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Antibody-guided Localization of Intraperitoneal Tumors following Intraperitoneal or Intravenous Antibody Administration

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ABSTRACT

Intraperitoneal tumors from a human cancer cell line (LoVo) were established in nude mice by i.p. inoculation of a single cell suspension. Two preparations of the same monoclonal antibody, radiolabeled with ^{125}I and ^{131}I were injected i.p. and i.v. into the same animals. Localization was assessed by dissection and counting the activity in tumors and normal tissues.

Tumor/tissue ratios 1 h after i.p. injection of antibody were approximately 50 times higher than after i.v. administration. This i.p./i.v. advantage fell to around 4 by 8 h and was just greater than 1 by 24 h. This effect was observed with both specific and nonspecific antibody, indicating that it is due to the route of administration. However, the absolute amounts of antibody bound to tumors depended on the specificity of the antibody. Twenty % of the injected dose of specific antibody was bound per gram to tumor 1 to 2 h after i.p. injection, falling to 10%/g by 24 h and remaining at this level up to 5 days after antibody administration. In contrast, less than 10%/g of nonspecific antibody was detected in tumors after 1 h; this fell rapidly to normal organ levels of less than 5%/g by 8 h.

This study demonstrates a major advantage when administering radiolabeled monoclonal antibodies i.p. for targeting intraperitoneal tumors.

INTRODUCTION

In many western countries, ovarian cancer has the highest incidence and mortality of any gynecological cancer (1). The initial spread of the tumor is often over the serosal surfaces of the peritoneal cavity; spread via the bloodstream occurs later and more rarely. A major cause of death in ovarian cancer patients is the inability to control disease within the peritoneal cavity. Overall survival has improved only marginally (2), as relapses occur even after surgically documented complete remission.

Administration of tumoricidal agents i.p. has a strong pharmacological and biological rationale for the treatment of intraperitoneally confined diseases such as ovarian cancer (3). Evidence has already been presented showing the pharmacological advantage of i.p. administration of chemotherapy agents (4–7) or other biological response modifiers such as interferon (8). A small number of patients has been treated by i.p. administration of radiolabeled antibodies, and encouraging results have been reported (9, 10).

Human tumors growing in athymic (nude) mice provide a model to test these observations in a more controlled system. The use of an established human cell line ensures genetically equivalent tumors in a large number of animals. The present study was conducted in order to assess the advantages, if any, of i.p. over i.v. administration of radiolabeled monoclonal antibodies in a xenograft model of intraperitoneally growing human tumors. This model has many features in common with clinical disease, including peritoneal spread, occasional ascites formation, and human cancer-associated antigen detectable by the monoclonal antibody AUA1.

MATERIALS AND METHODS

Mice

The animals used in this study were 2-mo-old female beige nude mice, bred at the Imperial Cancer Research Fund Animal Breeding Unit, South Mimms, Hertfordshire, United Kingdom. The mice were specific pathogen free, housed in sterile filter-top cages with sterile bedding, and maintained on irradiated diet and acidified water (pH 2.8) throughout the experiment.

Establishment of Tumors

The human carcinoembryonic antigen-producing colon adenocarcinoma cell line, LoVo, was established from a metastatic nodule in a patient with adenocarcinoma of the colon (11). The biological and cell kinetic properties of this cell line have been extensively studied (12).

Cells were cultured in RPMI 1640 medium plus L-glutamine supplemented with 10% fetal calf serum and antibiotics (100 units/ml) (Gibco, United Kingdom) in large tissue culture flasks (Nunc, Roskilde, Denmark). Cells were harvested by incubating with 0.02% EDTA solution for 5 min at 37°C and then washed with medium. A single cell suspension was achieved by repeat pipetting. After counting, cells were resuspended in medium to a concentration of 10^7 cells/ml.

Tumors were established in nude mice by an i.p. inoculation of 10^6 cells in 0.1 ml of medium. At this concentration, the incidence of subcutaneous tumors at the site of injection was minimized. Tumors were allowed to grow for 6 wk before injection of antibodies.

Monoclonal Antibodies

AUA1. Monoclonal antibody AUA1 was raised by immunizing BALB/c mice with the colon carcinoma cell line LoVo (11, 12). AUA1 is an IgG1 mouse immunoglobulin directed against a M_r 35,000 protein associated with the majority of human gastrointestinal, ovarian, and breast carcinomas, as well as proliferating epithelial cells in tissues such as the villi of normal colon (13).

