Radiolabeled RGD Peptides Move Beyond Cancer: PET Imaging of Delayed-Type Hypersensitivity Reaction

The arginine-glycine-aspartic acid (RGD) cell adhesion motif was discovered in fibronectin by Pierschbacher and Ruoslahti 20 years ago (*1*). Shortly thereafter, Smith and Cheresh (*2*) identified the vitronectin receptor, or $\alpha_v\beta_3$ integrin, as one of the adhesion molecules recognizing this sequence. The $\alpha_v\beta_3$ integrin is expressed on the luminal surface of neovasculature but is not found on the endothelial surface of mature capillaries. In addition, $\alpha_v\beta_3$ has been shown to be upregulated in tumor blood vessels that undergo continuous angiogenesis (*3*) and has been implicated in metastasis (*4*). Synthetic RGD peptide antagonists of $\alpha_v\beta_3$ were subsequently shown to inhibit growth of neovasculature and effect tumor regression in animal models (*5,6*), presumably by starving tumors of their blood supply.

Recent reports of an $\alpha_v\beta_3$-targeting, cyclic RGD peptide that specifically localized to tumor xenografts after in vivo phage display selection (*7*) and produced durable responses in tumor-bearing mice when coupled to doxorubicin (*8*) caused great excitement in the nuclear medicine community. These findings raised the possibility that radiolabeled cyclic peptide ligands for $\alpha_v\beta_3$ might become powerful new tools for molecular imaging and targeted radiotherapy of tumors undergoing angiogenesis. Haubner et al. (*9*), who synthesized the first such peptide, ^{125}I-c(RGDyV), demonstrated affinity of 2 nmol/L and high selectivity of this peptide for $\alpha_v\beta_3$ in vitro and showed specific tumor uptake in mouse models of melanoma and osteosarcoma. However, tumor targeting was modest in those studies, with high hepatobiliary clearance and intestinal uptake presenting major obstacles to adequate imaging contrast. Subsequently, van Hagen et al. (*10*) evaluated c(RGDyK) conjugated to diethylenetriaminepentaacetic acid (DTPA) and labeled with either ^{125}I or ^{111}In. These investigators found that the radioiodinated analog exhibited $\alpha_v\beta_3$-mediated uptake not only in tumor neovasculature but also in BON human carcinoid and CA20948 rat pancreatic carcinoma cells in culture. In vivo uptake of ^{111}In-DTPA-c(RGDyK) in CA20948 tumors was mediated by $\alpha_v\beta_3$ but remained relatively low, with most tumor-to-normal tissue ratios near unity. Although DTPA conjugation reduced liver accumulation of the ^{111}In tracer substantially, uptake and retention were 4–8 times higher in kidney than in any other tissue, also limiting the utility of this compound.

A breakthrough improvement in the in vivo distribution and imaging properties of radiolabeled RGD peptides was made by attachment of a sugar amino acid (SAA) moiety to a cyclic $\alpha_v\beta_3$ ligand (*11*). Compared with ^{125}I-c(RGDyV), the second-generation glycosylated derivative ^{125}I-c[RGDyK-(SAA)] (^{125}I-gluco-RGD) showed similar blood and renal clearance in melanoma- and osteosarcoma-bearing mice, greatly reduced liver uptake, increased tumor retention, and yielded higher tumor-to-blood ratios. It was concluded that glycosylation of the peptide improved its confinement to the vascular space early after injection, allowing improved tumor targeting and reduced liver uptake compared with the hydrophobic parent compound. A third-generation glycosylated peptide, c[RGDfK(SAA)], was labeled with 2-^{18}F-fluoropropionate to yield ^{18}F-galacto-RGD for PET imaging of $\alpha_v\beta_3$ (*12*). High-resolution PET of mice bearing both $\alpha_v\beta_3$-positive and $\alpha_v\beta_3$-negative melanoma xenografts demonstrated integrin-specific tracer uptake with high ratios of tumor to normal tissue at 60–120 min after injection, as well as blocking by $\alpha_v\beta_3$ antagonists. Preliminary results on the use of ^{18}F-galacto-RGD PET to image tumor angiogenesis in cancer patients have been reported (*13*). As clinical applications of this tracer develop, it may have significant potential utility for noninvasive evaluation of angiogenic and metastatic activity in cancer, as well as for monitoring tumor therapy using specific $\alpha_v\beta_3$ antagonists or other angiogenesis inhibitors.

