

EVALUATION OF TUMOR TARGETING WITH RADIOLABELED F(ab')₂ FRAGMENT OF A HUMANIZED MONOCLONAL ANTIBODY

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ABSTRACT

Humanized monoclonal antibody U36 and its F(ab')₂ fragment, radiolabeled with ¹²⁵I, were tested for tumor localization in nude mice bearing a squamous cell carcinoma xenograft line derived from a head and neck carcinoma. Monoclonal antibody IgG or F(ab')₂ fragment were injected in parallel and at days 1, 2 and 3, mice were dissected for determination of isotope biodistribution. IgG as well as F(ab')₂ showed highly specific localization in tumor tissue. The mean tumor uptake (n=3) is expressed as the percentage of the injected dose per gram of tumor tissue (% ID/g). % ID/g of IgG was 11.7% at day 1 and decreased to 10.9% at day 3 whereas % ID/g of F(ab')₂ was 2.9% at day 1 and decreased on following days. Tumor to blood ratios (T/B) at day 1 were 0.86 for IgG and 1.32 for F(ab')₂ and reached a maximum at day 3 with values of 4.41 and 1.84 respectively. These findings suggest that the superior tumor to non-tumor ratios in the day of 1 render the F(ab')₂ fragment more qualified for specific targeting radioisotopes to tumor xenografts in this experimental setting.

Keywords: Humanized monoclonal antibody, F(ab')₂ Fragment, I-125

INTRODUCTION

The intrinsic properties of immunoglobulin (IgG) molecules and their fragments regulate the in vivo biodistribution properties of these molecules in tumor bearing hosts (1). Monoclonal antibodies (MAbs) and fragments have been used in the diagnosis and therapy of hematopoietic and nonhematopoietic tumors in the humans and rodents (2). Intact IgG molecules are large (M_r = 150000) glycoproteins that exhibit a slow systemic clearance, low tumor blood flow, poor vascular permeability and high marrow toxicity, leading to poor tumor targeting specificity (3,4). In order to overcome these disadvantages, the use of antibody fragments including enzymatically produced M_r = 100000 F(ab')₂ and M_r = 50000 Fab fragments and engineered M_r = 25000 single chain Fv have been studied intensively (5). Compared to IgG, F(ab')₂, Fab and single chain Fv exhibit significantly improved tumor specificity and intratumor penetration in animal models (1). Generally, lower-molecular-weight agents provide better target to nontarget ratios due to their rapid background clearance (6,7).

Murine monoclonal antibodies can induce a human-antimouse antibody (HAMA) response

which may lead to anaphylactic shock and rapid clearance of injected monoclonal antibody (8).

Besides the use of monoclonal antibody fragments, other strategies to avoid HAMA response, are the development of chimeric, humanized and human monoclonal antibodies (9). Humanized monoclonal antibody U36 recognises an epitope encoded by variant exon V6 of the 200 kDa CD44 antigen which is expressed in 96% of squamous cell carcinoma of the head and neck (10).

The purpose of this investigation was to compare biodistribution characteristics of ¹²⁵I-labeled humanized monoclonal antibody U36 and its F(ab')₂ fragment in order to identify potentially useful radioconjugates for the diagnosis of squamous cell carcinoma.

MATERIALS AND METHODS

Cell line and Monoclonal Antibody (MAb)

UM-SCC-11B cell line was cultured under 5% CO₂ at 37 °C in DMEM supplemented with 2 mM L-glutamine, 16 mM NaHCO₃, 5% fetal calf serum, 1% penicillin, 1% streptomycin and 15 mM HEPES, pH 7.4. Humanized monoclonal antibody U36 was obtained from Dr. Dongen GAMS (Amsterdam, The Netherlands).

Purified humanized monoclonal antibody U36 was digested with 4% (w/w) pepsin (Sigma) for 16 hours at 37 °C in 25 mM sodium acetate buffer pH 4.0.

The reaction was terminated by addition of 2 M Tris to bring the pH at 8.0. The F(ab')₂ fragments were purified by Superose 12 column chromatography (Pharmacia, Uppsala, Sweden) followed by elution with PBS pH 7.4. The purity of MAb and F(ab')₂ preparation was evaluated by SDS-PAGE under nonreducing conditions and proved to be more than 95%.

