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Studies with Iodine-131-Labeled Antibody to Human Fibrinogen for Diagnosis and Therapy of Tumors

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Independent observations by Day and co-workers (1) and Spar *et al*, (2) have shown that ^{131}I rat fibrinogen as well as rabbit antibodies to this fibrinogen are selectively concentrated by some transplantable rat tumors. Similar studies have been done in dogs bearing spontaneous tumors (3). Bale (4) has demonstrated that the tumor-localizing radioactivity can be increased if such experimental animals are given epsilon aminocaproic acid, an inhibitor of the conversion of plasminogen to plasmin. Various authors, DiChiro (5), Spar (6), Dewey (7), and Bale (8) have reported that ^{131}I-labeled human fibrinogen and labeled and purified rabbit antibodies to human fibrinogen will concentrate in some human tumors.

It would seem that some transplantable and spontaneous tumors, as a result of being injurious to the invaded tissues and possibly as a defense mechanism by the host, have fibrin deposited in their stroma. Not only will systemically administered ^{131}I-labeled homologous fibrinogen concentrate in such lesions, but also antibodies to fibrinogen. This paper reports the use of ^{131}I labeled and purified rabbit antibody to human fibrinogen in human cancer patients to extend previous studies with it as a diagnostic agent. Two cases are also presented in which the concentration of the isotope in tumor was great enough to use highly radioactive preparations of antibody as a technique to attempt to deliver a therapeutic dose of radiation.

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METHODS

A total of 50 patients with various types of neoplasms were given intravenous tracer doses, in the range of 200-800 μC, of ^{131}I-labeled and purified rabbit antibodies to human fibrinogen. The techniques of antibody purification and iodination have been previously described (6,8). In brief, antibody reacting with human fibrin was isolated from the sera of rabbits immunized by intravenous injection of human fibrinogen. Antisera was mixed with human plasma and the mixture clotted by the addition of thrombin and calcium. The resultant fibrin clot contains most of the antibody, while other proteins are left in solution. Antibody is then eluted from the fibrin by stirring with a pH 11.6 buffer for five minutes at room temperature, and the insoluble fibrin separated by centrifuging.

A further purification is carried out by absorbing the antibody preparation with pulverized human fibrin, and after washing the fibrin-antibody complex, again eluting the antibody into solution at pH 11.6 Iodine-131 is coupled to 4 mg portions of antibody to give a preparation carrying about 25 mC ^{131}I/mg protein.

It is sterilized by filtration, diluted to a level of 1 mC/ml in a 2.5% solution of human albumin, and stored frozen until used. Portions are removed from each storage vial for sterility testing. It is thawed and administered intravenously to the cancer patients in tracer doses of 0.5 − 1.0 mC in a volume of 1-10 ml. Earlier all of the patients had had negative skin tests with 0.0035 mg of normal rabbit gamma globulin and were given Lugol's solution, 20-30 drops per day, and epsilon aminocaproic acid (EACA), 16-24 grams/day, for the duration of the study. The former was given to prevent ^{131}I accumulation in the thyroid and the latter to inhibit fibrinolysis and thereby decrease removal of fibrinogen from the tumor.

Blood samples were taken from the patients at frequent intervals after the administration of the ^{131}I preparation. Iodine-131 activities were determined to calculate blood disappearance curves as well as the percentages of the radioactivity that would be bound in clots formed from such plasmas.

Dot scans and photoscans were done on all patients using the Picker magnascanner with a 19-hole collimator and 2 × 3 inch sodium iodide crystal. Patients who had good tumor localization after the initial one and three day scans were restudied at appropriate intervals as long as qualitative or quantitative information could be derived from the additional data. *In vivo* isotope concentrations per gram of tumor were calculated, in many studies, from the dot scans based on the theoretical and experimental work of Beck (9). In a few instances, these values were checked by counts on surgical tumor samples. Tumor concentrations, in several studies, were determined using a head phantom.

Two patients, with sufficient tumor uptake of the isotope, were selected from this group and given therapeutic doses of ^{131}I-labeled antibodies with the hope that they would benefit from the resulting tumor radiation.

RESULTS

A general survey of the types and numbers of specific tumors scanned and the results of this procedure are shown on Table I.

