Use of Isotopic Immunoglobulin in Therapy¹

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Abstract

Antibodies raised in heterologous species against tumor-associated antigens such as ferritin and carcinoembryonic antigen may be utilized in diagnostic scanning and in cancer therapy. The radiolabeled (¹³¹I) antibodies have a mean effective half-life of 3 days. The tumor-bearing regions retain activity which was associated with objective evidence of remission in primary hepatic cancers. Major organ toxicity was not apparent in eight of nine patients treated with radioactive antibody. Objective evidence of clinical remission was documented by computer-assisted axial tomography scan remission in sequential studies that determine residual tumor in the same planar cuts. Future possible improvements in radioimmunoglobulin are discussed in light of the clinical findings.

Introduction

A number of laboratories have demonstrated in experimental models that xenogeneic tumors will concentrate radiolabeled antibodies to tumor-associated antigens as evidenced by nuclear scans in nude mice (8) and in the hamster cheek pouch (6). At least one syngeneic model system has demonstrated similar tumor targeting by radiolabeled antibody (12). More recently, clinical tumor visualization has been accomplished by scanning with radiolabeled anti-CEA³ and anti-ferritin antibodies (5, 17) (Fig. 1).

The therapeutic administration of antibody in cancer therapy has been applied in both ovarian and lung cancers (10, 14). The rationale for ovarian antitumor antiserum is founded in the studies of a syngeneic experimental ovarian cancer (13, 15). The possibility of using antibodies to tumor-associated antigens for the treatment of cancer by radioisotopic deposition offered a new possibility based on the successful clinical scanning of tumors containing ferritin and CEA antigens. These experimental and clinical results were the basis for the present pilot studies.

Materials and Methods

Antigens. Both ferritin and CEA have been isolated and purified as previously reported (3, 4, 7).

Antibody and Radiolabeled IgG: Production of Antisera to Ferritin and CEA. New Zealand White rabbits were immunized with approximately 100 to 200 µg of ferritin or CEA emulsified in complete Freund’s adjuvant i.d. in 4 sites in the thigh, with a repeat 2 weeks later. After an additional 2 weeks, 50 ml of blood were removed by sterile cardiac puncture and allowed to clot at room temperature for 1 hr, followed by refrigeration at 4° overnight to allow for clot retraction. The serum was removed in a sterile manner by centrifugation and frozen at −20° after a sample had been removed for testing. Additional bleedings were performed 2 weeks following booster injections.

The reactivity of the antisera was determined by immunoelectrophoresis on commercially prepared agarose plates (Corning Glass Works, Corning, N. Y.). Four µl of purified ferritin or of CEA in phosphate buffer (1 ml/mg) were placed in the sample wells, and the agarose plate was electrophoresed in EDTA: barbitol buffer, pH 8.6, at 15 V/cm for 35 min. Forty µl of antisera was placed in the trough, and the plate was incubated at 25° in a humidified chamber overnight to allow for immunodiffusion and the appearance of bands. The plates were then dialyzed overnight in 1% NaCl solution followed by 1 hr in deionized water, dried, and stained with Amido black.

Titration of Antisera. Antisera to CEA or ferritin is titrated by radioimmunoassay against the corresponding radiolabeled antigen. Purified antigen was labeled with ¹²⁵I using 100 µCi of Bolton-Hunter reagent (New England Nuclear, Boston, Mass.) per 100 µg of protein (2). The labeled antigen was separated...

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³ The abbreviations used are: CEA, carcinoembryonic antigen; i.d., intradermally; CAT, computer-assisted axial tomography.
from free iodine on a prepacked PD-10, C-25 column (Phar-
macia Fine Chemicals, Inc., Piscataway, N. J.) equilibrated with
dextrose gelatin-Veronal buffer (Grand Island Biological Com-
pany, Grand Island, N. Y.).

