Letters to the Editor

Biodistribution of Indium-111-Labeled Monoclonal Antibodies

TO THE EDITOR: In their contribution on the use of 1-(p-isothiocyanatobenzyl) diethylenetriaminepentaacetic acid) (SCN-Bz-DTPA) to label antibodies, Esterban et al. report reduced liver levels of indium-111 (^{111}In) with antibodies conjugated with SCN-Bz-DTPA over those conjugated with other agents such as the cyclic anhydride of DTPA (cDTPA) (*1*). In explaining this observation, the authors assume that conjugation with SCN-Bz-DTPA results in a more stable ^{111}In protein label. However, an alternative explanation should be considered arising from precisely the opposite assumption.

The label instability in question concerns leakage of ^{111}In from the antibody in blood with its subsequent transport to the liver. This transcomplexation phenomenon is one source of ^{111}In instability in serum which has been reported for proteins conjugated with cDTPA (*2*). Although Esterban et al. did not report serum stability measurements, reduced transcomplexation may be expected for antibodies conjugated with SCN-Bz-DTPA since reduced transcomplexation has been reported for antibodies with a Bz-EDTA versus a DTPA group (*3*). In that study the increased stability of the ^{111}In label was attributed to the presence of the bulky benzyl group in the former chelator. The same explanation may be applied in the case of SCN-Bz-DTPA and is more satisfying than that proposed by Esterban et al. The authors presume that it is the preservation of all eight coordination sites on DTPA in SCN-Bz-DTPA that is responsible for the increased stability despite the fact that indium forms six- or seven-coordinate complexes (*4*) and, after conjugation with cDTPA, seven coordination sites are available.

Regardless of the explanation of decreased transcomplexation in the case of antibodies conjugated with SCN-Bz-DTPA, the effect of this increased stability on ^{111}In liver levels is likely to be small. For proteins conjugated with cDTPA, the rate of ^{111}In transcomplexation has been measured to be ~9%/day and may be as little as 2% of the injected dose per day (*2*). Thus the explanation for reduced liver levels probably lies elsewhere.

An alternative explanation assumes that antibodies localize in the liver normally as one site of their catabolism. For antibodies radiolabeled with iodine, catabolism at this site can result in the release of the label in a form which diffuses from the organ (*6*). It is likely that the same phenomenon occurs for ^{111}In in the case of antibodies conjugated with SCN-Bz-DTPA but not cDTPA. No evidence of in vivo instability of the amide bond which results from cDTPA conjugation has been observed (*2*), however, it has recently been suggested that in vivo instability of the thiourea bond from the SCN-Bz-DTPA conjugate results in the release to urine of the chelated of ^{111}In (DeNardo, S., personal communication).

It is important to establish the reason for reduced liver radioactivity levels since if it is due to instability of the thiourea bond, then it is possible that other metabolizable linkages may be identified which are superior in this regard. In addition, if it can be demonstrated that the use of SCN-Bz-DTPA for conjugation results in an unstable linkage, then it will be important to examine carefully tumor tissue to establish whether and to what extent ^{111}In may be clearing by this mechanism from tumor as well.

References

1. Esterban JM, Schlom J, Gansow OA, et al. New method for the chelation of indium-111 to monoclonal antibodies: biodistribution and imaging of arthymic mice bearing human colon carcinoma xenografts. *J Nucl Med* 1987; 28:861–870.
2. Hnatowich DJ, Griffin TW, Kosciuczyk C, et al. Pharmacokinetics of an indium-111-labeled monoclonal antibody in cancer patients. *J Nucl Med* 1985; 26:849–858.
3. Goodwin DA, Meares CF, McTigue M, et al. Metal decomposition rates of ^{111}In-DTPA and EDTA conjugates of monoclonal antibodies in vivo. *Nucl Med Comm* 1986; 7:831–838.
4. Meares CF. Chelating agents for the binding of metal ions to antibodies. *Nucl Med Biol* 1986; 13:311–318.
6. Sands H, Jones PL. Methods for the study of the metabolism of radiolabeled monoclonal antibodies by liver and tumor. *J Nucl Med* 1987; 28:390–398.

Donald J. Hnatowich
University of Massachusetts Medical Center
Worcester, Massachusetts

REPLY: Dr. Hnatowich and others have shown that a major source of Indium-111 (^{111}In) instability in vivo is a result of the exchange of the metal from the chelate to transferrin (transchelation) (*1,2*); whether this is the only reason for the in vivo instability of the ^{111}In has not been demonstrated. Dr. Hnatowich has shown that when CA-DTPA was linked to MAb 19-9 ~10% of the ^{111}In was bound to an anti-human transferrin column in <12 hr in serum (see Fig. 5 in Ref. 1). Since MAb B72.3 has a long $T_{\frac{1}{2}}$ in vivo of ~5 days (*3,4*) this transchelation of the ^{111}In (10% in <12 hr) may indeed explain the increased levels of the indium we found in the liver with the CA-DTPA as compared with the SCN-Bz-DTPA, notwithstanding Dr. Hnatowich's statement that the transchelation of the ^{111}In in serum is too small to account for the differences. It must be noted that the transchelation is probably a function of the whole-body clearance of the MAb as well as the serum kinetics of the antibody. The antibody escapes the blood pool and a large percentage of the antibody is found in extravascular spaces where the MAb-chelate complex is probably also exposed to transferrin and any indium transchelated to transferrin will ultimately end up in the liver.