Of the 50 patients reported, all had malignant neoplasms. In the whole group 86% had carcinomas (including malignant melanoma), 46% of these being cranial and 68% of these brain tumors were diagnosed as primary lesions.

Scintiscanning has a general accuracy of 58%; of the sarcomas, 25% (2/8) were found and of the total carcinomas, 64% (27/42) were located. In this latter group, 55% (12/22) were positive studies on extracranial tumors, 62% (8/13) primary brain tumors, and 6 of 6 were positive studies on metastatic brain tumors.

Almost all of the patients were studied by scintillation scanning techniques with appropriate dot and photoscans being made. In addition, ^{131}I activities were determined in plasma and serum samples and determinations made of the per cent plasma ^{131}I bound in a washed fibrin clot made from the patient's plasma. This parameter served a check on the blood sampling technique, specificity of the ^{131}I antibody preparation, and possible changes in catabolism of the ^{131}I preparation. The average per cent clottable ^{131}I activity in these patients was 80 per cent with very little variation between patients and within consecutive determinations on the same patient.

Figure 1 shows the ^{131}I activity found in citrated blood samples of two typical patients drawn at various times after infusion of a tracer dose of ^{131}I antibody to human fibrinogen. Each patient had received a different ^{131}I preparation. All of the data are normalized to the radioactivity in an amount of blood equiva-

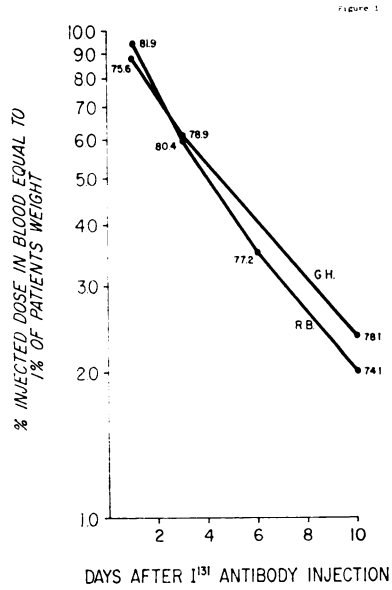

Fig. 1. Iodine-131 activity in blood of two patients at various times after injection of ^{131}I-labeled and purified antibody to human fibrinogen. Also given are the per cent of the radioactivity found in washed clots made from plasma samples.

lent to one per cent of the patient's weight. There is very little difference in the biological half-life after the first few days in these patients nor any correlation with the extent of localization of the ^{131}I preparation in their tumors. Both had metastatic brain lesions and patient RB later received a therapeutic dose. Also given is the per cent of the clottable radioactivity in individual citrated plasma samples. There is not much variation between the samples from these patients.

TABLE I

SCINTISCANNING RESULTS FROM PATIENTS WITH KNOWN NEOPLASMS AFTER INJECTION WITH ^{131}I ANTIBODIES TO HUMAN FIBRINOGEN

	Pos	*Neg*	*Total*
Abdominal tumor[1]	1	0	1
Brain tumor (primary)			
Astrocytoma	1	0	1
Ependymoma	0	1	1
Glioblastoma multiforme	3	0	3
Hemangioblastoma	1	0	1
Hemangioendothelioma	1	0	1
Meningioma	0	3	3
Pinealoma	0	1	1
Others	2	0	2
Brain tumor (secondary)			
Breast	1	0	1
Bronchogenic	2	0	2
Hypernephroma	1	0	1
Melanoma	2	0	2
Breast carcinoma	0	1	1
Bronchogenic carcinoma	2	1	3
Cervical carcinoma	3	1	4
Chondrosarcoma	0	2	2
Esophageal carcinoma	1	1	2
Hypernephroma	1	0	1
Kaposis' sarcoma	0	1	1
Melanoma	1	4	5
Myosarcoma	2	2	4
Neurofibrosarcoma	0	1	1
Ovarian carcinoma	3	0	3
Parotid carcinoma (muco-epidermoid)	0	1	1
Rectal carcinoma	1	0	1
Thyroid carcinoma	0	1	1
	29	21	50

[1]Primary unknown.