Radioiodination

Iodination of MAb and F(ab')₂ fragments was performed essentially as described by Haisma et al. (11). Solutions of 500 µg of MAb or F(ab')₂ fragment dissolved in 500 µl phosphate buffer saline, were mixed with 500 µCi ¹²⁵I (Amersham, Aylesbury, England) in a vial coated with 75 µg Iodogen (Pierce, Oud Beijerland, The Netherlands). After 3 min incubation at room temperature, free iodine was removed by gel filtration on a PD-10 column (Pharmacia, Uppsala, Sweden). For determination of the radiochemical purity by two methods. HPLC analysis of ¹²⁵I-labeled MAb or F(ab')₂ fragment was performed by a 10 × 300 mm Pharmacia Biotech Superdex 200 HR 10/30 column. The eluent consisted of 0.05 M sodium phosphate, 0.15 M sodium chloride plus 0.05% azide (pH 6.8) and flow rate was set at rate of 0.5 ml/min. TLC analysis of ¹²⁵I-labeled MAb or F(ab')₂ fragment was carried out on ITLC-SG (Gelman Sciences Inc.) with 0.1 M sodium citrate PH 5.0 as eluent. R_f values were 0.0 for the labeled MAb and its fragment and 1.0 for unbound label.

The binding characteristics of radioiodinated MAb and F(ab')₂ were analyzed by immunoreactivity. The immunoreactivity assay was performed essentially as described by Lindmo et al. (12). Data were graphically analyzed in a modified Lineweaver Burk Plot and the immunoreactive fraction was determined by linear extrapolation to conditions representing infinite antigen excess.

The immunoreactivity assay was performed in triplicate.

Biodistribution studies in nude mice

Female nude mice (Hsd, athymic nude-nu, 25-32 g; Harlan/CPB Zeist, The Netherlands) of 8-10 weeks old at the time of the experiments, were injected subcutaneously in the lateral thoracic region on both sides with 4 × 10⁶ UM-SCC-11B

cells. After two weeks, mice were injected, through eye cavity of 10 µCi of ¹²⁵I-MAb or ¹²⁵I-F(ab')₂ fragment. Mice were anaesthetized, bled, killed and dissected 1, 2 and 3 days after injection.

For each day, 3 mice were used. Organs were immediately removed, placed in 5 ml plastic tubes and weighed. Samples were taken from blood, urine, tumor, liver, spleen, kidney, heart, stomach, ileum, colon, bladder, sternum, muscle, lung, skin and tongue.

After weighing, all organs and tumors were counted in a dual isotope gamma counter (Wallac LKB-CompuGamma). The antibody uptake in the tumor and other tissues was calculated as the percentage of the injected dose per gram of tissue (%ID/g).

RESULTS

Radiolabeling of MAb and F(ab')₂ fragments

Labeling of 500 µg MAb and F(ab')₂ fragment with 500 µCi ¹²⁵I resulted in a specific activity of 0.882 and 0.848 µCi/µg respectively.

More than 97 % of the ¹²⁵I was bound to MAb and F(ab')₂, as revealed by TLC and HPLC. As determined by modified Lineweaver Burk Plot, the immunoreactive fractions of MAb and F(ab')₂ fragments at infinite antigen excess were 91.1% and 76.5% respectively.

Biodistribution

The amount of ¹²⁵I-MAb and ¹²⁵I-F(ab')₂ fragment in the xenografts and various organs, expressed as the average percentage of radioactivity of the injected dose per gram of tissue (% of ID/g), are shown in Fig. 1 and Fig. 2.

Table 1 and Table 2 show the tumor to tissue ratios of MAb and F(ab')₂ fragment that were calculated by dividing the percentage ID/g of tumor tissue by the percentage ID/g of various non-tumor tissues.

DISCUSSION

The radioimmunoscintigraphy (RIS) has found widespread clinical application in tumor diagnosis. Tumors of diameter 0.7 cm³ to 2.0 cm³ can be detected by this technique. A very important feature of RIS is that it can be of assistance in the diagnosis of metastases and therefore it can contribute to patient management concerning clinical decisions.

Furthermore, RIS can be explored as a scouting procedure to radioimmunotherapy (RIT), since the behavior of a novel product can be well characterized before its application in RIT (13).

F(ab')₂ fragments, potentially have several advantages over intact IgG. F(ab')₂ fragments have better accessibility to the tumor due to their smaller size, are cleared from the blood more rapidly. However, these advantages may all be controlled by a possibly decreased absolute uptake in the tumor and a reduced residence time of the F(ab')₂ fragment compared to the intact IgG (14).

In this study, we compared the characteristics of humanized monoclonal antibody U36 IgG and F(ab')₂ with regard to biodistribution parameters in nude mice bearing SCC xenografts. The digestion of human IgG for generation of F(ab')₂ significantly alter the immunoreactivity of the radiolabeled conjugate and F(ab')₂ showed a decrease in the percentage ID/g of tumor tissue as compared to intact IgG. It is possible that humanized

monoclonal antibody U36 IgG is sensitive to pepsin digestion. Humanized monoclonal antibody U36 F(ab')₂ fragment however showed specific localization in tumor tissue which the percentage ID/g was almost one third of the percentage ID/g of humanized monoclonal antibody U36 IgG. The overall tumor to non-tumor ratios of F(ab')₂ were several times higher than tumor to non-tumor ratios of IgG.