Janssen et al. used a dimeric peptide, E-[c(RGDfK)$_2$], conjugated to ^{111}In-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) or ^{99m}Tc-hydrazinonicotinamide (HYNIC), to target $\alpha_v\beta_3$ (*14*). These authors showed that $\alpha_v\beta_3$-specific tumor targeting, normal organ uptake, and tumor-to-blood ratios were generally superior for the ^{111}In-labeled compound. Moreover, the relative affinity of the dimeric tracer for $\alpha_v\beta_3$ was 10-fold higher than that of the corresponding monomer (*15*), suggesting that this compound offers the advantage of avidity in integrin binding. In the first reported radiotherapeutic studies of an $\alpha_v\beta_3$-targeting peptide, ^{90}Y-DOTA-E-[c(RGDfK)$_2$] delayed tumor growth in nude mice bearing human ovarian carcinoma xenografts, with minimal toxicity.

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Although these reports are encouraging, several significant limitations in radiopharmaceutical targeting of $\alpha_v\beta_3$ in tumors remain to be addressed. Both tumor cells and neovasculature overexpress $\alpha_v\beta_3$. Therefore, it is unclear what the relative contributions of each tissue are to tumor uptake in animal models and whether angiogenic activity is truly being imaged. In the mouse PET studies (*12*), static tumor-to-background ratios were analyzed and tumor $\alpha_v\beta_3$ density was not measured quantitatively. Quantitation of target concentrations will require dynamic studies to determine radiolabeled RGD tracer kinetics and correlation of these findings to independent measurement of $\alpha_v\beta_3$ expression by immunohistochemistry and Western blot analysis. Nonetheless, the results of these studies indicate that $\alpha_v\beta_3$ is a promising target for tumor imaging, radionuclide therapy, and noninvasive monitoring of other therapeutic interventions.

On pages 184–189 of this issue of *The Journal of Nuclear Medicine*, Pichler et al. (*16*) report a new application of radiolabeled RGD peptides targeting $\alpha_v\beta_3$: imaging of delayed-type hypersensitivity reaction (DTHR) in a mouse model of chronic inflammation. A substantial body of evidence now exists that $\alpha_v\beta_3$ plays a critical role in activated macrophage-dependent inflammation and inflammatory angiogenesis. Overexpression of $\alpha_v\beta_3$ has been documented in rheumatoid arthritis (*17*) and psoriasis (*18*), and the anti-$\alpha_v\beta_3$ monoclonal antibody MEDI-522 (Vitaxin; MedImmune) has been tested in patients for treatment of these autoimmune diseases (*19*). Given that MEDI-522 provided no benefit for therapy of rheumatoid arthritis and psoriasis, resulting in discontinuation of those clinical trials, molecular imaging of autoimmune processes holds great potential for improved evaluation of response to therapy.

Pichler et al. (*16*) developed a mouse model of DTHR for imaging $\alpha_v\beta_3$ expression in chronic inflammation because DTHRs are common in autoimmune diseases. By cutaneous application of the hapten 2,4,6-trinitrochlorobenzene (TNCB) to 1 ear of the mice in their studies, the authors elicited an acute contact hypersensitivity reaction. After several subsequent TNCB challenges, progressive chronic skin inflammation developed, which produced a clinically relevant model of autoimmune DTHR. Histology and immunohistochemistry of the angiogenesis marker cluster designation 31 (CD31) were used to distinguish acute inflammation, characterized by infiltration of polymorphonuclear leukocytes and lymphocytes, from chronic DTHR, exhibiting marked epidermal hyperplasia, hyperkeratosis, and neovascularization.

Autoradiography studies using ^{125}I-gluco-RGD demonstrated no significant differences between the TNCB-treated ear and the contralateral ear after the first challenge. In conjunction with the lack of detectable angiogenesis, this result indicated that $\alpha_v\beta_3$ expression was not significantly upregulated in the mouse model of acute inflammation. However, after 13 successive TNCB challenges, the ^{125}I-gluco-RGD uptake ratio of the challenged ear to the untreated ear reached a significant value of 2.3:1. Considering that neovascularization was clearly detected by CD31 immunohistochemistry by this point, the concentration of ^{125}I-gluco-RGD in the inflamed ear was likely mediated by binding to overexpressed $\alpha_v\beta_3$ in the chronic phase of DTHR.

