EDITORIAL

New Methods for Localizing Infection: A Role for Avidin-Biotin?

Recently nonspecific human IgG chelate conjugates labeled with ^{111}In have been proposed for localizing infection (*1*). This has been in response to a need for simplifying the current ^{111}In-WBC scan. The ^{111}In-WBC method requires laborious separation, labeling and reinjection of ^{111}In-oxine-labeled autologous leukocytes. With the increasing incidence of AIDS, there is now the possibility of misadministration of HIV-infected cells with disastrous consequences. Radiation exposure from a diagnostic misadministration pales into insignificance by comparison. There is also a small but finite chance of self-infection from a needle stick. These reasons, along with simplicity, are all persuasive arguments for the continued development of kit preparations wherever possible. Thus, new approaches amenable to kit formulations, such as human polyclonal immunoglobulin and anti-leukocyte monoclonal antibodies* labeled with either ^{99m}Tc or ^{111}In are very attractive.

Nevertheless, the high sensitivity and specificity of the ^{111}In-labeled WBC method make it the "gold standard" against which all the new methods must be measured. Teaching centers with cell labeling facilities and trained personnel that are currently using the ^{111}In WBC method will probably want to continue with this method until it is at least matched or surpassed by one of the new techniques. The ^{111}In- or ^{99m}Tc-labeled human polyclonal immunoglobulin technique has received much attention from publication of successful clinical trials showing both simplicity and sensitivity (*2,3*). However, other radiolabeled proteins have not been given comparable clinical trials; and because of logistic difficulties, it has not been possible to use them as the appropriate controls in the IgG studies. The necessary lack of controls in these clinical studies has given more easy acceptance to the idea that IgG has some specificity through the Fc receptor on granulocytes. This hypothesis has never been rigorously demonstrated to be true, and there are numerous reasons why it is probably not the mechanism of localization (*4*). The mechanism of uptake of nonspecific proteins remains obscure, but it is likely that increased capillary permeability, which is a hallmark of inflammation causing redness and swelling, probably plays the major role.

Methods based on anti-leukocyte monoclonal antibodies have a greater appeal because of the potential for high specificity, equal to ^{111}In-WBCs, as well as high sensitivity. The use of specific monoclonal antibodies therefore offers a significant advantage over the ^{111}In-IgG method, which cannot discriminate inflammatory from other diseases with increased capillary permeability. Set against these advantages is the lack of FDA approval in the U.S. for any commercial anti-leukocyte monoclonals compared to the ready availability of IgG already approved for human use.

To put the various nonspecific methods mentioned above in perspective, it is instructive to review a carefully controlled animal study carried out by McAfee et al. (*4*). This work focused on the degree to which a variety of nonspecific radioactive agents mimicked the biodistribution of ^{111}In-labeled mixed autologous leukocytes. These workers used the same animal model (dogs with acute soft-tissue *E. coli* abscesses and an acute arthritic lesion) to evaluate eight agents: ^{67}Ga-citrate, human and canine polyclonal IgG, rabbit anti-dog polyclonal IgG, serum albumin, monoclonal antibody TNT-1 F(ab')$_2$ against nuclear antigens, ^{57}Co-porphyrin and serum albumin nanocolloid. Pure leukocytes were harvested from joint effusion, thus enabling the stability and association of the label with leukocytes to be measured in vivo. The advantage of ^{111}In-WBCs was striking: none of the other agents achieved abscess concentrations even approaching those obtained with labeled leukocytes, which concentrated almost 10-fold higher. The target-to-background ratios were also much lower with the nonspecific agents. Nevertheless, the search for agents capable of direct intravenous injection, preferably labeled with ^{99m}Tc, is very important until one is found with comparable lesion concentration and contrast.

Rusckowski et al. (*5,6*), reasoning that the protein streptavidin will diffuse through leaky capillaries in a similar fashion to IgG, have proposed its use for localization of inflammation. Avidin and biotin form an extremely strong noncovalent complex (Ka ~ 10^{15}) (*7*). Due to this high affinity, only small amounts of avidin are required to bind biotin-linked radiopharmaceuticals in vivo. Rapid in vivo clearance and biodistribution of the radiolabeled biotin derivatives is crucial in producing a high target-to-background ratio in 3 hr or less following intravenous injection.

