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EDITORIAL

The Advantage of Protecting the Antigen-Binding Site During Antibody Labeling

Radioimmunotherapy and radioimmunoscintigraphy have emerged in recent years as fields of active research and development in nuclear medicine. There are large numbers of antibodies currently being evaluated in clinical trials for a variety of diseases. With several years of collected clinical experience, one limitation has consistently emerged; antibodies have low binding to target sites in vivo. Most investigators observe radioactivity concentrations in the range of 10^{-4}% of the injected dose per gram. Because of this low target binding, chemists are exploring methods for increasing antibody deposition in target sites, to improve the clinical utility of these materials. Since reduced immunoreactivity in vivo can result from effects of radiolabeling, approaches to minimize these detrimental effects may increase tumor binding and retention of radiolabeled antibody at the target site.

The article by Van den Abbeele et al. (*1*) presents a comprehensive study of the factors that can contribute to reduced immunoreactivity of radiolabeled antibody, as well as a unique approach to improving the quality of these new radiopharmaceuticals. After a clear presentation of the importance of conformational changes in the antibody tertiary structure associated with antigen binding, and a discussion of the effects of added radioiodine atoms on these critical conformational changes, the authors hypothesized that protection of the critical binding site during radiolabeling would improve the immunointegrity of the final product. To test this hypothesis, they designed an experiment where an antigen (a murine antibody) was adsorbed onto a stationary gel. The antibody to be labeled (goat or rabbit anti-mouse antibody) was allowed to bind to the stabilized antigen; thereby, protecting the critical binding site of the antibody from addition of radioiodine atoms. This was followed by standard radioiodination with chloramine-T. Following radioiodination, the labeled antibody was eluted from the antigen and critically assayed in a variety of ways. Antibodies labeled by this method were compared to those labeled without protection of the antigen-binding site. All antibodies were labeled at a series of iodine-to-antibody molar ratios that were purposely high to stress any adverse effects of increased substitution of radioiodine atoms per antibody.

Radiochemical purity was determined by thin-layer chromatography. Immunoreactivity assessed by Scatchard analysis was determined by the use of a cell binding assay at antigen excess, and antibody net charge was evaluated by isoelectric focusing techniques. Radiochemical purity for each group of antibody

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products was similar, but as predicted, immunointegrity of the antigen-protected antibody at all levels of iodination was unchanged. Comparable labelings of unprotected antibody showed progressive loss of immunointegrity with increasing amounts of radioiodine substituted per antibody molecule. Similarly, isoelectric focusing studies showed the protected antibodies to have isoelectric points indistinguishable from those of native antibodies, while unprotected antibodies showed progressive departure from the normal isoelectric point. Presumably, protection of amino acid residues critical to the antibody conformation structure related to antigen binding produced a radiopharmaceutical with characteristics indistinguishable from the native form. It would follow that these antibodies protected during labeling would have superior biologic behavior in vivo. The next logical step will be to utilize this technique in an in vivo model; these results are eagerly awaited.

This carefully described and well presented work brings into focus several important points about radiolabeled antibody pharmaceuticals alluded to by its authors. There are many determinants of antibody radiopharmaceutical quality (*2*). We generally consider radiochemical purity and binding avidity to antigen targets to be reliable predictors of radiolabeled antibody behavior in vivo. This article emphasizes more subtle alterations that interfere with the tertiary structure resulting from chemical bond rotation in the hypervariable region during complexation with antigen. These are a consequence of substitution reactions, nonspecific oxidation, and interchain cross-linkages. These regional chemical changes will probably result in reduced antibody binding to in vivo targets and to reduced retention at the target site. The study reported by Van den Abbeele describes radioiodination effects, but may be even more valid for chelate radiolabels where a larger molecule is covalently attached to the antibody.

The authors of this article also noted that the two antibodies used in their experimental system had markedly different susceptibilities to damage from the radioiodination procedures they employed. This reinforces the principle that each radiolabeled antibody is a unique radiopharmaceutical. Observations about the characteristics of one individual radiolabeled antibody or fragment do not predict the behavior of another. This is even true for fragments derived from a well-characterized whole antibody. For this reason, it is critical that each radiolabeled antibody or fragment be subjected to careful analysis and characterization before biodistribution experiments are reported. One antibody or fragment should not be substituted for another without extreme care in analysis and documentation of radiopharmaceutical quality control. Because indvidual antibody preparations may contain nonimmunoreactive proteins, small quantities of reducing agents, and other residue from the production process, there are many potential reasons for a radiolabeled antibody preparation to have less than optimum binding to targets. These also should be characterized thoroughly before radiolabel characterization can begin.

Careful attention to the many different aspects of antibody radiopharmaceutical quality, and consideration of the effects of radiolabeling on the molecular structure of the antibody as described in this article, may eventually lead to increased concentrations of radiolabeled antibody at the in vivo binding site. The consequence should be improved radioimmunotherapy. With this kind of detailed understanding of the effects of antibody alterations on biologic behavior, it can also be envisioned that antibodies and radiolabels can be specifically engineered for maximum binding and retention in targets. Then the full potential of radiolabeled antibodies for specific diagnosis and treatment may be realized.

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