Receptor-Mediated Tumor Targeting with Radiolabeled Peptides: There Is More to It than Somatostatin Analogs

In this issue of *The Journal of Nuclear Medicine*, Wild et al. report the binding and animal biodistribution data of [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-exendin-4 (Ahx is aminohexanoic acid; DTPA is diethylenetriaminepentaacetic acid), an analog of glucagon-like peptide-1 (GLP-1) (*1*). Their results raise high hopes of actually achieving selective and potent receptor-mediated targeting of tumors with a radiolabeled peptide but, at the same time, revive critical issues on the possibility of predicting the clinical success,

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usefulness, and indications of any newly developed radiopharmaceutical on the basis of preclinical in vitro and in vivo parameters. Indeed, the ^{111}In-labeled GLP-1 analog tested by Wild et al. is characterized by extremely high values of binding affinity (50% inhibitory concentration [IC_{50}] = 2.1 vs. 0.65 nmol/L for the unlabeled analog, and even 2.4 nmol/L for ^{125}I-labeled GLP-1 itself) and of specific tumor targeting in vivo (about 290 %ID/g [percentage injected dose per gram]). Even considering that the animal biodistribution study was performed under optimal conditions (because of the very high GLP-1 receptor density—about 17,000 dpm/mg in the tumor model used for the study—combined with the minute mass of the tumors), the results obtained by Wild et al. stand out as very promising in the perspective of developing a new radiopharmaceutical with high potential for diagnostic (and possibly therapeutic) applications in patients.

Nevertheless, common experience acquired while developing radiolabeled agents for molecular targeting based on the ligand−receptor interaction (especially for tumors) suggests a word of caution when trying to extrapolate from favorable preclinical localization data to similarly favorable results in humans. Thus, the basic questions in the mind of investigators remain: Is it possible to predict the pattern of biodistribution in humans (and therefore clinical success and indications) on the basis of in vitro and animal in vivo data? Conversely, is it possible to identify those preclinical parameters that best predict success or failure in human applications?

Indeed, it is not easy and probably not possible to define preclinical parameters that could predict human success of newly developed radiopharmaceuticals, at least not in a systematic manner. Radiolabeled analogs of somatostatin certainly represent a well-established paradigm of peptide radiopharmaceuticals for targeting neuroendocrine tumors (*2,3*). Since its introduction as a commercial radiopharmaceutical in 1994, a quite large "panel" of tumors and diseases has been investigated with [^{111}In-DTPA^0]-octreotide (OctreoScan; Mallinckrodt Medical, BV) scintigraphy, and extensive clinical studies have been performed primarily on neuroendocrine tumors (*4–6*) but also on other tumors, such as brain tumors (*7*), melanomas (*8*), lung cancers (*9*), and breast cancers (*10*). Despite the fact that only a relatively limited group of tumor types consistently express somatostatin receptors (SSTRs) with density sufficient for tumor targeting, the use of radiolabeled somatostatin analogs for diagnostic imaging (and also for therapy) has been quite successful, as witnessed during everyday practice in any nuclear medicine department.

Nevertheless, as also reviewed by Britz-Cunningham and Adelstein in 2003 (*11*), several analogs of small regulatory peptides different from somatostatin have been extensively evaluated as targeting ligands, as their receptors are overexpressed in various human tumors. These receptors represent promising targets for diagnostic imaging and for therapy because they are located on the plasma membrane and, on binding of the ligand, the receptor−ligand complex is quickly internalized and retained in the target cell. Furthermore, because of the short plasma half-life of these analogs, a high target-to-background ratio is rapidly reached. In this regard, the lessons learned from SSTR targeting have provided several useful parameters to be considered in preclinical studies. These parameters have been taken into account by investigators developing new radiopharmaceuticals for in vivo tissue characterization of different biochemical targets.

There is a vast body of published literature on radiolabeled peptides

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other than somatostatin analogs for cancer imaging. Especially when looking at preclinical data, a wide spectrum of different molecules has been described in the last decade, either in their native structure or modified to improve their target-binding affinity, labeling efficiency, or biodistribution. Nevertheless, most of these newly developed agents will never be used in patients, despite favorable in vivo biodistribution profiles in animal models of disease, now well documented also through noninvasive imaging achieved with dedicated γ-cameras (or PET) for small animals.

