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[54] **LOCALIZATION OF TUMORS BY RADIOLABELLED ANTIBODIES**

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[58] **Field of Search** ..... 424/1, 9, 12; 23/230 B; 250/303

[56] **References Cited**

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[57] **ABSTRACT**

A method of utilizing radiolabelled antibodies to carcinoembryonic antigens for determining the site of tumors which produce or are associated with carcinoembryonic antigen is disclosed.

3 Claims, No Drawings

LOCALIZATION OF TUMORS BY RADIOLABELLED ANTIBODIES

BACKGROUND

Carcinoembryonic antigen (CEA) is a mixture of several components, at least two of which have antigenic activity which is associated with human carcinoma. Methods such as those described in U.S. Pat. Nos. 3,663,684 and 3,697,638 have been developed for detecting the presence of CEA circulating in human blood. These known processes, however, do not provide a means for pinpointing the location and the source of the CEA. There is thus a need for a diagnostic method which will be satisfactory for locating the site of the tumor which produces or is associated with CEA.

DESCRIPTION OF THE INVENTION

We have discovered that when a measured amount of radiolabelled antibody specific to carcinoembryonic antigen is administered parenterally, preferably by intravenous injection, the location of carcinoma tumors which either produce or are associated with carcinoembryonic antigen can be determined. As used herein carcinoembryonic antigen (CEA) includes both a mixture of antigenic components with CEA activity or a single such component.

In order to localize the tumor, radiolabelled antibody specific to CEA is injected into the subject and during the following eight days the subject is scanned with a photoscanning device which locates the sites at which the radiolabelled antibody is complexed with CEA. It is at these sites where the tumors associated with CEA are located.

The radioisotope suitable for use in this invention is an atom which does not interfere with the acitivity of the antibody, is sufficiently stable to be detected after complexation with CEA, has a half-life of sufficient duration to enable detection within 8 days and is pharmaceutically inert, i.e., non-toxic and without pharmacological effect, in the amounts used and has a sufficient gamma intensity to be detected readily by the photoscanning techniques presently in use, i.e., 100 to 500 Kev (thousand electron volts). It has been found that while I^{125} or I^{131} are suitable for use in localizing tumors, I^{131} is preferred for use in humans because of its favorable half-life. As a practical matter, any radioisotope with a half-life up to about eight days and a gamma emission of 10 to 5000 Kev is suitable for use.

When radioactive iodine is used, the antibody is radio-iodinated by the procedure of Greenwood, Hunter and Glover, Biochem J. 89 114–123, (1963) as modified by McConahy and Dixon, Int. Arch. Allergy 29 185–189, 1966. The reaction is effected, for example, by using a 100 μl reaction mixture containing 50 μl. of chloramine-T (sodium p-toluenesulfochloramine); 40 μl. of goat anti-CEA Immunoglobulin G in a phosphate buffer of pH 7.5 (10 mg./ml. 0.05 M phosphate, pH 7.5) and 2 mCi of I^{125} or I^{131} in the form of NaI. The reaction takes place in about 1.5 minutes at room temperature and is stopped by the addition of sodium metabisulfite. The radioiodinated product can be separated from the unreacted radioisotope by chromatography in a cross-linked dextran gel column, e.g., Sephadex G-25 or Sephadex G-100 by eluting with Tris-NaCl or PBS. The labelled antibody is stabilized by mixing with a carrier protein such as serum or aqueous human albumin. The resulting product has a specific activity of about 4 to 6 μCi/μg. This specific activity can be modified by altering the reaction conditions. The level of the specific activity to be used should be below that which would interfere with the immunological activity of the anti-CEA antibody. It has been found that about 4 to 50 μCi/μg. are suitable with 4 to 6 μCi/μg preferred.

The antibody utilized is important to the operability of the process since it must be CEA specific and must be amenable to radiolabelling with sufficient activity per unit weight to result in a readily demonstrable effect.

It has been found that animal sources used for producing the antibodies vary greatly from species to species and between animals within a species in their ability to produce large concentrations of highly specific CEA antibodies per unit volume of serum. It has been found that rabbits and goats are suitable, with goats preferred. The antibodies are made by immunizing the animals against purified CEA, such as the CEA produced by the process disclosed in U.S. Pat. No. 3,697,638, and after a suitable time interval obtaining serum from the animal. The antibodies are isolated from the serum by column chromatography using, for example, Sephadex G-200 (Pharmacia, Uppsala, Sweden) and diethylaminoethyl (DEAE) cellulose (Whatman DE 52, H. Reeve Angel Inc., Clifton, N.J.). The purity and specificity of the antibodies is checked by immunoelectrophoresis and immunodiffusion.