In this issue Rusckowski et al (*5*) show encouraging results in their mouse model with *E. coli* infection in one thigh. Six hour uptake of ^{111}In-IgG, ^{111}In-streptavidin and 6 hr pretargeted streptavidin (sampled 3 hr post-^{111}In biotin) had equivalent uptake with target-to-blood ratios threefold higher for pretargeted streptavidin. This finding is in accordance with the view that all proteins will have approximately the same uptake in in-

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* Behringwerke BW 250/183; Immunomedics IMMU-MN3.

flammatory lesions via the common mechanism of leaky capillaries. It is in this situation of equivalent target uptake that the low background obtainable with pretargeting is a great advantage, as shown in this study. The much lower affinity of EB_1 ($K_A = 10^8$), in the antibody-hapten range, decreased the target concentration, but increased the target-to-background ratios, since normal tissue concentration also decreased. More recently, in collaboration with Paganelli and colleagues at San Raffaele Hospital in Milan, Italy, they have successfully extended the method to humans (*6*).

Increasing interest in pretargeting technology was shown at the recent 39th Annual Meeting of the Society of Nuclear Medicine, where 11 abstracts were presented concerning various aspects of the application of biotin and avidin. We reported the 24 hr whole-body retention and 3-hr organ distribution of six biotin-chelate conjugates in tumored mice, with and without avidin (*8*). Biotin-chelate derivatives formed stable, high specific activity complexes (1000 Ci/m*M*) with ^{111}In and ^{88}Y. Endogenous biotin did not block in vivo biotin-chelate binding. None of the conjugates of benzyl-EDTA, DTPA or DOTA concentrated selectively in any organ (other than kidney and liver—the major routes of excretion) or in mouse tumor or human tumor xenografts. Avidin and de-glycosylated avidin cleared rapidly from the circulation into the liver where it was not available for targeting biotin. Streptavidin circulated much longer, with 30% clearing into the kidney where it was available for targeting ~15% of injected biotin activity, making it the highest background organ. We showed pretargeting of a streptavidin-anti IA^k (B-lymphocyte determinant) monoclonal conjugate in antigen-positive C3H (spleen = 65%/g) versus antigen-negative balb/c (spleen = 19%/g) mice.

At the same meeting, the preparation and characterization of N_3S biotin conjugates for tumor targeting of ^{86}Re (or ^{99m}Tc) and its therapeutic use in tumored mice was reported. Overall delivery of rhenium to the tumor on a picomole-per-gram basis was greater than that of the preformed chelate ^{86}Re-labeled antibody for matched antibody doses (*9,10*).

The avidin-biotin system promises to be useful for constructing bispecific monoclonal antibodies having both target-specific sites and radiolabel binding sites for use in either leukocyte-specific or tumor-specific pretargeted immunoscintigraphy (*7,8,11*). A diagram of the proposed pharmacokinetics is shown in Figure 1. This schema includes a chase step of polybiotinylated protein capable of cross-linking and thus removing circulating avidin conjugates into the liver just prior to injecting labeled biotin. We have found that this greatly reduces the blood and liver background.

Paganelli et al. have recently reported results from 20 patients in whom pretargeted immunoscintigraphy was performed with biotinylated monoclonal antibody followed by avidin and imaged with ^{111}In-bis-biotinyl DTPA chelate (*12*). The images showed increased tumor contrast with low background especially in the liver (2%). Tumors and metastases (including liver) were seen at 3 hr in all patients. The excellent quality of the scans and high tumor-to-blood (5.5/1) and tumor-to-liver (6.7/1) ratios were compelling evidence that pretargeting will be a significant improvement over conventional immunoscintigraphy.