The vasoactive intestinal peptide (VIP), ligand of the most overexpressed receptor in human gastrointestinal adenocarcinomas, was first labeled with ^{123}I (*12*) and then its TP-3654 analog was developed for labeling with ^{99m}Tc (*13*). Preclinical data of these peptides clearly showed that the molecule is unstable in blood and degrades within a few minutes on injection. Moreover, VIP is pharmacologically very potent and doses in the submicrogram range will produce toxic effects requiring an efficient purification step before administration to reduce the administered dose to subpharmacologic levels. These drawbacks have so far limited the expected wide clinical applications of VIP-derived radiopharmaceuticals.

Various cholecystokinin (CCK)-A and CCK-B/gastrin–related peptides have been developed and tested in preclinical studies for targeting CCK-B/gastrin receptors in vivo (*14–16*). Radioiodinated human heptadecapeptide gastrin-I (*17*) and ^{111}In-DOTA/DTPA–conjugated (DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) CCK-B analogs (*18,19*) have been evaluated in an animal model of medullary thyroid cancer, exhibiting half-lives of several hours, prevalent urinary excretion, and specific uptake both in CCK-B–expressing organs and in tumors (~9 %ID/g of tumor at 1 h after injection). These parameters have been good predictors of favorable biodistribution profiles in a pilot clinical study (*20*), demonstrating efficient uptake in the receptor-positive organs as well as in the primary tumor lesions and in metastatic lesions.

The gastrin-releasing peptide (GRP) receptor is highly expressed in a variety of tumors such as cancers of the lung, breast, prostate, and pancreas. Prostatic cancer, in particular, is the focus of special attention, because of specific overexpression of the GRP receptor in the invasive and locally advanced forms of this tumor. In this regard, the 14-amino-acid neuropeptide bombesin (BN), characterized by high-affinity binding for the GRP receptors, is considered the prototype of a promising class of new ligands, and BN analogs are being developed as potential radiolabeled peptides for tumor targeting (*21,22*). The in vitro performance parameters of a hydrosoluble analog, ^{99m}Tc-BN(7–14), were very promising, with specific binding on rat brain cortex membrane in the nanomolar range (IC_{50} [mean ± SD] = 0.8 ± 0.4 nmol/L) (*23*). Unfortunately, despite their hydrophilic nature, these analogs maintain high hepatobiliary clearance, making scintigraphic exploration of the abdominal area problematic. Recently, a new peptide with low liver and intestinal uptake has been described (*24*), thus reviving interest in ^{99m}Tc-labeled BN analogs. DTPA-coupled BN agonists have demonstrated a high internalization rate in receptor-positive cell lines, translating into high in vivo specific uptake in BN-positive tissues and tumors. These molecules are of extreme interest not only for imaging purposes (if labeled with either ^{99m}Tc or ^{111}In) but also for receptor-mediated radiometabolic therapy, because the DTPA-chelator confers the possibility of labeling with ^{90}Y or ^{177}Lu. Finally, the development of ^{18}F-labeled and ^{64}Cu-labeled BN analogs has opened the scenario for PET, although so far only reported for animal models (*25,26*).

^{99m}Tc- and ^{111}In-DTPA/DOTA–labeled neurotensin (NT) has been evaluated both in vitro and in vivo because of its high potential for imaging endocrine pancreatic malignancies (*27*). Although the ^{99m}Tc-NT(8–13) analog exhibited a prolonged half-life in plasma without reducing its binding properties when compared with native NT, rapid degradation of the molecule at low concentration as used in biodistribution tests is still a critical factor interfering with the actual targeting potential of this "pseudopeptide" (*28*). On the other hand, some ^{111}In-labeled NT analogs are quite stable in serum and are rapidly internalized after binding to the NT receptor (when incubated with HT29 cells), and their biodistribution in animal models suggests a high potential for efficient tumor targeting (*29*).

A group of promising imaging probes deserves special attention, even though they have not yet reached the clinical setting. Recent advances in the knowledge of tumor-related neoangiogenesis and its modulation have attracted interest in biologic probes that can be used to image angiogenesis. Among the possible molecular targets, the markers of the extracellular matrix—such as α_v-integrins ($\alpha_v\beta_3$, $\alpha_v\beta_5$), vascular endothelial growth factor (VEGF) receptors (in particular, VEGFR-2 and neuropilin-1), and fibroblast growth factor (FGF) receptors (FGFR1 and syndecan-4)—could serve as selective targets.

^{123}I-Labeled $VEGF_{165}$ and $VEGF_{121}$ both bind more specifically to a variety of human tumor cells or tissues than to normal peripheral blood cells and adjacent nonneoplastic tissue, with some advantage of ^{123}I-$VEGF_{165}$ in terms of both binding to a higher number of different tumors when tested in animal models and higher binding capacity (*30,31*). This analog has also been tested clinically in a group of 40 patients with gastrointestinal tumors, with an overall 58% tumor detection rate for ^{123}I-$VEGF_{165}$.