The radiolabelled antibody is administered to the subject parenterally, preferably I.V., in an amount sufficient to provide radioactivity which can be readily detected, but not so much that the radioactivity is unsafe for the patient. This is accomplished utilizing a parenteral formulation containing about 300 to 1000 μCi per ml. Generally about 1 to 2 ml. are administered. A suitable formulation for injection contains a phosphate buffer at pH 7.0 and a stabilizer for the radiolabelled antibody.

In order to demonstrate that the radiolabelled antibody does localize tumors which produce or are associated with CEA, hamsters having such tumors implanted are simultaneously injected with an equal mixture of anti-CEA antibody labelled with iodine-125 and normal non-specific immunoglobulin G labelled with iodine-131. After a suitable time, generally from 1 to 8 days, the hamsters are sacrificed and various organs as well as blood are tested for radioactivity and the percent of injected dose found per gram of tissue is calculated for each isotope. By means of this technique, i.e., the paired-labelled antibody technique, there is demonstrated that tumors are preferentially radiolabelled by the labelled CEA antibody whereas the non-specific antibody is distributed randomly. This is shown by the localization ratio.

The following examples illustrate the invention.

EXAMPLE 1

Radioiodination of Antibodies to CEA

a. Two mCi of ^{125}I in the form of NaI are mixed with 40 μl. of goat anti-CEA IgG (10 mg./ml. of 0.05 M phosphate, pH 7.5). Fifty μl. of Chloramine-T (3 mg./ml. phosphate buffer) are added and the mixture allowed to react for 1.5 minutes at room temperature. Fifty μl. of $Na_2S_2O_5$ (6 mg./ml. phosphate buffer) is added to stop the reaction. The unbound radioisotpe is separated by filtration over a 2.6 × 35 cm Sephadex G-100 column employing Tris-NaCl as the eluting

buffer. The iodinated antibody is collected and stabilized in the carrier protein 5% aqueous human albumin (Albuspan - Parke, Davis).

The specific activity of the product is 4–6 μCi/μg.

b. Two mCi of ^{131}I in the form of NaI are mixed with 40 μl. of goat anti-CEA IgG (10 mg./ml. 0.05 M phosphate, pH 7.5). Fifty μl. of Chloramine-T (3 mg./ml. phosphate buffer) are added and the mixture allowed to react for 1.5 minutes at room temperature. The unbound radioisotope is separated by filtration over a 2.6 × 35 cm. Sephadex G-100 column employing Tris-NaCl as the eluting buffer. The iodinated antibody is collected and stabilized in the carrier protein 5% aqueous human albumin (Albuspan - Parke, Davis).

The specific activity of the product is 4–6 μCi/μg. For use in the paired labelled antibody technique normal goat IgG is radioiodinated in the same manner using it in place of the anti-CEA IgG.

EXAMPLE 2

CEA isolated from liver metastasis originating as adenocarcinoma of the colon was purified by column chromatography as described in U.S. Pat. No. 3,697,638. Goat antiserum to the thus prepared CEA was prepared by the s.c. injection into 2 sites of 500 μgm. of CEA in 1 ml. phosphate-buffered saline (PBS) containing 1 mg. of methylated bovine serum albumin and an equal volume of complete Freund's adjuvant. CEA was injected every 2 weeks for a total of 5 injections. The animals were terminally bled 10 days following the final injection.

Twenty ml. of the resulting goat anti-CEA antiserum is dialyzed against 0.1 M Tris-HCl, pH 7.0, containing 0.15 M NaCl and 0.02% sodium azide and applied to a 5 × 90 cm cross-linked dextran gel column (Sephadex G-200) equilibrated with the same buffer and maintained at 4°C. Isolated fractions are tested for anti-CEA activity by the radioimmunoassay of Hansen (U.S. Pat. No. 3,697,638) and the material eluting between 690 and 930 ml. of elution volume is pooled and concentrated by pressure filtration over a Diaflo UM 100 ultrafilter (retains 100,000 MW or greater). The concentrated material was dialyzed against 0.01 M phosphate buffer, pH 8.0, and applied to a 2.6 × 25 cm. diethylaminoethyl (DEAE)-cellulose column equilibrated with the same buffer. Following elution with 1 liter of starting buffer, the anti-CEA IgG appearing between 260 to 600 ml. of elution volume was pooled, dialyzed against distilled water, and lyophilized. Immunoelectrophoresis and immunodiffusion were performed to evaluate the purity of the anti-CEA IgG.