In normal tissues, neither humanized monoclonal antibody U36 IgG nor humanized monoclonal antibody U36 F(ab')₂ showed any non specific accumulation in vital organs.

In conclusion, our comparative study shows that humanized F(ab')₂ fragments are more suitable than whole human IgG molecules for rapid and reliable detection of tumors.

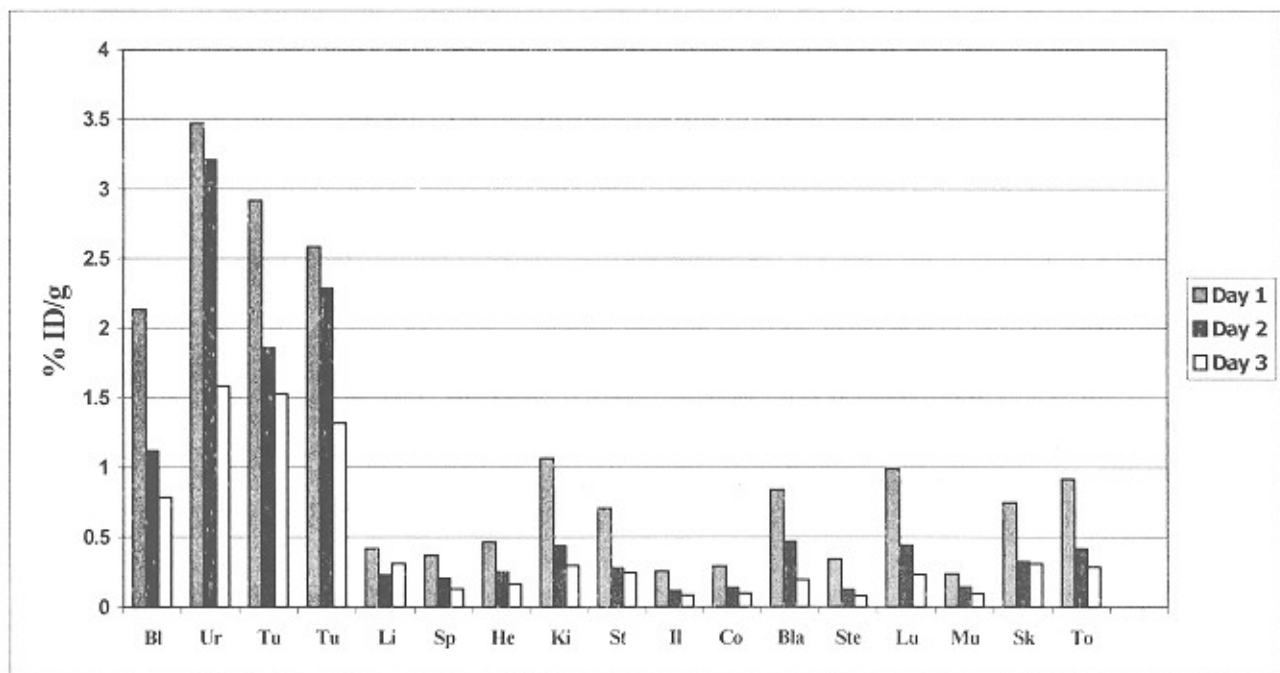


Fig. 1. Biodistribution data of ¹²⁵I-Humanized U36F(ab')₂ in nude mice bearing squamous cell carcinoma xenografts. The data are reported as mean % ID/g (Bl: blood, Ur: urine, Tu: tumor right and left, Li: liver, Sp: spleen, He: heart, Ki: kidney, St: stomach, Il: ileum, Co: colon, Bla: bladder, Ste: sternum, Lu: lung, Mu: muscle, Sk: skin, To: tongue).