HMFG2. This monoclonal antibody was developed in mice using as immunogen delipidated human milk fat globule and human mammary epithelial cells (14, 15). HMFG2 is an IgG1 class antibody directed to a large mucin-like molecule normally produced by the lactating human mammary epithelial cell. It reacts with carcinomas of the breast and ovary, but not with tumors from mesenchymal tissue (16, 17). It was shown to be negative for LoVo tumors by immunoperoxidase staining. Therefore, it was used as the *in vivo* control antibody in this study in order to determine nonspecific uptake.

Antibodies were produced in bulk by culturing hybridomas in Tissue Culture Medium RPMI 1640 containing 10% fetal calf serum. The antibodies were purified by affinity chromatography, using a sterile Protein A-Sepharose column (Pharmacia), and eluting with 0.1 M citrate buffer of decreasing pH (18). Purity was assessed by isoelectric focusing on agarose (19) and by polyacrylamide gel electrophoresis (20).

Radiolabeling of Antibodies. ^{125}I or ^{131}I (Amersham International IMS 30 and IBS 30, respectively) was used to label the antibodies using the iodogen method (21). Iodogen (Pierce and Warner, Ltd.) dissolved in dichloromethane (1 mg/ml) was aliquoted into polypropylene tubes, and the solvent was evaporated to dryness. These iodogen tubes (200 μg of iodogen per tube) were stored desiccated at 4°C for up to 6 mo.

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Five hundred μg of antibody (1 to 2 mg/ml in 0.1 M citrate buffer, pH 7.5) and 5 mCi of radioiodine were mixed in an iodogen tube for 10 min at room temperature. The radiolabeling efficiency was assayed by ascending paper chromatography of a 5-μl sample of reaction mixture using 10% (w/v) trichloroacetic acid as solvent. The point of origin (protein-bound radioisotope) and solvent front (free radioisotope) were counted in an ionization isotope calibrator [Scientific and Industrial Equipment (Reading), Ltd., Aldermaston, United Kingdom]. The reaction mixture was purified by sephadex G50 (Pharmacia) gel filtration on a 20-ml column using PBS[3] (pH 7.4) as elution buffer. Two-ml fractions were collected and measured in the isotope calibrator. The radioiodinated immunoglobulin fractions were pooled with 2 ml of 1% (w/v) human serum albumin in PBS (pH 7.4), sterilized by 0.22-μm Millipore filtration, and stored at 4°C.

Immunoreactivity of Antibodies. The reactivity of antibodies before and after iodination was tested as previously described (22). This involved the use of a direct radioimmunoassay and also a competitive assay against unlabeled antibody.

Administration of Antibodies. Mice were given injections i.p. or i.v. of 2 μg of two antibodies labeled with between 15 and 20 μCi of ^{125}I or ^{131}I. Mice received either the same antibody via two different routes of administration, each labeled with a different isotope, or two different antibodies i.p., the specific antibody and the negative control. Experiments were repeated, reversing the radiolabels.

Analysis of Tissue Counts

At times after antibody administration varying from 1 h to 5 days, groups of 5 mice were killed by exsanguination and immediately dissected. All the i.p. tumor nodules as well as samples of liver, kidney, spleen, lung, and other normal tissues were washed in PBS containing 250 units of heparin/100 ml, blotted dry, and weighed. Blood and urine samples were taken and weighed. ^{125}I and ^{131}I activity in the samples was counted using a 2-channel gamma counter (LKB Wallac 1280 Ultrogamma). After correction for interchannel cross-over, activity was expressed as cpm/g of wet tissue.

RESULTS

Antibodies. Purity of immunoglobulin preparations after supernatants were passed through a Protein A-Sepharose column was greater than 99%, as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing. Labeling efficiencies between 75 and 99% were obtained giving radiolabeled antibodies with specific activities between 7.5 and 10 mCi/mg. There was no significant loss of immunoreactivity when labeled antibodies were tested in radioimmunoassays and in competition with unlabeled antibodies (22).

Tumor Growth. Ninety-seven % of mice dissected 6 to 7 wk after inoculation of 10^6 LoVo cells had detectable tumors in the peritoneal cavity. The mean tumor mass was 0.116 g (mean of 54 mice), with a range of 0.004 to 0.669 g.

Tumors grew both as small unattached avascular nodules up to 1.5 mm in diameter and as larger poorly vascularized masses attached to the intestines, mesentery, or stomach. Metastatic spread above the diaphragm was not observed.

