Three-step immunoscintigraphy with the avidin-biotin system: state of the art and future perspectives


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INTRODUCTION

Specific targeting of radioactive agents to tumor cells has been a major goal of the in vivo use of monoclonal antibodies (Mab) for diagnostic and therapeutic purposes. However, only a relatively small amount of the injected dose of Mabs is bound by the tumor, while Mab conjugated to radioisotope keep circulating in the blood stream and in normal tissue. Also circulating Mabs, especially when bound to a beta emitting isotope, are obviously undesirable and should be limited in order to protect normal tissue such as bone marrow.
Antibody fragments, such as F(ab')\[2\] or Fab have a faster blood clearance than whole antibodies, to the extent of allowing the use of short-lived radionuclides such as \(^{99}\text{mTc}\) with good diagnostic sensitivity. This improvement, however, does not yield tumor to background ratios high enough to allow low-risk therapeutical applications.

Strategies of tumor pre-targeting, where Mab and radiolabel are administered separately have been proposed to reduce the background noise due to circulating antibodies (1-4). A 3-step immunoscintigraphy (3-S-ISG) using the avidin-biotin system has been used in cancer patients (5).

**MATERIALS AND METHODS**

Injection of biotinylated Mab (first step) is followed by avidin (second step) in order to precipitate circulating biotinylated Mab and at the same time to target the tumor cells allowing adequate homing in of the subsequently administered labelled biotin (third step). From early 1990 to November 1993 we have studied and followed up 127 patients according with the protocol described above using different Mabs specific for different tumors including colon and lung cancer, gliomas, melanomas and apudomas.

**RESULTS**

The method has shown to be safe, reliable and of clinical utility since an overall sensitivity of 88% with 94% specificity and 84% accuracy was demonstrated. Moreover, 38 unknown lesions in 25 patients were localized and 21 were confirmed in their follow-up. Of these patients, 16 had not evidence of disease at the time of 3-S ISG but only increased tumor markers. The immunoresponse against biotinylated mouse IgG (HAMA) and avidin (HAAR) was evaluated in 73 patients. None developed HAMA after the injection of 1-2 mg of whole biotinylated IgG and 8/73 patients developed a weak HAAR response. However, radioactivity delivered per gram of tumor was in the range of 0.01-0.001% i.d., still below the optimal dose for radioimmunotherapy. This was probably due to the fact that avidin blood clearance is very fast with a T1/2 of 82 minutes. Thus, we are now developing a recombinant avidin molecule in order to reduce the immunogenicity and improve it pharmacokinetic in view of a therapeutical application of this approach to cancer treatment.

**DISCUSSION**

Tumor pretargeting methods with the 3-step approach have been shown to offer several advantages over the administration of directly labelled Mabs. In particular since the label is a small molecule, with a fast blood clearance background radioactivity levels are drastically reduced and imaging can be performed shortly after injection of the radiolabel. The 3-step protocol is designed to remove the excess circulating biotinylated antibodies as
cold complexes and this is obtained prior to label injection.

The use of unlabelled, unfragment antibodies also avoids their damage by autoradiolysis and by enzyme treatments. Given that more than one molecule of avidin can bind to a single polybiotinylated Mab molecule localized on the tumor, and that up to three radioactive biotin molecules can bind to an avidin molecule this approach is also designed to provide an amplification of the signal from the tumor.

Moreover, one can use any Mab from a panel, or Mab mixtures: the second and third steps of the three-step protocol would be common to all studies and the use of a cocktail would enhance the possibilities of targeting more tumor cells by using different tumor antigens as target. For successful diagnosis as well as therapy the tumor must be covered as much as possible in avidin.

These protocols have now been optimized and can potentially be applied widely in the majority of solid tumors including breast, lung, colon, ovary and others malignacies.

Techniques employed in genetic engineering will be able to provide chimeric proteins made up of antibody fragments and recombinant avidin in order to obtain an antibody molecule conjugated with a modified avidin tetramer of low immunogenicity.

REFERENCES


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