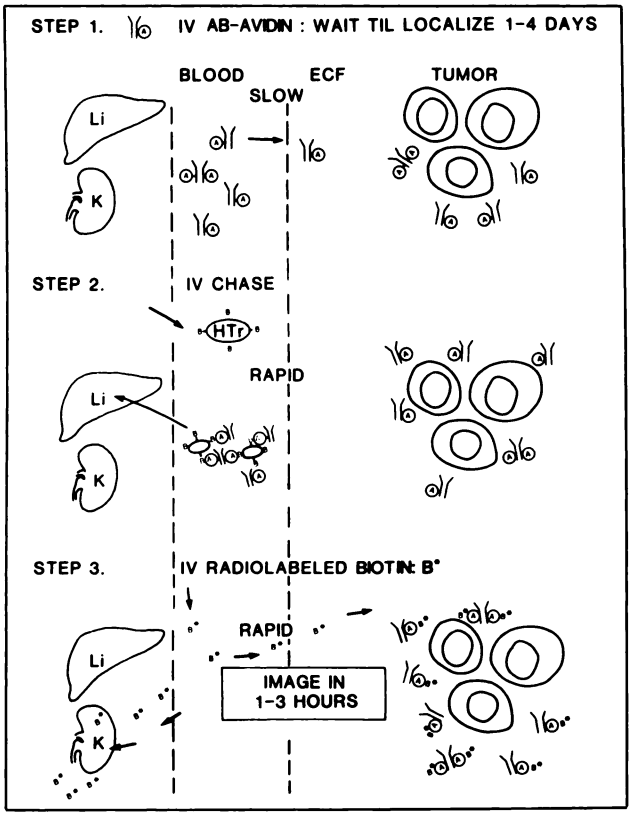

FIGURE 1. Scheme for pretargeted immunoscintigraphy. The dashed columns represent the capillary walls, with the major excretory organs, the liver and kidney, on the left and the tumor (or abscess) target surrounded by extracellular fluid (ECF) on the right. In this case an avidinated Mab is injected and slowly diffuses to the target. The chase is biotinylated human transferrin, shown cross-linking the avidin. Radiolabeled biotinylated chelate diffuses rapidly throughout the ECF and binds to the avidin in the target. (Reprinted with permission from: Goodwin DA. Strategies for antibody targeting. *Antibod Immunoconj Radiopharm* 1991;4: 427–434)

The widening interest in pretargeting technology following favorable reports such as those outlined here makes it likely that avidin-biotin will find broad application in this area of radiopharmaceutical development.

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SELF-STUDY TEST
Gastrointestinal Nuclear Medicine
ANSWERS

ITEMS 1–6: Effect of Drugs on Gastric Emptying

ANSWERS: 1, T; 2, T; 3, T; 4, T; 5, F; 6, F

Many drugs have been shown to slow gastric emptying, and their effects must be considered in reporting the results of gastric emptying studies. The nicotine associated with cigarette smoking has been shown to slow gastric emptying. In addition, calcium channel blockers have been shown to decrease the amplitude and duration of contractions of smooth muscle throughout the gastrointestinal tract. Calcium channel blockers either decrease the number of calcium channels (nifedipine, verapamil, diltiazem) and/or decrease the rate of calcium transport in the remaining channels (verapamil, diltiazem). Adrenergic agonists, especially beta agonists (such as isoproterenol), all tend to delay gastric emptying. Dopamine is a neural transmitter, which appears to be involved primarily in gastric relaxation. Dopamine agonists, such as levodopa, will slow gastric emptying. The D_2-receptor antagonist metoclopramide stimulates gastric contractions and, thus, increases the rate of gastric emptying. It is also felt to have a central antiemetic effect. Domperidone is another dopaminergic antagonist, which also accelerates gastric emptying and has been shown to increase gastric antral contractions.

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ITEMS 7–10: Barrett's Esophagus

ANSWERS: 7, F; 8, F; 9, F; 10, F

Much has been written about the clinical presentation and assessment of patients with Barrett's esophagus. Although Barrett's esophagus causes no symptoms per se, the clinical presentation is related to gastroesophageal reflux and covers the spectrum of regurgitation, heartburn, chest and abdominal pain, and dysphagia. It has been suggested that patients with Barrett's esophagus have less severe symptoms than do those with reflux esophagitis without Barrett's epithelium. The five major complications of Barrett's esophagus include: esophagitis, ulceration, stricture, bleeding, and adenocarcinoma (not squamous cell cancer). The frequency of adenocarcinoma of the esophagus in patients with Barrett's esophagus is approximately 10%. The risk of esophageal cancer with Barrett's esophagus is approximately 30 to 40 times greater than that in the general population. Once the diagnosis of Barrett's esophagus has been made on biopsy, periodic endoscopy with biopsy is recommended to monitor for malignant transformation. The radiographic appearance of Barrett's esophagus is not specific and includes gastroesophageal reflux, hiatal hernia, esophageal stricture, ulceration, irregular mucosal folds, granulating reticular mucosal pattern, and intramural pseudodiverticulosis. The findings of a benign-appearing stricture in the proximal esophagus or a deep esophageal ulceration should suggest the diagnosis and prompt endoscopic evaluation.

The scintigraphic assessment of Barrett's esophagus has not been widely explored or utilized. The accumulation of ^{99m}Tc pertechnetate in the lower esophagus after intravenous injection of this tracer is considered a positive examination and is related to mucous-secreting cells of Barrett's mucosa. The swallowing of free ^{99m}Tc in saliva and reflux of gastric activity can cause significant problems in scan interpretation, however.

Scintigraphy can identify possible areas of Barrett's esophagus, but plays no role in assessment for possible malignancy. Currently, scintigraphy plays no definitive role in the evaluation of patients with suspected Barrett's esophagus. A large prospective study with adequate controls will be necessary to define if any future role for scintigraphy exists.

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ITEMS 11–15: Peritoneovenous Shunt Imaging

ANSWERS: 11, F; 12, F; 13, T; 14, T; 15, T

Scintigraphic techniques for assessing patency of peritoneovenous shunts utilize tracers injected into the peritoneal cavity and/or directly into the efferent limb of the shunt. These imaging techniques monitor the transit

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