Several synthetic peptides containing the Arg-Gly-Asp RGD sequences have been radiolabeled with either single-photon or positron-emitting radionuclides and evaluated for their potential to visualize the $\alpha_v\beta_3$ receptor. ^{99m}Tc-RP593 exhibits high binding affinity for the $\alpha_v\beta_3$ receptor in an in vitro binding assay (*32*), whereas

the DTPA-coupled RGD analog cyclo(Arg-Gly-Asp-D-Tyr-Lys) has been radiolabeled with either ^{111}In or ^{125}I. Whereas the ^{125}I-labeled analog binds specifically and with high affinity to $\alpha_v\beta_3$ receptors on the neovasculature of prostate and breast cancer, the ^{111}In-labeled analog also accumulated in $\alpha_v\beta_3$ receptor–expressing pancreatic tumors (*33*).

The optimized glycosylated second-generation tracer I-GP2 exhibits high affinity for the $\alpha_v\beta_3$ receptor in vitro and specific binding to $\alpha_v\beta_3$ receptor–expressing tumors in vivo (*34*). The RGD-containing glycopeptide cyclo[-Arg-Gly-Asp-D-Phe-Lys(sugar amino acids)-] was radiolabeled with 1-nitrophenyl 2-^{18}F-fluoropropionate and evaluated in vitro and in tumor mouse models. High receptor-specific binding of the radiolabeled glycopeptide was demonstrated, yielding high tumor-to-background ratios (tumor-to-blood ratio of 27.5 and tumor-to-muscle ratio of 10.2 at 2 h after injection) (*35*). Finally, the $\alpha_v\beta_3$ receptor–targeting antibody vitaxin was labeled with ^{99m}Tc, but imaging with this agent in patients with metastatic cancer proved to be unsuccessful (*36*).

Several other peptides, already tested in animal models, are about to be evaluated in phase-I trials in humans—such as CXCR4 ligands (*37,38*), endoglin (CD105), epidermal growth factor (EGF), or even the very promising $\alpha_v\beta_3$ ligands.

On the basis of the experience gained from the examples mentioned here, we can consider the following parameters as predictors for successful clinical use: (a) receptor density on the target (particularly if overexpressed in a variety of cancers); (b) affinity of the ligand for receptor binding; (c) specific radioactivity of the labeled ligand; (d) plasma half-life (short to intermediate); (e) route of metabolic degradation or excretion (renal clearance preferred); (f) ex vivo counted %ID/g tumor in adequate animal models; (g) maximum tumor-to-background ratio achievable in vivo and time at which this is achieved; and (h) time course of the tumor-to-background ratios between an early time point (e.g., 2 h after injection) and a late time point (e.g., 24 h), expressed as the ratio. The latter parameter is especially important for the choice of the isotope for radiolabeling. In particular, if the tumor-to-background ratio is higher at 2 h than at 24 h after injection, then a positron-emitting radionuclide would be first choice for labeling, whereas a single-photon–emitting radionuclide would be more suitable if the tumor-to-background ratio continues to rise between 2 and 24 h after injection (the latter instance would also justify speculations about the potential for radiometabolic therapy after labeling with a suitable β-emitting radionuclide).

Several of these parameters favorably apply to the ligand–receptor system described by Wild et al. (*1*). In fact, the receptor density on the target (evaluated by quantitative autoradiography on insulinoma cells) is surprisingly high; the ligand affinity for the receptor is also very high (considering that the IC_{50} value ranges generally between 1 and 100 nmol/L for these peptides). The specific radioactivity of the ^{111}In-labeled exendin-4 was 90 GBq/μmol (~500 μCi/μg), thus justifying speculation on the possibility of obtaining good images in humans by injecting only 10 μg of labeled protein (185 MBq [5 mCi]). The in vivo mouse biodistribution studies revealed an exclusive renal metabolism (although with some unexpected lung uptake) and a short plasma half-life. The maximum tumor uptake was reached at 4 h after injection, with the maximum tumor-to-blood ratio at 48 h after injection (as high as 2,200, due to the combined effect of very fast plasma clearance and long retention of the radioligand at the tumor target). A long retention at the target site (with the tumor-to-background ratio at 48 h about 2-fold the ratio at 2 h after injection) suggests a high potential of this agent also for receptor-mediated radionuclide therapy. Therefore, radiolabeled exendin-4 represents a very promising diagnostic agent, with possible use also as a therapeutic agent, and we expect that this new radiopharmaceutical will rapidly progress through human studies.

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