Antibody Localization. The amount of radioactivity in intraperitoneal tumors, blood, and normal organs is shown in Fig. 1 as the percentage of injected dose per g. Liver and kidney activities are plotted as representative normal organs. Spleen and lung activities were approximately equal to those of liver and kidney, while in other normal tissues (heart, bone, stomach, intestine, and skin), activities were lower. Error bars on the normal organ curves have been omitted for clarity. The errors on these points were due to variability in the blood activity and were less than the percentage errors on the blood points. Some of the blood and urine samples were analyzed by trichloroacetic acid precipitation using ascending paper chromatography. This indicated that, at time points between 1 and 4 days, 93% of the radiolabel in blood was protein bound and that 96% in urine was free radioiodine. Thyroid uptake was not significantly greater than kidney and liver levels, although no thyroid blockage was used.

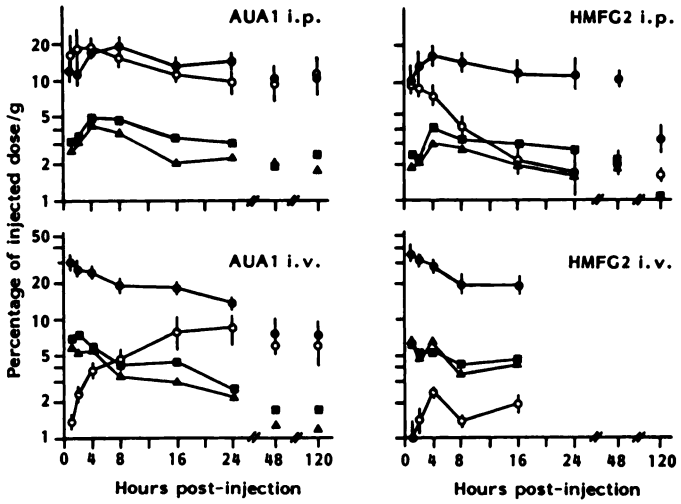

Fig. 1. Absolute amounts of injected antibody/g of intraperitoneal tumor (○), blood (●), kidney (■), and liver (▲) following i.p. or i.v. injection of specific (AUA1) or control (HMFG2) antibodies. *Points*, mean of between 5 and 12 samples; *bars*, SE, shown for tumor and blood only.

The *left-hand graphs* in Fig. 1 show the localization of the specific antibody AUA1, following i.p. (*upper graph*) or i.v. (*lower graph*) administration. Almost 20% of the injected dose was bound per gram of i.p. tumor 1 h after i.p. antibody injection and stayed at this level for up to 8 h after injection, before falling to around 10%/g by 24 h, and remaining at that level for up to 5 days. The tumor activity following i.v. antibody administration was 1.4%/g at 1 h, lower than normal organ levels of 5%/g. Eight h after injection, tumor activity was equal to that in normal tissues, and by 24 h, it had risen to 10%/g, similar to that achieved by i.p. administration. The *right-hand graphs* show the nonspecific binding of the control antibody. There was some (9%/g) nonspecific tumor uptake immediately after i.p. injection, but this fell rapidly to normal tissue levels by 8 h. The i.v. nonspecific antibody time course was only measured up to 16 h, since the normal organ and blood levels after i.p. or i.v. administration of AUA1 after this time were shown to be equivalent. Tumor uptake of HMFG2 was lower than normal tissue uptake at all time points.

The tumor/normal tissue ratios for the specific and control antibodies following both routes of administration are shown in Fig. 2, and in detail in Tables 1 and 2, for blood, kidney, and liver. The binding of AUA1 is far greater than that of HMFG2 in both cases, and the highest ratios are seen at very short times after i.p. injection of AUA1.

The *lower graphs* show that the peak tumor/tissue ratios occur 2 h after i.p. administration of AUA1. The control ratios are approximately 1 by 16 h after i.p. injection, whereas the AUA1 ratios start to rise after this, as nonspecific uptake by normal organs decreases. These tumor levels are maintained up to 5 days postinjection (as shown in Fig. 1). The *upper graphs* show the tumor uptake from the circulation of AUA1, but not HMFG2, increases until both routes of administration become almost equivalent by 16 h.

[3] The abbreviation used is: PBS, phosphate-buffered saline [Dulbecco's formula (modified) without magnesium and calcium (Flow Laboratories).