On each of these two patients, scans and also point counts with the scanner were done at various intervals. Figure 2 shows the ratio of radioactivity in the brain lesion to that in the heart pool. This method of presentation was chosen to negate the effect of physical decay on the ^{131}I activity and variations in the counting efficiency of the scanner. Absolute comparisons should not be made between these two studies since no compensation has been made for differences in the depth and size of the lesion.

A constant ratio of the ^{131}I in tumor to heart would indicate that the radioactivity in the lesion is decreasing at the same rate as the circulating ^{131}I protein. This seems to be occurring in patient GH during the interval between the 3 and 10 day scans. There is a definite increase in the ratio from one to three days. The determinations done on patient RB show an increase in the tumor/heart ratio with each succeeding study. The latter patient was given a therapeutic dose and will be described in detail later.

Many of the patients who had positive scans were studied several times over a 7-10 day period and from this data serial radiation dosimetry calculations were made using Beck's formula or by comparison with phantoms. These results are given in Table II. Even though all of these patients' photoscans are impressive, there is a very large variation in the isotope per gram of tumor. This is related to the size of the lesion being studied. A large mass with a low uptake of ^{131}I antibody can often be as impressive as a small lesion with a substantially higher concentration of radioisotope. For these determinations, it was assumed that the average three-to-seven day level of ^{131}I in the tumor would be maintained for a week and that the injected dose of radioactive antibody would be 160 mC. In these cases the calculated whole body dosage was less than 200 rads over this period.

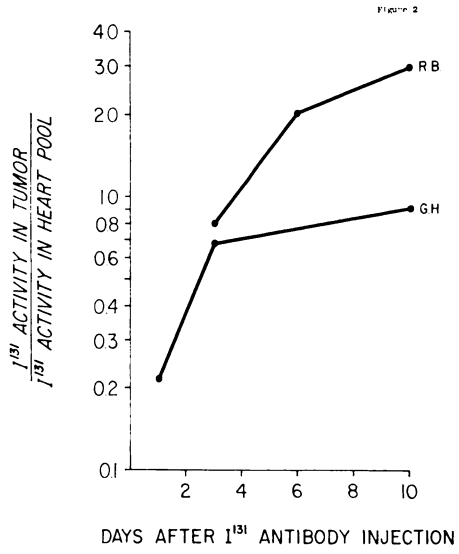

Fig. 2. Iodine-131 activity found in tumor relative to heart in two patients, one of whom was later given a therapeutic dose of ^{131}I antifibrinogen antibody (R.B.).

In three instances, samples of tumor were obtained and the radioactivity determined by NaI well-counting techniques. The per cent ^{131}I injected dose was calculated and compared with the values obtained by Beck's formula. The results are given in Table III. There is a very reasonable agreement between the experimental values and those calculated from the scintiscanning.

TABLE II

CALCULATED CONCENTRATIONS OF I^{131} IN HUMAN TUMORS OF VARIOUS TYPES AND SIZES. VALUES ARE EXTRAPOLATED TO GIVE β DOSE TO TUMOR IF 160 MC ^{131}I ANTIBODY WERE INJECTED

Patient	*Half-Life ^{131}I Blood or Plasma*	*Diagnosis*	*Lesion Radius (cm)*	*Maximum $\mu C/gm$ (for 400 μC injection)*	*β rad/ 160 mC*
J. L.	4.9	Ca abdomen (1° unknown)	6.5	1.5×10^{-2}	400
E. D.	4.0	Melanoma → brain	2.0	2.2×10^{-2}	590
N. C.	10.0	Ovary → epigastric region	6.5	4.7×10^{-2}	1510
M. B.	14.0	Glioblastoma multiforme	1.5	3.0×10^{-2}	810
E. S.	3.9	Ovary	5.0	6.2×10^{-3}	120
M. S. G.	5.1	Breast → brain	2.0	2.3×10^{-2}	620
R. V.	3.9	Melanoma (hip)	2.0	6.6×10^{-2}	1760
C. G.	7.0	Melanoma → brain	3.0	4.5×10^{-2}	1210
I. B.	6.0	Cervix (local recurrence in abdomen)	4.5	6.2×10^{-2}	1660
R. B.	3.0	Bronchogenic → brain	1.5	9.1×10^{-3}	2950