The antiserum to be titrated was serially diluted with phos-
phate-buffered saline containing 10% normal rabbit serum.
Twenty-five μl of diluted antiserum were added to 25 μl of
diluted labeled antigen in 6- x 50-mm tubes previously coated
with the fetal calf serum and incubated at 37° for 1 hr. Ten μl
of goat anti-rabbit IgG (Miles Laboratories, Inc., Elkhart, Ind.)
were added for maximum precipitation, and the tubes were
incubated at 4° overnight. The precipitates were washed 3
times with phosphate-buffered saline and were counted in an
automatic γ-counting system. The titer of the serum is that
dilution of antibody that binds 50% of the labeled antigen when
the latter is at a defined concentration. Antibody titers were
times with phosphate-buffered saline and were counted in an
automatic γ-counting system. The titer of the serum is that
dilution of antibody that binds 50% of the labeled antigen when
the latter is at a defined concentration. Antibody titers were
consistent greater than 1:5000 using 100 ng of 131I-labeled
protein per tube.

Preparation and Radiolabeling of IgG. The IgG fraction of
the serum was isolated by multiple precipitations with 33%
ammonium sulfate for 2 hr at room temperature. The solution
was poured into sterile centrifuge tubes and spun down at
3500 rpm at 4° for 30 min. The white precipitate was redis-
solved in one-third to one-half the original volume in sterile 0.1
m NaPO4:0.15 m NaCl, pH 7.5, and dialyzed against the same
buffer overnight at 4°. Insoluble particles were removed by
centrifugation.

The IgG fraction was labeled with 131I by the lactoperoxidase
method (9). In a sterile serum bottle for every 5 mg of IgG, 25
μg lactoperoxidase, 1 μCi of Na131I, and 10 μl of 8 mM hydrogen
peroxide were added in that order. The mixture was stirred
vigorously for 30 min at room temperature, and the labeled
protein was separated from unbound label using a Pharmacia
PD-10, C-25 column (Pharmacia). Sterility and pyrogen testing
was carried out prior to administration.

Patient Selection. Patients initially selected for isotopic im-
munoglobulin therapy had failed conventional treatment. Pa-
ients were not accepted into the program if they were over 75
years old, had active infection, or had a past history of asthma
or eczema.

All patients given radioimmunoglobulin were required to be
capable of self-care with a Karnofsky scale of greater than
60% functional status. The histological diagnosis was con-
firmed by biopsy.

Following initial pilot studies in diverse cancers, a Phase I
and II protocol was designed for the treatment of intrathoracic
cancer including biliary carcinoma and hepatoma. Treatment
required 2100 rad external beam irradiation integrated with
alternating Adriamycin (15 mg) and 5-fluorouracil (500 mg)
followed by Flisyl (6 g/sq m), a known radiosensitizer. At the
end of this treatment course, patients received 2 monthly
maintenance courses of Adriamycin (50 mg/sq m i.v.) plus 5-
fluorouracil (600 mg/sq m i.v.). One month following these 2
cycles, the patients received isotopic immunoglobulin. A WBC
of 4,000 or more, platelet count of greater than 100,000, blood
urea nitrogen less than 25 mg/100 ml, and creatinine less than
1.5 mg/100 ml were required prior to administration of immu-
noglobulin.

Tumor Evaluation. Physical examination, isotopic scanning
and quantitation, and the CAT scan were used where appro-
priate in evaluating tumor response.

In the hepatic cancers, the CAT scan was utilized prior to
initiation of therapy and 1 month following each sequence of
treatment as well as monthly during the follow-up course.

Preparative Regimen for Patients Receiving 131I. Patients
received 10 drops of Lugol’s solution twice a day p.o. 7 days
prior to radiolabeled antibody administration, throughout hos-
pitalization, and at least 2 weeks following treatment. Skin
testing with radiolabeled antibody and eye testing with unla-
beled antibody were carried out 1 day prior to radiolabeled
antibody administration. Independent laboratory verification of
sterility and lack of pyrogenicity of the radiolabeled antibody
was confirmed prior to administration. The patients had an i.v.
catheter line established and a 3-way stopcock in place for
administration of radiolabeled antibody. All patients were
taught temperature recording as well as recording of intake
and output.

Radioactive Monitoring and Dose Calculation. The effective
half-life of 131I-labeled antibody was determined from serial
measurements of the radioactivity in blood samples by scintil-
lation counting (cpm/ml blood) and by monitoring the 131I
exposure rate at the bedside with an unshielded Geiger counter
at a distance of 1 m from the patient. Measurements com-
cibed 1 day following radioimmunoglobulin infusion and were
recorded daily for the duration of the patient’s hospitalization.