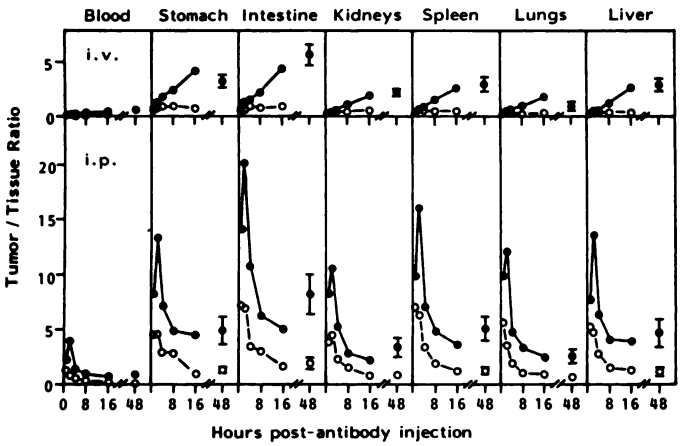

Fig. 2. Tumor/normal tissue uptake ratios following i.v. (*top*) or i.p. (*bottom*) injection of specific AUA1 (●) or control HMFG2 (○) antibodies. *Points*, mean of between 4 and 10 samples; *bars*, SE, shown for the 48-h only.

Table 1 *Tumor/tissue ratios following i.v. or i.p. injection of specific (AUA1) antibody in mice bearing i.p. human tumors*

h postinjection	Tumor/blood	Tumor/kidney	Tumor/liver
Intravenous			
1	0.04 ± 0.00[a]	0.19 ± 0.03	0.21 ± 0.03
2	0.11 ± 0.02	0.46 ± 0.07	0.59 ± 0.10
4	0.15 ± 0.01	0.71 ± 0.08	0.69 ± 0.08
8	0.24 ± 0.04	1.2 ± 0.2	1.4 ± 0.2
16	0.5 ± 0.2	1.9 ± 0.7	2.7 ± 0.7
48	0.6 ± 0.1	2.2 ± 0.3	3.0 ± 0.6
Intraperitoneal			
1	2.2 ± 1.1	8.2 ± 3.3	7.7 ± 2.8
2	3.9 ± 1.4	10.5 ± 4.2	13.5 ± 6.2
4	1.5 ± 0.2	5.3 ± 0.9	6.3 ± 1.0
8	0.9 ± 0.2	2.8 ± 0.8	4.1 ± 1.0
16	0.6 ± 0.1	2.3 ± 0.4	4.0 ± 0.7
48	0.9 ± 0.1	3.3 ± 0.8	4.7 ± 1.3

[a] Mean ± SE of between 4 and 10 mice per point.

Table 2 *Tumor/tissue ratios following i.v. or i.p. injection of control (HMFG2) antibody in mice bearing i.p. human tumors*

h postinjection	Tumor/blood	Tumor/kidney	Tumor/liver
Intravenous			
1	0.03 ± 0.01[a]	0.14 ± 0.03	0.14 ± 0.03
2	0.05 ± 0.01	0.29 ± 0.06	0.29 ± 0.04
4	0.08 ± 0.01	0.53 ± 0.20	0.37 ± 0.04
8	0.08 ± 0.01	0.39 ± 0.06	0.37 ± 0.06
16	0.09 ± 0.03	0.48 ± 0.16	0.42 ± 0.07
48	N/D[b]	N/D	N/D
Intraperitoneal			
1	1.3 ± 0.3	3.9 ± 0.8	5.1 ± 0.6
2	0.8 ± 0.2	4.6 ± 1.3	4.7 ± 1.3
4	0.6 ± 0.1	2.2 ± 0.4	2.8 ± 0.6
8	0.32 ± 0.08	1.5 ± 0.4	1.5 ± 0.2
16	0.21 ± 0.03	0.8 ± 0.1	1.4 ± 0.3
48	0.18 ± 0.04	0.9 ± 0.2	1.2 ± 0.4

[a] Mean ± SE of between 4 and 10 mice per point.
[b] N/D, not determined.

This is further indicated in Fig. 3, which is a plot of the tumor/tissue ratios following i.p. injection divided by those following i.v. injection, what we called the "i.p./i.v. advantage." The advantage of i.p. over i.v. is similar for all normal organs, falling from around 70 at 1 h to just greater than 1 by 24 h when the advantage is lost. The short-term advantage appears similar in the case of the nonspecific binding, indicating that it is due to the route of administration of the antibody. However, in the case of nonspecific antibody, the drop is steeper between 1 and 4 h, and the absolute amount of bound antibody is much lower (Fig. 1).