TABLE III

COMPARISON OF PER CENT DOSE ^{131}I IN TUMOR SAMPLES WITH VALUES CALCULATED FROM SCANNING

Patient	*Diagnosis*	*Method of Obtaining Sample*	*Days After ^{131}I Injection*	*Per Cent Dose ^{131}I In Sample*	*Calculated*
C. G.	Melanoma to brain	Surgery (several specimens obtained)	7 days	$.14 \times 10^{-2}$ 3.4×10^{-2}	1.38×10^{-2}
I. B.	Carcinoma of cervix	Biopsy	4 days	$.91 \times 10^{-2}$	1.56×10^{-2}
D. M.	Carcinoma of rectum	Autopsy	9 days	$.96 \times 10^{-2}$	$.92 \times 10^{-2}$

For various reasons, such as the clinical condition of the patient and availability of ^{131}I of high specific activity, only two patients were treated with therapeutic doses of ^{131}I antibody to human fibrinogen.

Patient NC (U. of C., No. 314852). A 49-year-old white female who underwent, in September, 1961, a hysterectomy and bilateral salpingo-oophorectomy for papillary adenocarcinoma presumably arising in the ovary. Shortly after surgery she received 3900 r of external radiation to the tumor site and lower abdomen. In September, 1962, she sustained a fracture to the left hip while riding on a train. X-rays indicated this was due to tumor invasion at this site.

Upon physical examination in October, 1962, the patient had a 12 × 15 cm hard, firm, fixed mass in the epigastrium. She received a diagnostic dose of 400 μC of ^{131}I fibrinogen antibody. Uptake was substantial in the area of the mass (Figure 3) and she was given a therapeutic dose of 160 mC of antibody in November of the same year. Calculations indicated about 2000 rads was delivered to the epigastric tumor. Subsequent to this she healed her fractured hip. She developed signs of an enteric fistula eight months later. In July, 1963, she underwent a subtotal gastrectomy and partial transverse colectomy for the x-ray proven gastrocolic fistula. At operation the main metastatic tumor was approximately 30 per cent of the size estimated from palpation and x-ray nine months earlier. Since regrowth of the tumor may have occurred after the antibody therapy, the actual reduction in mass due to this treatment may have been quite substantial. Pathological specimens showed carcinoma in the stomach and colon as well as the para-aortic nodes. The patient was readmitted in January, 1964, after which time she had a gradual progressive downhill course expiring March, 1954. Permission for autopsy was not obtained.

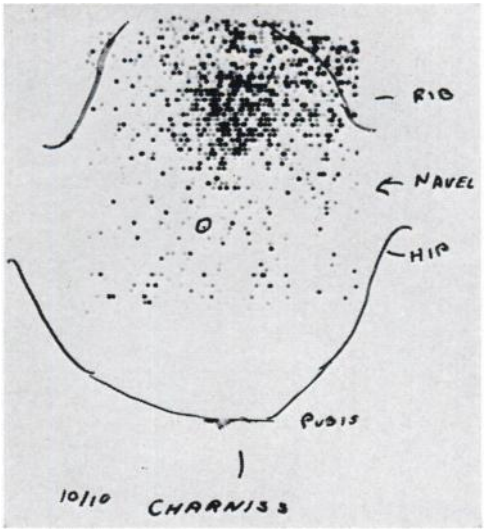

Fig. 3. Photoscan of patient N.C. made 4 days after an injection of 400 μC of ^{131}I anti-fibrinogen antibody.

Patient RB (U. of R., No. 336509). A 57-year-old white female who was seen in consultation one year after receiving 5000 r to a brain lesion, presumably a metasis from carcinoma of the lung. Diagnosis was made by thoracentesis and chest x-ray. Three months before being seen in consultation, the patient had a recurrence of the brain lesion. At the time of the diagnostic study with ^{131}I antibody, the patient was incontinent and ataxic. Good localization in the brain (Fig. 4) and lung lesions was obtained and the patient was given 100 mC therapeutic dose with a calculated tumor dose of about 2000 rads based on scanning studies and assuming uniform ^{131}I distribution in the tumor. There was no significant clinical improvement and she died three months later without postmortem examination.

