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EDITORIAL

Targeted Proteins for Diagnostic Imaging: Does Chemistry Make a Difference?

The imaging of occult infection is an important area of nuclear medicine. Vehicles for abscess localization have ranged from ^{67}Ga-citrate to radiolabeled leukocytes to radiolabeled immunoglobulin G (IgG) of current interest. Although ^{111}In labeled polyclonal IgG is probably the most widely cited protein being evaluated for focal infection scintigraphy (*1–4*), the mechanism of radiolabel accumulation remains unclear (*5–8*). In this issue of *The Journal of Nuclear Medicine*, Oyen et al. examined the roles of protein carrier and radiolabel in targeting of abscesses (*9*). They evaluated three different protein carriers and went on to assess the contribution of three different radiolabels and their associated chemistries in imaging experimental infectious foci in a rat model.

In the first part of the study, the authors compared radiolabeled IgG with immunoglobulin A (IgA) and human serum albumin (HSA) controls since these proteins lack specificity for abscess. In each case ^{111}In labeling via a bifunctional chelate served as the standard radiolabel so that localization differences could be ascribed to individual protein distribution properties. Layered upon the targeting properties of the protein was the contribution of the radiolabels and their chemistries. In the second part of the study, the authors compared various radiolabels with IgG serving as the standard protein vehicle.

This study was well-conceived and designed to determine the role of protein carrier and radiolabel. However, an accurate interpretion of the role of the protein assumes the radiolabel serves as a radiotracer. Furthermore, an interpretion of the role of the radiolabel requires an analysis of its chemistry and an appreciation for the pharmacokinetics of its radioactive metabolites. Since these properties direct the biodistribution of radioactivity, it is instructive to briefly review relevant factors such as attachment stability, metabolic fate, and route of excretion characteristic of radioiodines, ^{111}In and ^{99m}Tc as used in this study.

IODINE AS RADIOTRACER

Radioiodine isotopes continue to be the most widely used protein radiolabels: ^{123}I for imaging, and ^{125}I and ^{131}I for preclinical studies with their convenient longer half-lives and ready availability. The "easy" direct radioiodination approach in which the radioiodine is added to the activated ortho position of tyrosine is most often used, as was done in the Oyen et al. study (*9*). Label stability is usually sufficient to follow proteins in circulation or bound to cell surfaces. Once internalized by cells, however, catabolism releases peptide fragments or free amino acids with further metabolic processing ultimately releasing radioiodide (*10*). Deiodination may occur rapidly as in the example of the T-101 antibody in which imaging of cutaneous T-cell lymphoma is virtually precluded by rapid loss of radioactivity from tumor cells (*11*). Metabolically stabilized ligand chemistry has been developed which substan-

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tially reduces the loss of radioiodide by the attachment of the radiolabel to non-activated para and meta positions on the aromatic ring, as the *p*-iodophenyl (*12*) or *m*-iodophenyl (*13*) derivative. While this avoids post-metabolic accumulation of radioiodide in the stomach and thyroid, retention of the iodobenzoate metabolites in target cells may be only slightly extended unless an approach such as the tyramine-cellobiose linker is used to enhance intracellular trapping of radiolabel following internalization and catabolism (*14*).

Oyen et al. (*9*) attribute the persistence of radioiodine relative to ^{111}In in the blood to deiodination and subsequent washin of radioiodide and radioiodinated fragments. This explanation seems unlikely since the iodine blood values were higher from two hours onward and the blood disappearance pharmacokinetics of small radioiodine species would be expected to be more rapid than that of IgG. Relative to ^{111}In, the ^{123}I radiolabel may in fact more accurately reflect the actual IgG blood disappearance rate. The abscess accumulation of ^{123}I-IgG was also correspondingly higher than that seen with ^{111}In-IgG at early times, but decreased more rapidly. Perhaps higher initial blood levels produced better early uptake of ^{123}I-IgG but deiodination resulted in diminished retention of the iodine radiolabel as described above.

INDIUM-111 AS RADIOTRACER

The ^{111}In labeling of IgG, IgA and HSA in the Oyen study (*9*) was accomplished using diethylenetriaminepentaacetic acid (DTPA) linked via the bicyclic anhydride derivative according to Hnatowich et al. (*15*). Analysis indicated 2–3 DTPA ligands per protein molecule.

Much effort has been expended and multiple strategies have been developed to improve ^{111}In protein labeling. The Hnatowich method utilizes one donor carboxylate group to link the chelating group to protein (*15*). Newer methods have been developed that allow chelation with all donor groups and utilize a single linkage avoiding the potential for cross-linking leading to aggregation or altered protein tertiary structure. Gansow et al. (*16*) and Carney et al. (*17*) couple via carbon backbone derivatized with phenylisothiocyanate linkages, while Meares et al. (*18*) use a carbon backbone derivative of ethylenediaminetetraacetic acid (EDTA). The molar substitution ratio of chelator to antibody has been shown to be an important parameter affecting both immunoreactivity and uptake in non-target organs (*15,17,19*) with caution suggested when ratios exceed 2 or 3:1.