Tumor Radiation Dose. The deposition of radiolabeled an-
tibody was established in those patients with primary hepatic
cancer. Relative measurements of the uptake and decay of
131I-labeled antibody in the liver were accomplished with a
Geiger tube shielded with a slotted collimator, 4 half-value
layers (lead) thick. When obtaining counts, the shielded detec-
tor was placed reproducibly on the patient’s skin with the slot
directly over the liver.

An absolute determination of the 131I activity deposited in the
liver and tumor tissue was made by the method of conjugate
γ-camera views (16). Transmission measurements were re-
quired 3 to 4 days prior to injection of radiolabeled antibody
and were carried out for each patient with an external flood
source of 2 mCi of 131I. The patient was positioned reproducibly
under a γ camera utilizing line lasers and skin ink marks over
the regions of interest. A parallel-hole 400-keV collimator was
used for the 364-keV emissions, and a 20% energy window
was selected to reduce scattered radiation effects. Anterior
and posterior transmission measurements of the liver and an
image of the flood source were obtained. Data were recorded
on magnetic tape using a minicomputer interfaced with the
scintillation camera. The computer permitted definition of the
area of interest and provided anterior and posterior count rate
information for each area reviewed.

The calibration factor of the system was determined from a
standard of known activity. Anterior and posterior count rate
information for liver and tumor tissue was obtained from the
tumor scans and was obtained for identical regions in the trans-
mision and flood source images. Average values of the 131I
activity in liver and tumor tissue were computed from these
data. These results provided quantitative calibration for the
relative uptake measurements made with the shielded Geiger
counter.

Tumor:Liver Ratio. The ratio of tumor-bearing to normal liver
tissue was estimated from representative slices of CAT scans
using the most involved view as the cut to be reproducibly
evaluated prior to, during, and following each form of integrated
therapy. The normal and tumor-bearing regions of the liver were contoured manually and digitized on a treatment-planning computer, and the ratios of the areas were determined.

Specialized Room Requirements. The patient's room and the nuclear scanning area were protected by portable shields designed in cooperation with the Radium Chemical Company and our physics department to minimize the allowable exposure rate at any point in time to below 2 mR/hr.

Results

The effective half-life of radioimmunoglobulin for total body irradiation as determined by external counting and blood sample determinations had a mean value of 3.0 days (Table 1; Chart 1). Our estimated dose of total body irradiation was 1 rad/mCi administered radioimmunoglobulin.

Table 1
Effective half-life of radiolabeled antibody in 9 patients receiving radioimmunoglobulin

<table>
<thead>
<tr>
<th>Cancer pilot studies</th>
<th>Effective half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonhepatic</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
</tr>
<tr>
<td>Hepatic</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td>Mean</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The 4 patients with nonhepatic cancers had colon cancer (2), lung cancer, and a floor-of-the-mouth primary. The only toxic reaction to antiserum was a transient fever seen in the patient with lung cancer. A late radiation reaction was noted in the patient with the floor-of-the-mouth tumor consisting of a radiation mucositis and could be viewed as a preferable reaction since it was a reflection of the absorbed radiation dose rather than uncontrollable toxicity. In fact, the mucositis cleared in a reasonably short period of time. The 4 patients with nonhepatic cancer had demonstrable tumor localization, perhaps the most dramatic being that in the patient with the floor-of-the-mouth lesion (Fig. 2). In addition, this patient stopped taking the Lugol’s solution and was the only patient in the series to demonstrate thyroid uptake. Considering the combined modalities utilized in the treatment of these patients, it is remarkable that no significant toxicity was recorded (Table 2).

Further, in over 20 patients who were given radiolabeled anti-CEA, this was the only patient demonstrating floor-of-the-mouth localization. No significant therapeutic effect, however, was noted in any of the nonhepatic cancers treated with radiolabeled antibody up to a dose of 100 mCi.

Of the 4 patients treated with primary hepatic cancer, 2 had intrahepatic biliary carcinoma (CEA), and 2 had hepatoma (ferritin). Three of the 4 patients demonstrated significant tumor regression as evidenced by CAT scan analysis (Table 3; Fig. 3; Chart 2).

The only patient not benefiting from radioimmunoglobulin had a 3-month delay in administration because of hematopoietic depression and also had ascites at the time of radioimmunoglobulin infusion.