Scans on both patients after the therapeutic dose indicated concentrations of ^{131}I in the lesions in good agreement with the values that had been predicted from the diagnostic studies. There was also no difference in the biological half-life of the therapeutic preparation from that obtained with the earlier tracer studies. Essentially all of the excreted ^{131}I could be accounted for in the urine.

DISCUSSION

The favorable results obtained with the group of 50 patients places scinti-scanning with radioactive antihuman fibrinogen as an adjunct in the diagnostic localization of human neoplasms. The favorable evaluation of the merit of this preparation is based on the 58 per cent general diagnostic accuracy for all tumors in this series and particularly on the very high per cent positivity on scanning of brain metastases. It is recognized that the selection in this series is skewed in that 14 per cent of the tumors were sarcomas and that many of the more frequently encountered carcinomas were not scanned.

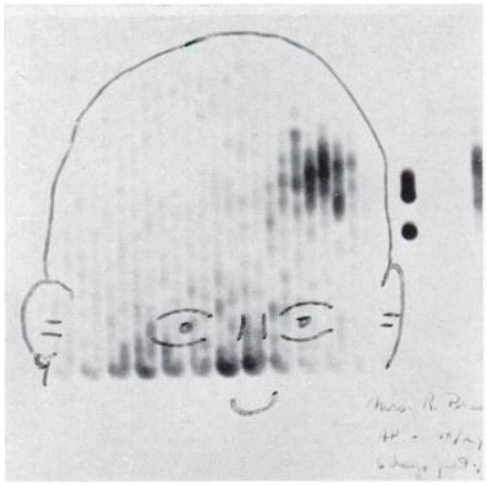

Fig. 4. Photoscan of patient R.B. made 6 days after an injection of 600 μC of ^{131}I anti-fibrinogen antibody.

The configuration of the tissues in the collimator field of view are measured from the scan. This together with the gamma-ray attenuation coefficients in tissue permit estimations, from the collimator-detector response, of the isotope concentrations in the tumor and surrounding tissue. From such calculations we were able to predict those patients in this study who might benefit from therapeutic doses of ^{131}I antihuman fibrinogen.

An interesting immunological observation was made on therapy patient NC which showed that on the fourteenth day after the therapeutic injection of ^{131}I, her plasma radioactivity rapidly decreased to one-twentieth of its predicted level. This was seen in one other patient early in this study and is believed to be a sensitization to the fibrinogen antibody even though skin testing remained negative on all occasions for both patients. Circulating antirabbit gamma globulin antibodies were demonstrated by immunodiffusion. Other cooperating groups using this preparation have occasionally encountered an unexpected rapid decrease in blood-borne ^{131}I protein that resembles an immunological clearance of an ^{131}I-labeled antigen. In several cases, the patients had been exposed to rabbits in their environment, i.e. as a laboratory technician and as an avid hunter.

Before this procedure of tumor therapy can be considered as a realistic tool for many patients, means must be found to increase the concentration of ^{131}I in the tumor relative to the normal tissue. Several experimental approaches have been tried that are amenable to human use. Some involve induction of fibrinolysis before infusion of the ^{131}I preparation (10), or the use of fever during the period of localization of ^{131}I to increase its deposition (11).

Another approach to this is to decrease the ^{131}I rabbit antihuman fibrinogen present in blood with minimal loss of tumor-bound isotope. Such a technique will be useful if a means is found, such as fever, for producing rapid localization in the tumor of some of the administered radioactive antibody, but leaving another large fraction still in general circulation. This circulating fraction will add to the whole body radiation dose without adding very much to the tumor radiation effect. A large portion of this circulating fraction can be removed rapidly by injecting goat antiserum to rabbit gamma globulin since the ^{131}I preparation being used in these studies is in reality a purified rabbit gamma globulin. Using amounts of antiserum to rabbit gamma globulin that are in immunological equivalence with the originally injected antigen (^{131}I rabbit antibody to human fibrinogen), about 75% of the circulating ^{131}I-labeled preparation are rapidly removed in rats with little loss of tumor-bound isotope (12).

