Experimental Approaches to Increase Radiolabeled Antibody Localization in Tumors

Donald J. Buchsbaum

Department of Radiation Oncology, University of Alabama at Birmingham, Birmingham, Alabama 35233

Abstract

Approaches have been developed to improve the localization of radiolabeled monoclonal antibodies (MAbs) in experimental tumors, to reduce their uptake in normal tissues, and, thus, to improve the time-dependent tumor: normal tissue (T:NT) ratio so that higher and more frequent doses of radionuclide could be used for radioimmunotherapy. These approaches involve three general strategies: (a) modifying antibodies or radiolabeling techniques; (b) increasing the clearance of radiolabeled MAbs; and (c) modifying tumor delivery, tumor antigen expression, or increasing tumor vascular permeability or blood flow. The use of animal models permits the assessment of a wide range of MAbs, radiolabeling conditions, and the efficacy of administration methods before their initial use in clinical trials.

MAbs with specificity for binding to tumor-associated antigens or growth factor receptors expressed on tumor cells have been utilized in experimental studies of radiolabeled antibody targeting. Tumor-associated targets present on endothelial cells should be highly accessible to systemically administered radiolabeled MAbs. The use of indirect radiiodination techniques and labile linker-chelates may provide an improvement in tumor retention and T:NT ratios.

The addition or deletion of glycosylation to MAbs by alteration of recombinant immunoglobulin genes or by biochemical modification can alter the pharmacokinetics of blood and whole body clearance of radiolabeled MAbs. Genetically engineered chimeric or humanized MAbs have shown equivalent or greater tumor localization compared to murine MAbs. By using MAbs with greater affinity and avidity, an increase in the uptake and retention of radiolabeled MAbs in tumors and an increase in their therapeutic efficacy may be achieved.

Several approaches in the administration methods of MAbs have been developed in an attempt to improve tumor localization and therapeutic results and to reduce toxicity. These approaches include: (a) predosing with unlabeled antibody before administering a radiolabeled MAb; (b) using a mixture or “cocktail” of MAbs rather than a single radiolabeled antibody; and (c) administering multiple doses of radiolabeled MAbs.

Various approaches have been tested for increasing the clearance of radiolabeled MAbs and, thus, for increasing the T:NT ratio. It has been found that compared to intact antibody, the smaller antibody fragments (F(ab')2, Fab, or single-chain Fv) can bind to tumor cells with a more homogeneous distribution. The antibody fragments and domain deletions often have a more rapid catabolism in blood, in tumors, and in normal tissues than an intact antibody does. In general, the use of antibody fragments leads to higher T:NT ratios but a lower percentage of injected dose delivered to the tumor.

In an attempt to maximize MAb deposition into tumor sites while minimizing radionuclide exposure to the bone marrow, investigators have designed several pretargeting strategies to separate these two components. These systems have produced preferential tumor targeting in animal models and in radioimaging studies in man.

Regional administration of radiolabeled MAbs may lead to increased tumor localization if the antibody binds rapidly to the target antigen. Experimental studies have shown that IFN increases the localization of radiolabeled MAbs in tumors, resulting in greater tumor growth inhibition than with radiolabeled antibody alone. Several approaches that affect tumor vascularity, blood flow, and vascular permeability have resulted in increased tumor localization of radiolabeled MAbs. These include external beam irradiation, hyperthermia, and the use of vasoactive conjugates.

The prospects of radiolabeled antibodies in cancer detection and therapy remain promising because of their specificity for binding to antigens on tumor cells. It appears that methods that increase the localization of radiolabeled MAbs in solid tumors while reducing the uptake in normal tissues will be required to deliver a sufficient radiation-absorbed dose for curative treatment of radioresistant tumors in clinical radioimmunotherapy.