Concerns surrounding ^{111}In as a protein radiotracer have focused on chelate stability and reticuloendothelial system clearance. Hepatic uptake and retention of ^{111}In radioactivity can result from either transchelation to transferrin which traffics to the liver, spleen and bone marrow or from protein degradation with retention of residual ^{111}In metabolites.

The ^{111}In biodistribution results presented by Oyen et al. (*9*) are generally consistent with previous studies characterizing the disposition of ^{111}In-labeled protein. In sum, significantly more uptake and retention in liver and spleen is seen with ^{111}In relative to ^{99m}Tc or radioiodine radiolabels. Somewhat surprisingly, renal uptake of ^{111}In labeled IgG was prominent. This observation and the more rapid blood disappearance of ^{111}In-IgG compared to ^{123}I-IgG suggest inherent serum instability or extraction of derivatized protein by liver and kidneys due to an excessive DTPA to IgG ratio.

TECHNETIUM-99m AS RADIOTRACER

The final protein radiolabel evaluated by Oyen et al. (*9*) was ^{99m}Tc. The combination of superior imaging properties, widespread availability, and low cost indicate preference for ^{99m}Tc when possible. Since proteins are typically slow to target and slow to disappear from blood, their distribution properties may not be complementary with the 24-hr ^{99m}Tc window of imageability given its 6-hr half-life.

In this study, ^{99m}Tc labeling of IgG was accomplished by exchange to thiol derivatized protein prepared by reaction with 2-iminothiolane (*20*). This modification converts lysine side chain amine groups to amidines linked to a 3-carbon chain terminating in sulfhydryl suitable for binding technetium. While it is generally appreciated that sulfur is a major contributor to chelated technetium stability, the contribution of a single sulfhydryl donor is questionable without invoking assistance from collateral donor groups forming appropriately sized chelate rings. The metal donor contribution from the amidine group is not known. Even if strong donor capability was assumed, the bidentate unit forms a 7-membered chelate ring and would not be expected to be highly stable based on the findings of Davison et al. who investigated stability in a 5- and 6-membered chelate ring series of tetradentate N, S chelating agents (21). In that study, the tetradentate N_2S_2 system was significantly less stable with three 6-membered chelate rings.

Given the stability considerations raised above, it can be appreciated that ^{99m}Tc-IgG radiolabel disappears from the blood more rapidly than ^{123}I or ^{111}In-IgG radiolabels. In contrast to ^{111}In, a significant drop in ^{99m}Tc radioactivity retained at the abscess was seen. These results may be rationalized by assuming rapid initial accumulation at the site of inflammation (maximal uptake was reached by 6 hr postinjection with all of the variously labeled IgG) and subsequent loss of ^{99m}Tc due to inherent instability. Interestingly, useful imaging with ^{99m}Tc direct labeled proteins has been demonstrated. In some cases, only relative stability may be required for targeting with label loss actually contributing to blood disappearance and attainment of favorable target to background ratios for imaging. The 2-iminothiolane or similar direct labeling approach depends on achieving a balance of stability and instability, which seems ul-

timately more limited than a stable chelate and linkage approach with defined modifications to targeting proteins. Along this line, promising results have been reported with hydrazino nicotinamide linked ^{99m}Tc-IgG by Abrams et al. (*22*).

ROLE OF PROTEIN IN TARGETING INFECTIOUS FOCI

In the Oyen et al. study (*9*), IgA was used as a control immunoglobulin since it lacks Fc-gamma receptor affinity, in order to evaluate the role that receptor plays in IgG uptake and retention in infectious foci. Liver and spleen uptake of ^{111}In-IgA was higher than for ^{111}In-IgG indicating that the protein did alter the biodistribution properties. However, the abscess-to-background ratios were comparable for both IgG and IgA, which does not support a major role for specific receptor interactions. In addition, control ^{111}In-HSA showed comparable abscess uptake and retention. Thus, the localization of protein agents in abscesses may be governed largely by relative blood flow and the maintenance of local concentration dependent on circulation half-life. The target-to-background ratio attained in abscess may be governed more by factors relating to nonspecific recognition of labeled-altered proteins, internalization, catabolism, and either retention (^{111}In) or release (radioiodine, ^{99m}Tc), depending on radionuclide chemistry.

SUMMARY

The Oyen et al. study (*9*) is valuable in that it systematically evaluates several of the factors involved in radiolabeled protein uptake and retention in infectious foci. The role of particular proteins and their receptor specific interactions seems to be inconsequential in agreement with the findings of other (*23*). However, the role of the radiolabel was shown to be important and significant differences were delineated from comparisons of the radionuclides and their associated chemistries.