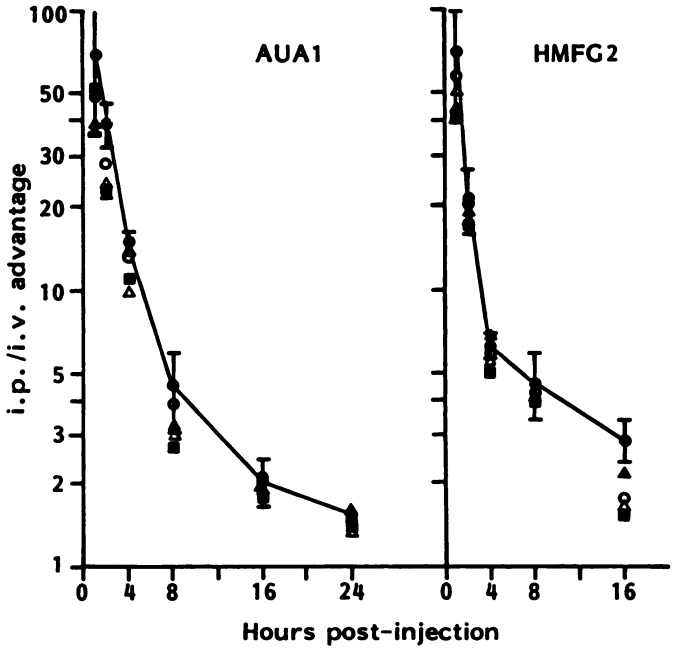

Fig. 3. Tumor/normal tissue ratios following i.p. injection relative to those following i.v. injection (the i.p./i.v. advantage) for specific (AUA1) and control (HMFG2) antibodies. The *symbols* refer to ratios of tumor to blood (●), kidney (■), liver (▲), spleen (△), and lungs (○). *Points*, mean of 4 to 5 samples; *bars*, SE, shown for blood only.

DISCUSSION

We and others (22–27) have previously shown that, although good tumor/nontumor ratios can be achieved when radiolabeled monoclonal antibodies are administered i.v. for tumor imaging in patients with malignant disease, the absolute amount of labeled antibody located in the tumors is very small (0.01 to 0.001% of the injected dose per gram of tumor) (22, 27, 28). Calculations performed on these total amounts of radioactivity in the tumor indicate that i.v. administration of high doses of radiolabeled antibody would result in untoward toxicity to the host without achieving radiotherapeutic benefit (22, 28, 29). Data indicate that the percentage of injected dose in the tumor must be increased at least 10-fold if lethal whole-body irradiation is to be avoided and a satisfactory radiotherapeutic benefit is to be achieved (22, 28, 29).

Dedrick and coworkers (3) first presented a mathematical model demonstrating the rationale for the i.p. administration of chemotherapy for the treatment of diseases, such as ovarian cancer, that are principally to be confined to the peritoneal cavity (1). Furthermore, pharmacokinetic modeling studies indicated (3) that large molecules (*e.g.*, immunoglobulin or albumin) can penetrate deeper into tissues than smaller molecules (*e.g.*, chemotherapy), because larger molecules are removed more slowly from a cavity. This, in theory, should allow for a further therapeutic advantage of large molecules over small molecules when given i.p.

In this paper, we demonstrate that i.p. administration of radiolabeled antibody to tumors confined to the peritoneal cavity can achieve about a 50-fold advantage over i.v. administration. The advantage was more marked in the first few hours, up to 8 h after injection, and in mice was virtually lost by 24 h. This should allow higher tumor and lower normal tissue doses leading to a favorable therapeutic effect, particularly if radioisotopes with shorter half-lives are used. ^{90}Y, with a 64-h half-life, has been successfully used to label antibodies (30, 31). ^{90}Y has the added advantage of being a pure β-emitter, so it should

increase tumor dose and reduce systemic toxicity still further. Previous studies using ^{131}I-labeled antibodies have shown that 10% of the whole-body dose was calculated to be due to the γ-emission from the radioisotope (10). Furthermore, pure β-emitters are safer to handle by laboratory, medical, and nursing staffs. The use of F(ab')$_2$ antibody fragments could further increase the tumor/normal tissue ratio, since there is less nonspecific uptake, particularly by the reticuloendothelial system, and fragments are cleared more rapidly from the circulation than whole antibody (32). However, F(ab')$_2$ fragments accumulate to a significant extent in the kidneys (33), and thus careful consideration has to be exercised prior to their therapeutic application, in order to avoid untoward renal toxicity. In addition, the absolute amount of F(ab')$_2$ fragments accumulating in tumor may be less than intact antibody (33).

Following i.p. administration, peak blood levels in mice were reached by 2 h, while in clinical studies, we have observed peak blood levels after 24 to 48 h (34). This indicates that the pharmacological advantage of i.p. administered radiolabeled antibodies may be even higher in patients than in nude mice.

In conclusion, our study supports the theory in favor of i.p. administration of agents for the treatment of ovarian cancer. Clinically, however, technical and theoretical problems associated with this form of drug delivery remain to be resolved (34, 35).

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