Introduction

This paper will review the approaches to increase the localization of radiolabeled MAbs in tumors that have been performed in experimental animal models. The use of animal models permits the assessment of a wide range of MAbs, radiolabeling conditions, and the efficacy of administration methods before their initial use in clinical trials. This approach provides the maximum opportunity to develop successful protocols for imaging and treating patients. Other reviews have been published on experimental studies of antibody targeting (1–6), but this paper will place an emphasis on the relationship of increased antibody uptake in tumors to improved therapeutic results after RAIT. To improve the therapeutic efficacy of radiolabeled MAbs, it is necessary to increase the localization in the tumor while at the same time minimize uptake in normal tissues, thereby achieving a maximum therapeutic ratio.

It is important to recognize that murine MAbs do not have the same biodistribution and pharmacokinetics in animal models as humans because of differences in the volume of distribution and concentration of antibody, the cross-reactivity of the MAbs with normal tissues that express the target antigen in humans, and the fact that the immunogenicity of the antibodies in mice is different than in humans. However, animal models are useful for developing and evaluating new experimental approaches to increase the localization of radiolabeled antibodies in tumors. Historically, most of the studies of antibody targeting were first tested in animal models. Those that looked promising were then tested in clinical trials.

Discussion

A variety of approaches are available to improve the localization of radiolabeled MAbs in experimental tumors, to reduce the uptake of radiolabeled MAbs in normal tissues, and, thus, to improve the time-dependent T:NT ratio so that higher and more frequent doses of radionuclide could be used for RAIT (Tables 1–3). The limitations of the human tumor xenograft models in terms of lack of cross-reactivity of murine MAbs with normal murine tissues, and differences in pharmacokinetics of the antibodies and other reagents in animals versus humans, including volumes of distribution and the number of times the antibody passes through the tumor (5, 7), must be recognized. Nonetheless, many of the approaches developed in animal models have shown promise in clinical trials.

MAbs with specificity for binding to tumor-associated antigens or growth factor receptors expressed on tumor cells have been radiola-

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2 To whom requests for reprints should be addressed, at the Department of Radiation Oncology, University of Alabama at Birmingham, 619 South 19th Street, Birmingham, AL 35233.

3 The abbreviations used are: MAb, monoclonal antibody; RAIT, radioimmunotherapy; sFv, single-chain Fv; T:NT, tumor/normal tissue ratio.
beled and utilized in experimental studies of antibody targeting. Using MAbs with greater affinity may increase the uptake and retention of radiolabeled MAbs in tumors, thereby increasing their therapeutic efficacy. However, if the circulating tumor-associated antigen is present, the higher affinity may then result in greater normal tissue uptake and less tumor localization than if a lower affinity MAb was used. Although studies in animal models support the hypothesis that greater tumor localization results from increased MAb affinity, studies in humans have not yet produced experimental data to support this hypothesis (8, 9). Predosing with unlabeled antibody before administration of the radiolabeled MAb has shown a biodistribution advantage in animal studies and in a clinical trial (10, 11). The use of indirect radioiodination techniques (12, 13) and labile linker-chelates (14) may provide an improvement in tumor retention and T:NT ratios.

Several investigators have demonstrated a relationship between tumor antigen content in human tumor xenografts and localization of radiolabeled antibody (15, 16). A number of experimental studies has shown that IFN increases the expression of tumor-associated antigens on tumor cells in vivo, leading to increased localization of radiolabeled MAbs in tumors in animal models (17—20) and in patients (21—23). The use of IFN has led to improved therapeutic results with radiolabeled MAbs in animal models (17, 19). However, this approach may result in higher levels of circulating tumor-associated antigen, which may interfere with the localization of the radiolabeled antibody in the tumor and produce a higher uptake of radioactivity in normal tissues. Clinical RAIT trials with IFN and 131I-labeled MAbs have shown tumor uptake, some antitumor effects, and increased myelosuppression (22, 23).