Using high-resolution rodent PET, Pichler et al. (*16*) also detected marked focal uptake of ^{18}F-galacto-RGD in the ears of mice treated with 10 TNCB challenges. After a single challenge, mice in the acute phase of inflammation showed barely detectable tracer uptake in the challenged ear, whereas after 10 challenges, the tracer uptake ratio in the chronically inflamed ear versus the contralateral ear was 4.7:1. Furthermore, pretreatment with a large excess of unlabeled c(RGDfV) reduced ^{18}F-galacto-RGD uptake in the challenged ear to near background levels, suggesting that high-resolution mouse PET of chronic DTHR was the result of specific $\alpha_v\beta_3$-mediated uptake.

As in the case of tumor imaging, several limitations in imaging chronic DTHR suggest additional studies are needed to obtain conclusive proof that selective accumulation of radiolabeled RGD peptides truly represents molecular imaging of $\alpha_v\beta_3$ expression. The major limitation of the work of Pichler et al. (*16*) is that tracer uptake was not correlated with $\alpha_v\beta_3$ integrin concentration. This shortcoming was due to the current unavailability of specific antibodies against murine $\alpha_v\beta_3$. Therefore, angiogenic activity in this model was detected by CD31 immunohistochemistry rather than by an independent, direct assay for $\alpha_v\beta_3$ expression. In the future, antibodies against murine $\alpha_v\beta_3$ or PET studies in combination with immunohistochemistry and Western blotting in humans may address this limitation and validate molecular imaging of $\alpha_v\beta_3$.

In addition, activated macrophages, granulocytes, and lymphocytes can express $\alpha_v\beta_3$. Infiltrating leukocytes and lymphocytes were detected in TNCB-treated tissue in this mouse model; therefore, uptake of ^{125}I-gluco-RGD and ^{18}F-galacto-RGD by such cells cannot be excluded or distinguished conclusively from inflammatory angiogenesis. However, sizable infiltrates of immune cells were detected by histology well before increased focal uptake of the radiopharmaceuticals was observed, suggesting that $\alpha_v\beta_3$ expression on leukocytes and lymphocytes was probably not a major factor in tracer accumulation. The lack of ^{125}I-gluco-RGD and ^{18}F-galacto-RGD uptake in acute DTHR and the nearly complete blocking of ^{18}F-galacto-RGD by c(RGDfV) in chronic DTHR point to the likelihood that, for the most part, these imaging agents are specific for $\alpha_v\beta_3$ upregulation in inflammatory angiogenesis.

As with many reports on high-resolution mouse PET, the studies of Pichler et al. (*16*) were subject to physical limitations of the imaging modality. The authors acknowledged that the thinness of normal mouse ear intro-

duced substantial partial-volume effects in their model of chronic DTHR, leading to overestimation of ^{18}F-galacto-RGD uptake in the TNCB-treated ear relative to the untreated ear. In contrast, the autoradiography experiments were performed on cryosections of uniform thickness, precluding partial-volume effects. The treated-to-untreated ratio of ^{125}I-gluco-RGD uptake was 2.3:1 by autoradiography, whereas the corresponding ratio for ^{18}F-galacto-RGD by PET was approximately twice as high at 4.7:1.

Future development of relevant and appropriate modeling algorithms in high-resolution rodent PET will be necessary for accurate correction of partial-volume effects. Aside from the limitations of the noninvasive imaging modality, differences in target tissue uptake of ^{125}I-gluco-RGD and ^{18}F-galacto-RGD may very well depend on differential metabolism of the 2 tracers. Further studies, including analysis of ^{125}I and ^{18}F metabolites in target and clearance tissues and quantitative autoradiography of ^{18}F-galacto-RGD in uniform cryosections of TNCB-treated and untreated ears, are needed to correlate with PET data and to compare the 2 tracers directly.

The findings of Pichler et al. (*16*) give rise to the possibility of extending applications of $\alpha_v\beta_3$-targeting radiolabeled RGD peptides from imaging tumor angiogenesis to noninvasively detecting inflammatory processes. In particular, ^{18}F-galacto-RGD PET might offer a new means to distinguish acute and chronic T-cell immune responses. The future use of nuclear medicine procedures to identify these processes conclusively in humans may ultimately lead to substantially improved detection and diagnosis of autoimmune disorders, as well as to new molecular imaging tools for monitoring the outcomes of emerging therapeutic interventions in these diseases.

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