Table 1. Tumor to tissue ratio of ¹²⁵I- Humanized U36F(ab')₂ in xenograft bearing nude mice

Organs	Day 1 (X ± SD)	Day 2 (X ± SD)	Day 3 (X ± SD)
Blood	1.32 ± 0.25	1.52 ± 0.79	1.84 ± 0.41
Sternum	8.40 ± 2.19	10.66 ± 2.80	18.69 ± 6.87
Liver	6.76 ± 1.34	9.52 ± 1.39	11.02 ± 2.77
Spleen	7.40 ± 0.43	11.31 ± 1.16	11.81 ± 3.17
Kidney	2.69 ± 0.68	5.02 ± 0.63	4.77 ± 0.43
Colon	10.19 ± 3.56	12.00 ± 0.93	16.35 ± 4.65
Skin	3.72 ± 0.51	6.48 ± 0.23	4.93 ± 1.50
Tongue	3.04 ± 0.46	5.25 ± 1.03	4.88 ± 0.52

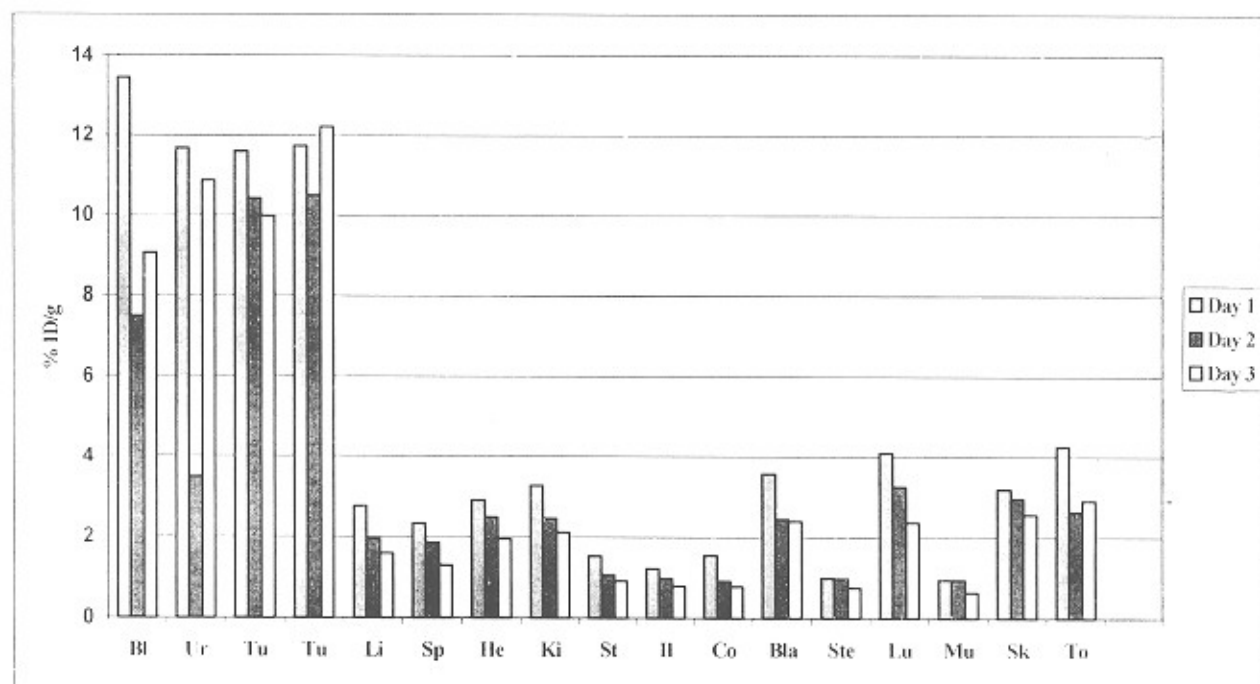


Fig. 2. Biodistribution data of ^{125}I - Humanized U36IgG in nude mice bearing squamous cell carcinoma xenografts. The data are reported as mean % ID/g (Bl: blood, Ur: urine, Tu: tumor right and left, Li: liver, Sp: spleen, He: heart, Ki: kidney, St: stomach, Il: ileum, Co: colon, Bla: bladder, Ste: sternum, Lu: lung, Mu: muscle, Sk: skin, To: tongue).

Table 2. Tumor to tissue ratio of ^{125}I - Humanized U36IgG in xenograft bearing nude mice

Organs	Day 1 (X \pm SD)	Day 2 (X \pm SD)	Day 3 (X \pm SD)
Blood	0.86 \pm 0.10	0.86 \pm 0.16	1.23 \pm 0.21
Sternum	9.30 \pm 2.91	10.86 \pm 2.00	14.80 \pm 1.69
Liver	4.31 \pm 0.93	5.35 \pm 0.71	7.06 \pm 1.82
Spleen	5.02 \pm 0.27	5.57 \pm 0.44	9.03 \pm 1.80
Kidney	3.55 \pm 0.32	4.21 \pm 0.84	4.53 \pm 1.98
Colon	7.48 \pm 0.55	8.48 \pm 1.98	16.04 \pm 5.98
Skin	3.64 \pm 0.55	3.56 \pm 0.63	3.71 \pm 0.99
Tongue	2.72 \pm 0.37	3.10 \pm 0.78	3.78 \pm 0.60

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