It has been postulated that low-affinity MAbs or antibody fragments would penetrate tumors better than high-affinity or intact MAbs (15, 20, 24—26). Potential advantages of antibody fragments include faster removal from the circulatory system, resulting in a lower radiation dose to bone marrow, and the fact that these smaller molecules may be less immunogenic. On the other hand, the fragments clear from tumors faster and may produce higher doses to the kidneys. Genetic engineering of MAbs to produce chimeric or humanized constructs, domain deletions, altered glycosylation, Fv molecules, and alterations in the hinge region or binding site and incorporation of radiolabeling sites, drugs, or biological response modifiers in the constant region offers prospects for improved diagnostic and therapeutic reagents with increased tumor localization and less normal tissue uptake and toxicity than intact murine MAbs (27, 28). Some of these modifications may permit fractionated RAIT in humans, which may result in improved therapeutic efficacy and less toxicity (29, 30).

Tumor vascularity, blood flow, and vascular permeability are all factors that affect the localization and distribution of radiolabeled MAbs in tumors, as well as influence their therapeutic effectiveness (15, 16, 26, 31—35). Several approaches that affect these physiological parameters, including external beam irradiation, hyperthermia, and the use of vasoactive conjugates have resulted in increased tumor localization of radiolabeled MAbs (36—41). Regional administration has shown improved tumor localization in some instances (42, 43).

### Table 1  Antibody strategies for increasing tumor localization

<table>
<thead>
<tr>
<th>MAbs reactive with new tumor antigens or vascular endothelium</th>
<th>New labeling techniques</th>
<th>Antibody isotype</th>
<th>Genetically engineered antibodies</th>
<th>Biochemically modified antibodies</th>
<th>High affinity antibodies</th>
<th>Unlabeled antibody predosing</th>
<th>Cocktails of antibodies</th>
<th>Fractionated administration</th>
</tr>
</thead>
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Table 2  Strategies for increasing the clearance of radiolabeled MAbs

<table>
<thead>
<tr>
<th>Antibody fragments</th>
<th>Pretargeting approaches</th>
<th>Metabolizable chelating agents</th>
<th>Biochemical modification</th>
<th>Second clearing antibody</th>
<th>Plasmapheresis</th>
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Table 3  Tumor strategies for increasing localization of radiolabeled MAbs

<table>
<thead>
<tr>
<th>Regional administration</th>
<th>Biological response modifiers to increase antigen expression</th>
<th>Increase tumor vascular permeability or blood flow</th>
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An area for potential investigation is the use of radiolabeled MAbs against tumor vascular endothelium (44, 45). Such antibodies should be highly accessible to targets present in blood vessels following i.v. injection. Prospects exist for improving tumor localization and therapy and for reducing normal tissue activity and toxicity by using pretargeting approaches (46—61).

The prospects of radiolabeled antibodies in cancer detection and therapy remain promising because of their specificity for binding to antigens on tumor cells. One of the major reasons RAIT has not been more successful in patients is due to the relatively low uptake of radiolabeled antibody in tumors (62—65). The low uptake of antibody in tumors (<0.1% of injected dose/g of tissue) is a result of the large volume of distribution after systemic administration, the degree of tumor vascularization and vascular permeability, the heterogeneity and sometimes low expression of tumor-associated antigens in tumors, and the relatively short circulating half-life of the antibody (65). Several methods with the potential to increase tumor uptake of radiolabeled MAbs have been investigated, with results varying from significant to no detectable improvement. The goal of all the studies exploring experimental approaches to increase radiolabeled MAb targeting in tumors is to develop information on the best way to apply these and future techniques to the improvement of localization and treatment of tumors clinically. It appears that methods that increase the localization of radiolabeled MAbs in solid tumors, while reducing the uptake in normal tissues, will be required to deliver a sufficient radiation-absorbed dose for curative treatment of such radioresistant tumors in clinical RAIT, and that combination with other biological regimens, chemotheraphy, or radiotherapy regimens will be likely (62—65).

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### References


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