Results obtained with two patients who had received ^{131}I antibody to human fibrinogen three days before the infusion of goat antiserum are shown in Figure 5. One of the studies involved a subject with no tumor (Fig. 5A), and the other was a cancer patient who had ^{131}I concentration in peritoneal metastases from carcinoma of the ovary (Fig. 5B). There is rapid removal of the ^{131}I from the blood of both individuals, with a much slower loss of bound isotope from the tumor as shown by the discrepancy in the whole body radioactivity values when compared with the blood levels. It is hoped that by the use of systemic techniques such as this, a larger proportion of tumor patients will be able to be given thera-

peutic doses of ^{131}I antibody to human fibrinogen with a greater hope of some long-term remissions.

SUMMARY

Fifty patients with neoplasms were injected intravenously with purified and ^{131}I-labeled rabbit antibodies to human fibrinogen and were evaluated with scintiscanning for diagnostic reliability. Fifty-six per cent of all tumors showed positive scans and a much higher percentage was obtained in scans of patients with metastases to the brain.

Using Beck's formula or head phantoms, *in vivo* concentration of ^{131}I in the lesions was calculated and from these values estimations were made for 10 patients of the β radiation dose that would be delivered to the tumor if 160 mC of ^{131}I antifibrinogen were given. These ranged from 123 to 2900 rad. Whole body radiation (β and γ) was less than 200 rad. Reports are presented for two patients who received therapeutic trials, one of whom showed a regression of the lesion.

Figure 5

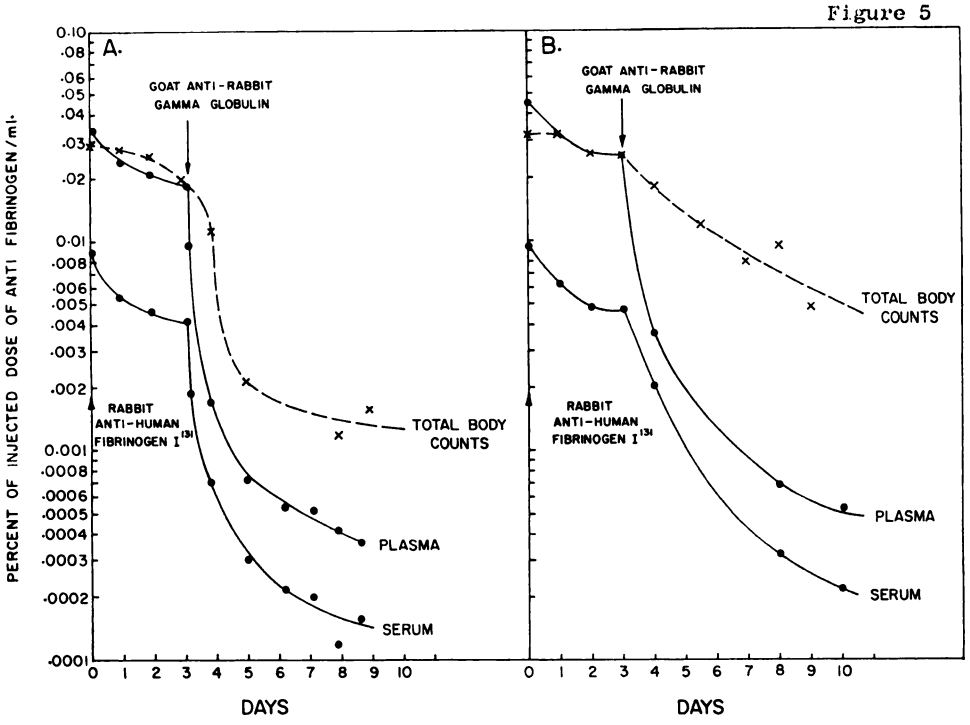

Fig. 5 Plasma and serum disappearance curves after intravenous administration of rabbit antihuman fibrinogen labeled with ^{131}I together with total body counts for the two patients. Patient A had no tumor; patient B had extensive metastatic malignancy in the peritoneal cavity from carcinoma of the ovary. Total body counts are expressed in relative units and normalized to the plasma curves at time of injection of goat anti-rabbit gamma globulin. Retention of radioactivity outside the blood pool is clearly demonstrated in patient B.

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