EXAMPLE 3

An injectable formulation was prepared containing 0.200 gms. of radiolabelled anti-CEA antibody with an activity of 400 μCi/ml. of a phosphate buffered saline solution at pH 7.0 containing, by weight, 0.5 to 2.5% hepatitis free-human albumin.

EXAMPLE 4

Demonstration of Localization of Tumors

Male Syrian hamsters weighing 50–60 gms., were heterografted intramuscularly with a CEA producing human signet-ring cell carcinoma of the colon, designated in the laboratory as GW-39. At the time of transplantation, 2.5 mg. of cortisone acetate was given subcutaneously. The resulting tumor-bearing hamsters were administered an intracardial injection of the radiolabelled antibody and normal IgG mixture 1–4 weeks after implantation of tumor. The anti-CEA antibody and normal IgG were labelled with ^{125}I and ^{131}I, respectively. The radioactivities were mixed 1:1 on an activity basis and sterilely filtered. 10–12 μCi of each were given in a final volume of 0.2–0.3 ml. Animals were administered Lugol's solution (a stable KI—I_2 aqueous solution) in their drinking water to block thyroid uptake. At timed intervals, each animal was exsanguinated by cardiac puncture and various organs (liver, spleen, kidney, lung, stomach, muscle, tumor) were removed and radioactivity was determined in each organ as well as in 1 ml. of blood using a gamma scintillation counter. Using appropriately diluted injection mixture standards, the percent of injected dose found per gram of tissue was calculated for each radioisotope. In addition, a localization ratio was derived using the formula:

$$\frac{\text{Anti-CEA Antibody/Normal IgG recovered in tissue}}{\text{Anti-CEA Antibody/Normal IgG injected}}$$

The level of significance between tumors and reference tissues was calculated by the Student's t-test.

The results showing the tumor localization ratio at different time intervals after injection as set forth in the following Table:

TABLE I

Localization Ratio Obtained in Tumor Following Injection of Anti-CEA — Normal IgG Mixture

Day After Injection	Tumor Localization Ratio	± Standard Error (S.E.)	Tumor Wt. Gms. ± S.E.
1	1.6151	.0355	0.49 ± 0.33
2	1.9607	.0826	0.58 ± 0.32
4	2.4825	.1289	0.94 ± 0.46
6	3.5392	.2156	1.2 ± 0.72
8	4.1434	.6506	2.23 ± 1.2

The tumor localization ratio indicates that the anti-CEA antibody is localized on the tumor when compared to the nonspecific antibody. The data show that this ratio increases with time, at least up to 8 days.

The measurement of the ratio of labelled anti-CEA antibody in a tumor compared to the amount in blood increased with time while labelled normal antibodies show a relatively stable ratio. This indicates that at day 1 after injection of the labelled anti-CEA antibody, a tumor can be localized by photoscanning.

The following Table shows the ratios:

Table 2

Tumor/Blood Ratios Obtained After Injection of I^{125} Labelled Anti-CEA Antibody and I^{131} Labelled Normal Antibody

Antibody Days After Injection	Anti-CEA Antibody Tumor/Blood Ratio	Normal Antibody Tumor/Blood Ratio
1	.8132	.4866
2	.9960	.5380
4	1.1420	.5080
6	1.7516	.5800
8	2.3016	.5350

We claim:

1. A method for determining the location of a tumor which either produces or is associated with carcinoem-

bryonic antigen which comprises injecting a subject parenterally with an antibody radiolabelled with a pharmacologically inert radioisotope having a half-life up to about 8 days and a gamma emission of 10 to 5000 Kev said antibody being specific to carcinoembryonic antigen and subsequently scanning the subject with a photoscanning device to determine the location of the

resulting uptake of radiolabelled antibody by said tumor.

2. The method of claim 1 wherein the antibody is radiolabelled with iodine-131.

3. The method of claim 1 wherein the subject is injected intravenously.

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