

ments the measurement of reversibility, we suspect it will be of assistance in detecting ischemia.

We continue to believe that one of the best uses of this approach to data-based quantification is by diagnosticians with limited experience, since they can be reminded of the abnormal patterns of tracer concentration and change. In all cases, physicians should use the detection or characterization of abnormalities by these quantification programs as flags to point out regions of concern to be verified by the physician's own expertise. The development of these tools requires an extensive effort to make them as robust and accurate as possible and to understand their limitations. The ultimate reward for this effort is the widespread acceptance of the use of these tools by the nuclear medicine field.

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Tumor Imaging with Indium-Labeled Biotin

TO THE EDITOR: I read with interest the paper by Kalafonos et al. in which the authors reported positive imaging in 8 out of 10 lung tumors (1). A blinded observer found improved image quality in three of these eight when prior antibody conjugate had been given. Three were negative images with or without antibody-avidin conjugate. Rapid internalization of the conjugate is cited as the reason for nonspecific visualization (positive visualization but no improvement with Ab conjugate). A marginal difference in urine excretion, $71\% \pm 9\%$ s.d. versus $83\% \pm 7\%$ s.d., is presented as evidence for specific binding in patients receiving conjugate. It is reported that increasing the ^{111}In -biotin injected from $50 \mu\text{g}$ to $1000 \mu\text{g}$ did nothing to improve specific binding. The authors add that the positive images may be due "in part" to localization of labeled biotin in tumor.

Interestingly the authors have shown specific biotin uptake in the nude mouse tumor model and two tumor cell types in vitro over DTPA controls. This in itself may be a very important observation that must be carefully controlled. This control must now be applied to other interesting positive human tumor imaging results with the avidin-biotin systems reported recently (1,2).

However, I would like to put forward an alternate explanation for the lack of specific targeting not discussed by the authors. This is simply that in all cases there was not enough avidin administered to bind specifically more than 15% of the ^{111}In -biotin injected, if 100% of the conjugate had localized in the target. Since we know that at best only 1% or less of the injected antibody dose localizes in human tumors, this amount is reduced to 0.15% maximum. This calculation is based on the following assumptions: the 1 mg of protein injected was based on IgG MW = 150,000 and not the conjugate MW 210,000; bis-biotinyl

DTPA MW = 1,102; Ab-avidin conjugate is a monomer (see Materials and Methods p. 1792) capable of binding one molecule of ^{111}In -bis-biotin DTPA (reduced from the native valency of 4 by steric hindrance due to the use of one site for conjugation, blocking one neighboring site, and the use of bis-biotin DTPA, which uses two sites to bind one ^{111}In . At the $50\text{-}\mu\text{g}$ biotin level, the molar ratio of biotin/streptavidin = 6.8/1. This ratio, which determines the amount of specific biotin binding possible, becomes much less favorable (136/1) if the amount of bis-biotin is increased to $1000 \mu\text{g}$, as the authors did in an attempt to improve targeting. Under this condition, localization by specific binding becomes 0.74%–0.0074% maximum.

My main point is that pretargeting is a form of receptor binding, in which the concentration of receptor is very low, usually pM. In this situation, high-specific activity is mandatory for adequate specific localization of radiopharmaceuticals.

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REPLY: We wish to thank Dr. D.A. Goodwin for his thoughtful comments concerning our recent paper on patient imaging with ^{111}In -labeled biotin and streptavidin-conjugated anti-tumor antibodies (1). Dr. Goodwin has referred to our observation that specific targeting, as judged by image quality, was achieved in only three of eight patients. In addition to possible explanations for this phenomenon described in our report, Dr. Goodwin has added another: since only a limited concentration of streptavidin may be expected in tumor under the best of circumstances, the available biotin binding sites may have become saturated at the doses of biotin administered.

Dr. Goodwin is certainly correct that the localization of streptavidin-conjugated antibody in tumor is likely to be limited since tumor accumulation of this conjugate, as with any antibody, will be influenced by poor tumor perfusion, restricted vascular permeability, limited antigenic expression, etc. We also agree with his calculation that the 1 mg of conjugated antibody administered would bind approximately $5 \mu\text{g}$ of labeled biotin. By assuming a reasonable value for the percentage of administered antibody conjugate which localizes in tumor, it is possible to calculate the weight of labeled biotin required to achieve saturation of the biotin-binding sites therein. However, it is incorrect in our view to argue further that the biotin dose administered should be reduced to that which is approximately equivalent to this value. This ignores an important property of biotin, namely its rapid clearance from circulation. Since approximately 50% of administered biotin appears in urine in 1 hr (1), only a small fraction

of the administered biotin may have sufficiently rapid access to streptavidin in tumor. If so, to achieve saturation, labeled biotin must be administered at greater, and possibly much greater, doses than that suggested by stoichiometry alone. Nevertheless, the localization of only a small fraction of the administered biotin at the tumor site is not as serious as it would otherwise be since, as we have shown, unbound biotin clears from the circulation and from normal organs (except kidneys) so that positive tumor/non-tumor radioactivity ratios may be achieved.

We agree with Dr. Goodwin that the use of high-specific activity biotin would be helpful since the limited number of biotin binding sites in tumor should not be occupied with unlabeled biotin administered along with labeled biotin. Furthermore, increasing the dose of conjugated antibody administered should

also improve specific targeting. The effects of these improvements on patient imaging must await future clinical trials.

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