Distribution of Injected $^{131}$-labeled Antibody to Dog Fibrin in Tumor-bearing Dogs*

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SUMMARY

Radiodine-labeled antibody to dog fibrin was prepared and injected intravenously into 28 dogs with spontaneous tumors. The $^{131}$ content of tumor tissue was determined 3 days later. Most tumors showed no preferential concentration of $^{131}$. In a few instances preferential $^{131}$ localization was found, and in at least one tumor it was great enough to suggest that, if similar localization occurs in some types of human cancer, highly radioactive antibody of this type might be a useful tool for selective radiation of malignant tumor tissue.

Previous work, summarized elsewhere by the present authors (1), has shown that $^{131}$-labeled antibody to rat fibrin, injected intravenously in rats bearing certain types of transplanted tumors, will localize with a high degree of preferentiality in tumor tissue. In that paper it is also shown that a highly radioactive antibody of this type can be used for consistently successful radiation therapy of one of those transplanted rat tumors, the Murphy-Sturm lymphosarcoma. It therefore seemed worthwhile to investigate whether a similar localization of antibody to fibrin would occur in spontaneous tumors in an animal in size and life span more like man—namely, the dog.

MATERIALS AND METHODS

Antibody reacting with dog fibrin and labeled with $^{131}$ was prepared from serum obtained from rabbits immunized with dog fibrin or fibrinogen by methods similar to those described for making antibody to rat fibrin (1). However, each preparation was further purified after $^{131}$ labeling by being mixed with citrated dog plasma, clotting of the mixture with calcium plus thrombin, and subsequent elution of the labeled antibody from the washed, homogenized clot at a pH of approximately 11.6 or with 32 per cent urea.

With each preparation tests were run in vitro with dog, rat, and rabbit plasma as a measure of species specificity and as an indication of the fraction of $^{131}$ in the antibody preparation that would bind to freshly formed fibrin. The test was carried out by mixing dilutions of the final purified $^{131}$-labeled antibody preparation with CaCl$_2$, thrombin, and citrated plasma. The clot was beaten with a glass rod, centrifuged, and washed with saline; $^{131}$ content was measured; and the percentage of the initial added radioactivity was calculated. In the preparations used for dog injections the amount of the initial radioactivity found in dog plasma clots varied from 69 to 90 per cent, in rat plasma clots from 6.8 to 15.2 per cent, and in rabbit plasma clots from 4.7 to 9.8 per cent.

In total, 28 dogs with 30 spontaneous tumors were used. Most of these were household pets submitted to cooperating veterinary surgeons for surgical removal of their tumors or were for other reasons not available for sacrifice experiments.

These dogs were given injections intravenously of 2–10 ml. of $^{131}$-labeled purified antibody containing 5–20 $\mu$g. of $^{131}$. Potassium iodide, 136 mg., was administered orally on the day before, the day of, and the day after antibody injection to reduce thyroid accumulation and promote excretion of $^{131}$ released by catabolism of protein. Blood samples were taken 5–10 minutes after antibody injection, 1 or 2 days later, and on the 3d day. In instances in which the dog was treated at our laboratory, the tumor or biopsy material was divided into fractions of a gram weight or less, the $^{131}$ con-
tent was determined, and the material was fixed for subsequent histological study. When dogs were treated at other locations, several pairs of corresponding tumor samples were obtained, and one of each pair was used for $^{131}I$ determination and the corresponding sample for histological study. From five dogs available for sacrifice 3 days after antibody injection, tissue samples of most major organs were obtained plus, in each instance, tissue from one or more sites containing metastatic tumor lesions. None of the dogs showed any untoward reactions as a result of the injection of the $^{131}I$-labeled antibody.

RESULTS AND DISCUSSION

The portion of injected $^{131}I$ found in blood equal to 1 per cent of the injected animals’ weight averaged 11.9 per cent in the blood samples taken shortly after radioactive antibody injection. Average values found at 1 day were 7.6 per cent; at 2 days, 6.5 per cent; and at 3 days, 5.56 per cent. There was no consistent variation in these findings with any of the seven labeled antibody preparations used in these experiments. On the basis of the rate of disappearance of $^{131}I$ from the bloodstream from the 1st day after injection to the 3d, an average half-life of 4.4 days in the blood was estimated for this 2-day period.

Chart 1 shows in graphic form the degree of $^{131}I$ localization found in tumor tissue in the 30 tumors of 28 dogs used in this experimental study. Data are reported as the percentage of the injected $^{131}I$ dose that would have been found in an amount of tissue equal to 1 per cent of the animal’s weight with the same radioactivity per unit weight as the tissue measured. Values are averages for all tumor tissue measured unless it is specifically stated otherwise in the text.
equivalent in $^{131}$I content per gram to the samples actually measured and equal to 1 per cent of the animal's weight. These normalized values permit comparisons of $^{131}$I distribution in animals of different size. If the injected $^{131}$I, initially attached to antibody, were evenly distributed and none excreted, the value for each tissue would be one. Thus, tumor values greater than unity represent $^{131}$I concentrations in tumor tissue greater than the average found for other tissues in that animal, even without allowance for $^{131}$I excretion that had occurred in the 3 days intervening between antibody injection and the time of obtaining tumor tissue by surgical removal, biopsy, or sacrifice and autopsy.