In our paper (*5*) we did not speculate as to the reason for the decreased liver uptake with the ^{111}In-SCN-Bz-DTPA-B72.3 IgG because we did not have rigorous proof that the differences seen in liver activity were due to the transchelation

rates between the chelates, although that is a likely explanation. Dr. Carrasquillo and co-workers (personal communication) have examined the stability in human serum of the SCN-Bz-DTPA and the CA-DTPA chelates linked to a different MAb (T101) and have found the SCN-Bz-DTPA to be more stable.

Dr. Hnatowich would attribute the difference in stability of the ^{111}In in the SCN-Bz-DTPA versus the CA-DTPA chelate linked to MAb B72.3 to the difference in linkage chemistry, i.e., the thiourea link formed by the isothiocyanate versus the acid amide formed by the anhydride with protein amines. However, we have controlled for this by using the same linkage group with an ethylenediaminetetraacetate (EDTA) chelate. MAb B72.3 linked to the SCN-Bz-EDTA and labeled with ^{111}In gave a favorable biodistribution at 8 hours post-inoculation of the radiolabeled MAb, but after 72 hr there was 15.85% ID/g in the liver. If the thiourea linkage was unstable, we would expect the ^{111}In-SCN-Bz-EDTA-B72.3 IgG to clear at the same rate as the ^{111}In-SCN-Bz-DTPA-B72.3 IgG and more rapidly than the ^{111}In-CA-B72.3 IgG. Moreover, Meares and colleagues have shown that an antibody labeled with the use of ^{111}In-SCN-Bz-EDTA was more stable than the same antibody labeled with the CA-DTPA (*6*). The more favorable biodistribution of the ^{111}In in the DTPA chelate versus the EDTA chelate and the CA-DTPA when linked to B72.3 IgG are, therefore, not at all a result of the linkage group but must be due the kinetic inertness to the loss of indium by the MAb linked with the SCN-Bz-DTPA chelate.

The preferential biodistribution of the ^{111}In-SCN-Bz-DTPA-B72.3 IgG as compared to ^{111}In-CA-DTPA-B72.3 IgG is due in large part to the chelate used, whether the "bulky" linkage group further improves the biodistribution can only be determined by synthesizing different linkers that still maintain the integrity of the DTPA molecule. Similarly, the influence of the coordination number differences between the ligands can only be assessed by further kinetic and thermodynamic studies.

References

1. Hnatowich DJ, Griffin TW, Kosciuzyk C, et al. Pharmacokinetics of an indium-111-labeled monoclonal antibody in cancer patients. *J Nucl Med* 1985; 26:849–858.
2. Gobuty AH, Kim EE, Weiner RE. Radiolabeled monoclonal antibodies: radiochemical pharmacokinetics and clinical challenges. *J Nucl Med* 1985; 26:546–548.
3. Keenan AM, Colcher D, Larson SM, et al. Radioimmunoscintigraphy of human colon cancer xenografts in mice with radioiodinated monoclonal antibody B72.3 *J Nucl Med* 1984; 25:1197–1203.
4. Colcher D, Keenan AM, Larson SM, et al. Prolonged binding of a radiolabeled monoclonal antibody (B72.3) used for the *in situ* radioimmunodetection of human colon carcinoma xenografts. *Cancer Res* 1984; 5744–5751.
5. Esteban JM, Schlom J, Gansow OA, et al. A new method for the chelation of 111-In to monoclonal antibodies: biodistribution and imaging of athymic mice bearing human colon carcinoma xenografts. *J Nucl Med* 1987; 28:861–870.
6. Goodwin DA, Meares CF, McTigue M, et al. Metal decomposition rates of ^{111}In-DTPA and EDTA conjugates of monoclonal antibodies *in vivo*. *Nucl Med Comm* 1986; 831–838.

David Colcher
Otto Gansow
Laboratory of Tumor Immunology and Biology and the Radiation Oncology Branch
National Cancer Institute
Bethesda, Maryland

Attenuation Correction Equations for SPECT

TO THE EDITOR: We read with interest the article by Bailey, Hutton, and Walker (*1*). The equation for calculating attenuation correction factors given on p. 846 appears to be similar to that proposed by Chang (*2*) for a homogenous media and cannot account for spatially varying attenuation. We are currently using a variation of the Chang equation to correct for attenuation in inhomogenous media. This has the form:

$$C(x, y) = \left[\frac{1}{M}\sum_{i=1}^{M} A_i(x, y, \theta_i)\right]^{-1}$$

$$A_i(x, y, \theta_i) = \prod_{j=1}^{N} \exp(-\mu_j(x, y, \theta_i, r_j) l_j(x, y, \theta_i, r_j)).$$

The correction factor C for each point (x, y) in the transaxial image is the average of the attenuation factors (A_i) for projections at M angles (θ_i) over 360° around the point. Each attenuation factor is the product of the attenuation due to each of N voxels defined by the radial distance from the point (r_j) along the projection angle (μ_j is the attenuation coefficient for the jth voxel, l_j is the length of the projection through this voxel). We wonder if the investigators used this implementation of Chang's algorithm, the original equation published, or a different equation.

References

1. Bailey DL, Hutton BF, Walker PJ. Improved SPECT using simultaneous emission and transmission tomography. *J Nucl Med* 1987; 28:844–851.
2. Chang LT. A method for attenuation correction in radionuclide computed tomography. *IEEE Trans Nucl Sci* 1978; NS-25:638–643.

James R. Galt
D. Rex Hamilton
Ernest V. Garcia
Emory University School of Medicine
Atlanta, Georgia

REPLY: Galt and his colleagues are correct in suggesting that the equation given in our article (p. 846) (*1*) for calculating attenuation correction values is similar to the original Chang equation (*2*), however, we use the attenuation map values