The conclusion implicating radionuclide chemistry and associated linkages underscores the need to optimize the attachment and labeling chemical modifications of protein carriers. Evaluation criteria should include serum stability, determination and assessment of the effect of molar substitution ratio, and potential for improving blood clearance without reducing the target-to-non-target ratio. Important areas for future study include characterization of radioactive metabolites and the design and synthesis of new ligands which direct the disposition of metabolites reducing retention in normal organs or accelerating renal excretion. Additionally, intracellular processing of radiolabel, compartmental distribution and strategies for augmenting internalization and retention within the target cell merit detailed exploration.

For each radionuclide of interest, ^{111}In, radioiodines, ^{99m}Tc and others, improved chemical moieties exist for controlling radiolabel fate. When carrying out mechanistic and evaluative studies, clear-cut conclusions will only be reached when defined and controlled chemistry is used. Having established a "gold standard," simplifications in radiolabeling and other chemical refinements can then be pursued with a quantitative understanding of the trade-offs in targeting agent performance versus other considerations such as cost reduction, simplicity, and convenience.

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(continued from p. 344)

SELF-STUDY TEST
Radiobiology and Radiation Protection
ANSWERS

exposure and conception (it has been observed that avoiding conception for a time interval after irradiation greatly reduces the production of mutations).

ITEMS 25-29: Multistage Development of Radiation-Induced Cancer

ANSWERS: 25, T; 26, F; 27, T; 28, T; 29, T

For many types of radiation-induced cancer, the epidemiologic evidence suggests that events subsequent to irradiation are required to produce a cell that is capable of uncontrolled proliferation. For example, in irradiated populations no excess risk of breast or lung cancer has been seen until the exposed individuals have reached ages at which these cancers usually are observed in nonirradiated populations. This suggests that induction of these cancers requires one or more time-dependent factors in addition to whatever role ionizing radiation plays in their causation.

Bone cancer and leukemia, on the other hand, have appeared in excess within a few years after exposure, suggesting that the multiple stages must occur rapidly or that they may not be required to complete the carcinogenic process. These observations do not support the concept of multistage tumor induction by radiation.

Another marked contrast that distinguishes radiation-induced leukemia and bone cancer is the return of risk to near normal levels within a period of 30 yr or less after irradiation, whereas, with other cancers the risk period may extend to the end of life. These long latent periods again imply a multistage process. The literature of experimental carcinogenesis abounds with examples in which cocarcinogens or promoting agents modify the dose–response curve and the latent period for radiation carcinogenesis. This reduction in latent period by "promoters" indicates that the process (promotion) is at least a second step after initiation.

Recent studies of malignant transformation by viral oncogenes and activated cellular oncogenes suggest that cellular malignant transformation may require activation by more than one cellular oncogene. It is possible, thus, that the long latent periods that characteristically elapse between irradiation and clinical appearance of the cancer may result from the need for activation of recessive oncogenes or other sequential steps.

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ITEMS 30-33: Radiation-Induced Cancer in Humans

ANSWERS: 30, F; 31, T; 32, T; 33, F

The presence of radiation-induced cancers in a human population is difficult to detect and to quantitate because the cancers induced by radiation are indistinguishable from those occurring naturally. Their existence can be detected only on the basis of a statistically significant excess in irradiated individuals above the natural incidence. Detection of radiation-induced cancers is also difficult because of the long latent periods (typically 10 yr or more for solid tumors) between irradiation and detection, as shown in the following table.

Approximate Latent Periods (yr) for Radiation-Induced Cancers in Humans

Type	Minimum	Mean	Total Period of Expression
Leukemia	2–4	10	25–30
Bone	2–4	15	25–30
Thyroid	5–10	20	>40
Breast	5–15*	23	>40
Other solid tumors	10	20–30	>40

*Varies with age at exposure
Adapted from Ref. 2, below.

Radiation-induced cancers are considered to be the most important late somatic effect of radiation. Leukemia induced by radiation stands out because of the natural rarity of the disease, the relative ease of its induction by radiation, and its short latent period (2–4 yr). When the *total* risk of radiation-induced cancer is considered, however, it is clear that the risk of induced solid tumors exceeds that of leukemia. For the A-bomb survivors, the ratio of radiation-induced solid tumors to leukemias is now approximately 4:1. The major sites of solid cancers induced by whole-body radiations are breast (in women), thyroid, lung, and some digestive organs.

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ITEMS 34-38: Sex Dependence of Radiation Carcinogenesis

ANSWERS: 34, T; 35, F; 36, F; 37, T; 38, F

The incidence of radiation-induced human breast and thyroid cancer is such that the total cancer risk is greater for women than for men. Breast cancer occurs almost exclusively in women, and absolute-risk estimates for thyroid cancer induction by radiation are higher for women than for men (as is the case for the natural incidence). With respect to other cancers, the radiation risks in the two sexes are approximately equal.

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