Values for tumor of unity or greater do not necessarily indicate antibody localization by binding to antigen. Some tumors are relatively vascular, and others without obviously large blood content have a large extracellular volume in which plasma proteins, including $\gamma$-globulin, can accumulate by nonspecific means. Values for labeled albumin or $\gamma$-globulin concentrations in tumors have been reported up to one-half the radioactivity of an equal amount of blood in human patients (2). It is perhaps realistic in interpreting data from Chart 1 to assume that tumor radioactivity slightly or moderately lower than the blood value at the time of tumor removal cannot unequivocally be interpreted as evidence for or against specific antibody localization in tumor and that values much lower than blood are good evidence of little or no antibody localization. On these premises Chart 1 certainly indicates that deposition of antibody to fibrin in tumor tissue does not usually occur in spontaneous tumors, at least in amounts of any therapeutic significance in dogs, since of 30 tumors studied 22 had values below the average blood value of 5.57 per cent, including sixteen below half this value.

On the other hand, Chart 1 indicates that there was a group of tumors in which $^{131}$I localization occurred in or within the immediate environment of tumor tissue in amounts certainly suggestive of a specific type of localization. Five of these tumors appeared benign on histological examination; and, of this group, No. 24, a 0.5-gm. squamous papilloma on the metacarpus; No. 29, a 1-gm. fibroepithelioma on the hind limb of the same dog; and No. 27, an interdigital fibroepithelioma, about 1 gm., each had regions of marked inflammatory reaction. Tumor No. 26 was a fibroma estimated at 30 gm. in the chest region, with a little involvement of mammary duct epithelium. Tumor No. 25, apparently a hemangioma, showed nearly uniform $^{131}$I localization in the six specimens into which the 5-gm. tumor was divided.

Of the other tumors that showed highest $^{131}$I localization, tumor No. 28 was a 0.5-gm. biopsy specimen from a moderately vascular malignant melanoma of considerable but undetermined extent on the upper palate of a 10-kg. cocker spaniel. This lesion had recurred after surgical removal a year earlier. The $^{131}$I uptake value for tumor No. 28 given in Chart 1 is the average for all five approximately 0.5-gm. specimens obtained on biopsy of an estimated 50-gm. tumor in a 25-kg. spayed springer spaniel. Radioactivity values for the five specimens ranged from 11.1 to 19.9 per cent of injected dose per unit weight, 250 gm. tissue. This tumor, diagnosed on all five specimens as a fibrosarcoma with hemangiomatoid differentiation, occurred at the site of a tumor removed surgically 2 years earlier and diagnosed from microscopic ex-
amination as an intracanalicular fibroadenoma of mammary gland. Tumor No. 30 was obtained from an 18.6-kg. part poodle, in which a "bone" tumor had been present and growing at the proximal end of the left humerus for 2 months. This dog was sacrificed, and specimens from a tumor weighing an estimated 75 gm. and from major organs were obtained for I\(^{131}\) measurement 3 days after labeled antibody injections. On pathological examination the tumor was a synovial sarcoma with hemangiomatoid differentiation. Chart 2 shows the high radioactivity of the three specimens of viable tumor, ranging in weight from 0.5 to 0.8 gm., compared with the relatively low radioactivity of normal organs and tissues. The lung specimen obtained showed microscopically many small synovial cell metastases equaling in bulk a few per cent of lung tissue. The relatively high I\(^{131}\) concentration in lung, 9.4 per cent, compared with tissues not containing tumor, suggests that the metastatic tumor had retained a tendency to concentrate antibody.

Four other dogs, those with tumors No. 3, 17, 19, and 22, were also sacrificed 3 days after antibody injection and showed I\(^{131}\) concentration values for organs uninvolved by tumor closely similar to the organ values of Chart 2. Average value for lung was 2.1 per cent. Various metastatic lesions in dogs 3, 17, and 22 showed low I\(^{131}\) values similar to those in the primary tumors. Tumor No. 19 specimens, all metastatic from a primary tumor removed at an earlier operation, were low except for one liver metastasis value of 31 per cent, which was omitted from the average used in preparing Chart 1. However, most other tumor specimens from this dog appeared largely or completely necrotic, and this high value may conceivably be representative of viable tumor tissue.

The small number and selected nature of these tumors, largely skin or surface growths noticed by the owners, and their often benign nature make possible only the most general conclusions regarding uptake of antibody to fibrin in human cancer—even on the unproved assumption that human tumors would behave like similar dog tumors. One definite finding is that most dog tumors of the sort referred by dog owners to veterinarians for treatment do not concentrate antibody to dog fibrin in amounts great enough to be of therapeutic significance as a means of concentrating radioactivity specifically in tumor tissue. On the other hand, the demonstration that radioactive antibody to fibrin does localize with substantial preferability in some dog tumors shows that this phenomenon is not limited to transplanted tumors in rodents and thus suggests that it may be worthwhile to investigate whether there exist types of human cancer in which this technic of localizing radioactivity preferentially in tumor tissue can be applied as a useful treatment in cases unresponsive to more conventional therapeutic techics.

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REFERENCES

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