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Re:Activity and Protein Levels in Studies of Monoclonal Antibody Imaging

The recent imaging article by Khaw et al. (*1*) is to be recommended for its thoroughness regarding monoclonal antibody research with the nude-mouse model, but there are pertinent questions that the authors fail to discuss. These concern the relatively large amounts of indium-111 and protein given to the animals. It seems that their experiments pertain more to monoclonal-associated radiation therapy than to diagnosis.

Khaw et al. (*1*) report injection of 200 μCi of In-111-labeled antibody into ~20 g of mouse. The activity was attached, in the case of their intact monoclonal 10-3D2, to 20 μg of IgG. If one assumes a 70-kg human and scales these values upward by a factor equal to the ratio of human to mouse masses, rather unacceptable levels of activity and protein are obtained. Amounts approaching a curie of In-111 and 100 mg of mouse-derived protein would probably not be permitted in a human imaging study. A reduction in these levels by approximately two orders of magnitude would be clinically realistic.

The obvious question arises as to the resultant murine biodistributions with such appropriately lowered amounts of activity and tracer. With a relatively small number of antibodies and no sloughing of tumor-associated antigens (*1*), it is possible that the tumor uptake, measured in % injected dose/g, could be significantly increased. On the other hand, if the antigen does enter the circulation to some degree, it is likely that lowering the amount of injected antibody would reduce the injected dose/g lodging in the tumor (*2*). Other opposing effects can occur. In the related therapy situation, Order (*3*) has described prolonging by several days the human tumor retention of a labeled monoclonal IgG by raising the total protein dose to approximately 200 mg. The additional carrier protein appeared to retard sloughing of labeled monoclonal complexes and thus to enhance radiotherapeutic effects. At any rate, it is unclear that if the amounts of In-111 and IgG were reduced, there would be consistency in the levels of tumor uptake and figures of merit reported in the recent article.

Questions regarding dose-level effects on pharmacokinetics are also involved in the conclusions reached by Khaw et al (*1*). Using signal-to-noise criteria, they report optimal BT-20 tumor localization of 10-3D2 at 4 days after i.v. injection in the nude-mouse model. This result might not recur, however, with decreased amounts of activity and protein. In fact, imaging out to 7 days, as reported by these authors, may not even be possible with In-111 levels reduced by a factor of 100.

It seems that drug-dose effects need to be addressed by researchers in monoclonal imaging. One cannot simply assume that the amount of radiopharmaceutical given to a particular species has no effect on the biodistribution. Differential tissue uptake is generally going to be a function of the amount of material injected per unit mass of the animal used in the experiment. Clearly, diagnostic animal research should concern activity levels less than 10 mCi/70 kg or 140 μCi/g—i.e., be equivalent to the maximum human dose levels. Similarly, the amount of protein should be restricted to less than 500 μg/70 kg or 7 ng/g of test animal so as to reduce the likelihood of antimouse antibody production with serial studies. Published reports involving polyclonal (*4*) and monoclonal (*5*) human imaging trials are in good agreement with these limits.

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Reply

My colleagues and I thank Dr. Williams for his thoughtful comments on our article (*1*). We disagree, however, with his suggestion that the concentration of monoclonal antibody and the amount of In-111 reported in our manuscript were excessive, amounting to what he describes as "monoclonal-associated radiation therapy." These quantities were selected for the following reasons: (a) the amount of antigen expected on the surface of the tumor; (b) the physical constraints involved in imaging small animals; (c) the radionuclide used; and (d) the assumption that use of approximately 0.2% murine antibody in a murine model would not interfere with the circulation of the injected antibody.

Dr. Williams suggests that one can extrapolate from the antibody concentration and radiation doses used in our nude-mouse model to those used for a 70-kg patient with mammary carcinoma.

However, if such a direct scale-up were used, the In-111 activity and the amount of antibody protein, approaching 1 Ci and 100 mg, respectively, are certainly excessive. Under this system, a 20-g nude mouse with a 100-mg tumor would equate to a 70-kg patient with a 350 g mammary tumor. Since the total number of antigenic determinants present in 350 g of tumor mass would be large, it would not seem unreasonable to use 50–100 mg of antibody. However, if the mammary tumor were this large, imaging for diagnosis would be unnecessary.

In our experience, the radioactivity and antibody concentrations needed for animal studies (*2*) are not directly applicable to those needed for human subjects (*3*). Approximately the same concentrations of antibodies have been used for both experimental and clinical imaging studies of acute myocardial infarcts. In their initial studies of nude mice, Mach et al. (*4*) used 16 μCi of I-131 labeled to 2 μg of specific antibody, mixed with 200 μg of normal IgG as carrier. In their subsequent clinical report in the *New England Journal of Medicine* (*5*), these investigators used 1 mCi labeled to 1 mg Ab per patient, whereas a direct scale-up dose would have required 56 mCi of I-131 labeled to 7 mg Ab, with 700 mg carrier IgG.

Although we are not advocating the administration of large amounts of antibody, recent studies suggest that this may be beneficial for diagnostic applications. Recently it has been suggested (personal communication, Samuel Halpern) that investigators should consider increasing the amount of antibody administered, a concept supported by the results of Belitsky et al. (*6*), who used 100 mg of immune globulin fraction for diagnostic imaging. Diagnostic doses of 50 mg monoclonal antibodies and 5 mCi In-111 have been used (unpublished data, Larson SM). Furthermore, gram amounts of Fab fragments of goat antibody have been used to reverse digoxin toxicity in man, without adverse effects (*7*).

Other important considerations for the choice of activity levels and dosages were the requirements of the pinhole collimator used to record images of the nude mice. The low sensitivity of the pinhole collimator constrains the dose that can be used when images must be recorded in a practical time interval. To enable us to image the animals for 20 min/view, we found it necessary to administer 200 μCi of In-111 activity labeled to 20 μg 10-3D2. Since each group of four to six mice had two views (one whole-body and one close-up) each imaging session took 2 to 3 hr. If we had used 2 μCi of activity labeled to 0.2 μg, as suggested by Dr. Williams, it would have been difficult to image within a reasonable amount of time.

If biodistribution data were the sole goal of this study, then a dose of 2 μCi In-111 would have been sufficient. Although the amount of antibody protein may affect biodistribution by inducing antimouse antibodies, this should not be a problem when only a single administration of the antibody is contemplated. Production of antimouse antibody is unlikely to occur in our murine tumor model, since only soluble murine antibody was used.

As indicated in the article, the 126-kilodalton phosphoglycoprotein antigen associated with mammary tumor is not shed by the tumor cells. Therefore the argument that antigens entering the circulation would interfere with tumor targeting is invalid in our model system.

In light of the above considerations, we believe that the dose level of In-111 activity and the concentration of antibody protein used in our experimental studies of nude-mouse tumor imaging are valid for the demonstration of the feasibility of mammary-tumor visualization for diagnosis.

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