Acute toxicity was recorded in only 1 of 9 patients treated

Table 2
Integrated or previous treatment

Pilot use of radioimmunoglobulin following conventional therapy failures

<table>
<thead>
<tr>
<th>131I-antibody (mCi)</th>
<th>Other treatment</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>Chest and supraclavicular irradiation</td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>(lung)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Surgery (colon)</td>
<td>None</td>
</tr>
<tr>
<td>115</td>
<td>Abdominal irradiation</td>
<td>None</td>
</tr>
<tr>
<td>117</td>
<td>Surgery (colon)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irradiation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3 times)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irradiation (floor of mouth)</td>
<td>Radiation mucositis</td>
</tr>
</tbody>
</table>

Table 3
Primary hepatic cancer

CAT scan data of remission in 4 patients with primary hepatic cancer. A planar cut is followed throughout the treatment course and a computer program determines the percentage of residual tumor.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Irradiation</th>
<th>Chemo-therapy</th>
<th>Isotopic IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (sq cm)</td>
<td>356</td>
<td>366</td>
<td>371</td>
</tr>
<tr>
<td>% of tumor</td>
<td>68%</td>
<td>30%</td>
<td>42%</td>
</tr>
<tr>
<td>Liver (sq cm)</td>
<td>351</td>
<td>321</td>
<td>426</td>
</tr>
<tr>
<td>% of tumor</td>
<td>47%</td>
<td>35%</td>
<td>40%</td>
</tr>
<tr>
<td>Liver (sq cm)</td>
<td>540</td>
<td>460</td>
<td>490</td>
</tr>
<tr>
<td>% of tumor</td>
<td>69%</td>
<td>54%</td>
<td>15%</td>
</tr>
<tr>
<td>Liver (sq cm)</td>
<td>462</td>
<td>468</td>
<td>466</td>
</tr>
<tr>
<td>% of tumor</td>
<td>53%</td>
<td>51%</td>
<td>34%</td>
</tr>
</tbody>
</table>

* Three-month delay (hematopoietic) period.
* Regression continued to 5%.
The patient had spontaneous hematopoietic recovery and required no chemotherapy occurring 3 weeks after immunoglobulin. The patient expressed late toxicity as evidenced by significant hematopoietic depression following 3 weeks after immunoglobulin. Not only was this evident by clinical status and physical examination but it was also clearly documented by serial CAT scans with objective evidence of remission of tumor. These unmaintained remissions were of 2, 9, and 12 months duration. Two of the 3 patients achieved a 90% Karnofsky status and returned to normal activities. The patient with the shortest remission had the most advanced hepatic cancer as evidenced by tumor replacement of a massively enlarged liver and intrahepatic jaundice which reversed only transiently during the course of therapy.

**Discussion**

In the early development of a potential new modality for cancer therapy, the first and critical question is the toxicity of the administered agent. In the combined experience of those exploring radioimmunoglobulin for diagnostic purposes and our own experience with radioimmunoglobulin in therapeutic applications, the evidence supports the relative safety of radioimmunoglobulin in the doses administered to date. Obviously, the need for blockade of the thyroid (if radioactive iodine is used), the careful historical evaluation of asthma and eczema, the exclusion of potentially hypersensitive patients, and finally the proper precautions for sterility, pyrogenicity, and environmental control of the patient were all necessary preparative features of the therapeutic application of radioimmunoglobulin.

The mean effective half-life of 3 days was also determined to be representative of the i.v. administration of radioimmunoglobulin. The effect of total body irradiation and potential hematopoietic depression will be features requiring further evaluation in future administration.

It must be realized that the irradiation dose absorbed both in the total body and at the tumor-bearing site was at a low dose rate, which has a radiological effectiveness different from that of conventional dose irradiation both in tumoricidal effectiveness and in potential complications and toxicity. As the modality is presently utilized, it would seem that ultimate integration with chemotherapy with such agents as Adriamycin which causes sensitization to radiation and/or radiosensitizers could amplify the tumoricidal effectiveness of such agents. The relatively slow biological accumulation of radioimmunoglobulin at the tumor-bearing site which takes 3 to 4 days in these preliminary studies seems to have an effective half-life of decay of 7 to 7.5 days. This illustrates further the potential differential dose deposition between the total body radiation and the radiation at the tumor-bearing site.

