, HOLLINGSWORTH JW, CASHMORE AR, et al: Methotrexate displacement in man. *J Clin Invest* 43: 621–629, 1964

5. WAN SH, HUFFMAN DH, AZARNOFF DL, et al: Effect of route of administration and effusions on methotrexate pharmacokinetics. *Cancer Res* 34: 3487–3491, 1974

6. RASO V, SCHREIBER R: A rapid and specific radioimmunoassay for methotrexate. *Cancer Res* 35: 1407–1410, 1975

7. BRONSKILL MJ, BUSH RS, EGE GN: A quantitative measurement of peritoneal drainage in malignant ascites. *Cancer* 40: 2375–2380, 1977

8. KLEIN G, RÉVÉSZ L: Quantitative studies on the multiplication of neoplastic cells *in vivo*. I. Growth curves of the Ehrlich and MC1M Ascites Tumor. *J Natl Cancer Instit* 14: 229–277, 1953

9. EGE GN: Internal mammary lymphoscintigraphy. The rationale, technique, interpretation and clinical application: A review based on 848 cases. *Radiology* 118: 101–107, 1976

10. WARBICK A, EGE GN, HENKELMAN RM, et al: An evaluation of radiocolloid sizing techniques. *J Nucl Med* 18: 827–834, 1977

11. SUBRAMANIAN G, MCAFEE JG, MEHTER A, et al: ^{99m}Tc-stannous phytate: A new in vivo colloid for imaging the reticuloendothelial system. *J Nucl Med* 14: 459, 1973 (Abst)

12. DAVIS MA, KAPLAN ML, AHNBERG DS, et al: A modified ^{99m}Tc-phytate colloid for liver-spleen imaging. *Int J Appl Radiat Isot* 28: 123–130, 1977

13. ARZOUMANIAN A, ROSENTHALL L, SETO H: Clinical comparison of ^{99m}Tc-labeled preformed phytate colloid and sulfur colloid: Concise communication. *J Nucl Med* 18: 118–120, 1977

14. DAVIS MA, JONES AG, TRINDADE H: A rapid and accurate method for sizing radiocolloids. *J Nucl Med* 15: 923–928, 1974

15. FEKETE E, FERRIGNO MA: Studies on a transplantable teratoma of the mouse. *Cancer Res* 12: 438–440, 1952

16. ALLEN L: On the penetrability of the lymphatics of the diaphragm. *Anatomical Record* 124: 639–657, 1956