The use of conventional cytotoxic reductive agents prior to the administration of radioimmunoglobulin reduces the tumor volume, thereby allowing greater accumulation in the tumor-bearing site and, for geometric reasons, a higher radiation dose. From our previous experience in direct radioactive implantation in tumor-bearing sites, we have learned that the greater the concentration of isotopes in a tumor-bearing region and the greater the restriction of the tumor-bearing region, the higher the potential dose achieved. In addition, ideally, tumoricidal doses have been in the range of 40 to 60 rad/hr, significantly greater than the present dose rate achieved by radioimmunoglobulin of 2 to 5 rad/hr.

The tumor-normal tissue ratio for scanning purposes was high and on review of the effective half-life was preferentially distributed in favor of a tumoricidal effect. However, it was realized that localization may be due to immunospecific binding directly related to the Fab portion of the IgG molecule as well as Fc fragment binding due to the fact that solid tumors have been demonstrated to have Fc receptors (1). In addition, although there was no direct evidence that the sludging of the blood volume in the hypervascular tumor bed had an effect on antibody binding, there must certainly be some differential deposition which may be related to blood sludging, antigen concentration, or some other feature which causes the localization and preferential binding of antibodies to tumor-associated antigens. Antibodies to tumor-associated antigens could cross-react in other sites but are not demonstrable in significant concentration by nuclear scanning of these sites, i.e., colon-CEA and marrow-ferritin.

The potential of increased dose deposition, with affinity chromatography-prepared antibody and possibly hybridoma-generated high-specific-activity antibody are applications that await future trials. If we could achieve a dose rate of 20 to 40 rad/hr in the tumor-bearing site, this would represent to radiotherapists a "biological implant" similar to the mechanical and direct implantation that we presently carry out in accessible tumor-bearing sites.

Heterologous antibody, although binding by antibody characteristics, remains antigenic to the host, thereby causing autosensitization, and thus may amplify the cytotoxic effect of
deposited antibody in addition to radiation effects. Presently, we are utilizing nonradioactive antibody in ovarian cancer with the basis being model work carried out in the experimental laboratory and translated to the clinical program. This randomized prospective study may elucidate potential benefit of unlabeled antibody, but our present experience of a 92% disease-free remission for the entire group of patients studied will require further observation (14).

The need for further study and the possibility of varied applications of antibodies to tumor-associated antigens also increases the consideration of radioactive chemotherapeutic and other biological agents integrated with standard chemotherapy and/or radiotherapy in the treatment of disseminated cancer. Presently, we are restricted in the use of radiation therapy to regional treatment and only in the applications of total body or half-body irradiation to systemic disease. Even in these approaches, the regions treated and dose deposited in normal tissue sites were not preferentially located. The use of radioimmunoglobulin is to be expected in future applications which will allow amplification of tumor deposition relative to background and further improvement in these agents.

The traditional points of discussion that have been raised when therapeutic radioimmunoglobulin was first being considered for possible cancer therapy, i.e., that heterologous antibody could not be safely administered, that no significant dose deposition would be achieved, that renal shutdown would occur as a result of such administration, and that tumor localization could not be achieved, have now been shown not to be major barriers. Rather, we now face the scientific task of describing the best immunoglobulin agents, and the most highly active antigens, and of learning more about dose and dose deposition, as well as the integration of radioimmunoglobulin in cancer therapy. The targeting of tumor-bearing sites by radioimmunoglobulin has been clearly demonstrated by multiple laboratories at different institutions. Future programs should integrate immunology, nuclear medicine, physics, medical oncology, and radiotherapy in what will truly be multimodality treatment programs (11).

References


Fig. 2. A patient scanned with 131I-anti-CEA IgG with a floor-of-the-mouth lesion with tumor uptake (arrows). The patient did not take Lugol’s solution and thus had thyroid uptake. The blood pool in the heart was seen in the base of the scan.

Fig. 3. A. CAT scan of a hepatoma prior to treatment; B, radioactive scan with the heart and blood pool above and the tumor scan below (arrows); C, remission of disease following radioimmunoglobulin (anti-ferritin).
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