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Renal Toxicity of Radiolabeled Peptides and Antibody Fragments: Mechanisms, Impact on Radionuclide Therapy, and Strategies for Prevention

Erik Vegt[1,2], Marion de Jong[3], Jack F.M. Wetzels[4], Rosalinde Masereeuw[5], Marleen Melis[3], Wim J.G. Oyen[1], Martin Gotthardt[1], and Otto C. Boerman[1]

[1]Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [2]Department of Nuclear Medicine, GROW School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands; [3]Department of Nuclear Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands; [4]Department of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; and [5]Department of Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Learning Objectives: On successful completion of this activity, participants should be able to describe (1) mechanisms of renal accumulation of radiolabeled peptides, proteins, and antibody fragments; (2) different techniques to reduce kidney damage in peptide-receptor radionuclide therapy and antibody fragment radioimmunotherapy; and (3) factors determining the risk of nephrotoxicity in radionuclide therapies.

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Peptide-receptor radionuclide therapy (PRRT) with radiolabeled somatostatin analogs such as octreotide is an effective therapy against neuroendocrine tumors. Other radiolabeled peptides and antibody fragments are under investigation. Most of these compounds are cleared through the kidneys and reabsorbed and partially retained in the proximal tubules, causing dose-limiting nephrotoxicity. An overview of renal handling of radiolabeled peptides and resulting nephrotoxicity is presented, and strategies to reduce nephrotoxicity are discussed. Modification of size, charge, or structure of radiolabeled peptides can alter glomerular filtration and tubular reabsorption. Coinfusion of competitive inhibitors of reabsorption also interferes with the interaction of peptides with renal endocytic receptors; coinfusion of basic amino acids is currently used for kidney protection in clinical PRRT. Furthermore, nephrotoxicity may be reduced by dose fractionation, use of radioprotectors, or use of mitigating agents. Decreasing the risk of nephrotoxicity allows for administration of higher radiation doses, increasing the effectiveness of PRRT.

Key Words: kidney; peptide; antibody fragment; radionuclide therapy; PRRT

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For correspondence or reprints contact: Erik Vegt, Maastricht University Medical Centre, Department of Nuclear Medicine, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands.
E-mail: ErikVegt@gmail.com
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The potential to irradiate tumors using radiolabeled antibodies that target tumor-associated epitopes was first reported in the 1950s. A drawback of conventional radioimmunotherapy is the slow extravasation and clearance of intact antibodies from the blood due to their size (~150 kDa). The optimal tumor-to-background ratio is reached only after several days, and the radiosensitive bone marrow receives a considerable radiation dose. Radiolabeled antibody fragments and analogs, such as Fab fragments and Affibody molecules, were developed to improve pharmacokinetics and increase tumor–to–normal tissue dose ratios.

An alternative approach to tumor targeting is provided by the overexpression of specific cell-surface receptors by certain tumors. For example, somatostatin receptors can be targeted by somatostatin analogs such as octreotide. The use of radiolabeled somatostatin analogs for imaging and therapy of neuroendocrine tumors was first studied in the 1990s (*1*). Nowadays, peptide-receptor radionuclide therapy (PRRT) with somatostatin analogs has an established role in the therapy of metastasized neuroendocrine tumors (*2*). Many other radiolabeled peptides have been studied, such as gastrin and cholecystokinin analogs, which target the cholecystokinin-2 or gastrin receptor, and ex-

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endin, which targets the glucagonlike peptide receptor (*3,4*).

Hydrophilic radiolabeled peptides, including small antibody fragments, are excreted mainly via the kidneys and are partly reabsorbed in the proximal tubular cells. Renal retention of therapeutic radionuclides causes a relatively high radiation dose to the kidneys, which can lead to kidney failure (*1,5*). This toxicity precludes the use of higher doses, thus limiting the efficacy of therapy.

Insight into the mechanisms of renal retention and toxicity of radiolabeled peptides is necessary for the study, design, and use of strategies to reduce nephrotoxicity in PRRT and radioimmunotherapy. Several such strategies have already been studied, including structural modification of radiolabeled peptides, competitive inhibition of their renal reabsorption, and use of radioprotectors. Reduction of nephrotoxicity may enable the administration of higher-activity doses, thus improving the efficacy of therapy.

This article presents an overview of the mechanisms of renal handling, retention, and toxicity of radiolabeled peptides and antibody fragments and discusses strategies to reduce nephrotoxicity.

PHYSIOLOGY OF RENAL HANDLING AND RETENTION OF RADIOLABELED PEPTIDES

In the human kidneys, approximately 180 L of plasma are filtered through the glomeruli each day, forming the ultrafiltrate that enters the lumen of the proximal tubules. About 99% of the filtered water and a large part of the solutes is actively reabsorbed from the ultrafiltrate and mostly transported back into the bloodstream, to prevent the excessive loss of water and useful nutrients and to preserve homeostasis. The major part of the filtered load of proteins and peptides is reabsorbed in the proximal tubules. Other solutes are actively cleared from the blood into the ultrafiltrate by tubular secretion (*6*). Figure 1 presents an overview of glomerular and proximal tubular handling of peptides and proteins.

Glomerular Ultrafiltration

The degree of filtration of solutes is largely dependent on their size, charge, and protein binding. Molecules with an effective radius of less than 1.8 nm (or weighing $<\sim$12 kDa) are freely filtered through the pores in the glomerular membrane, whereas molecules larger than 4.2 nm (weighing >70 kDa) are not filtered or are filtered to only a very limited extent. Small molecules that are bound to larger transport proteins such as albumin will not pass the glomerular filter. Evidence indicates that cationic and neutral molecules are filtered more efficiently than anionic molecules (*6*), although this concept of charge selectivity has been challenged in recent years (*7*). Most radiolabeled peptides are not bound to proteins and weigh less than 12 kDa; consequently, they are cleared efficiently via glomerular filtration.

Proximal Tubular Reabsorption and Processing

In the proximal tubule, approximately 50%−60% of filtered water and solutes is reabsorbed by active and passive transport processes. The main driving force for these processes is the Na^+-K^+-adenosine triphosphatase in the basolateral membrane, which pumps Na^+ out of the cell and K^+ into the cell. The ensuing concentration gradient stimulates reentry of Na^+ through various symporters and antiporters, providing energy for the cotransport of other molecules among which most are amino acids (*6*).

Multiple transport mechanisms are involved in the proximal tubular reabsorption of proteins and peptides. Some oligopeptides are hydrolyzed at the brush border of proximal tubular cells, after which the resulting amino acids and di- or tripeptides are transported into the cells by amino acid transporters and the oligopeptide transporters, respectively (*6*). Larger peptides and proteins are internalized via endocytosis. Most peptides are almost quantitatively reabsorbed in the proximal tubules.

Receptor-Mediated Endocytosis. Endocytosis is triggered by the binding of ligands to endocytic receptors, providing a positive feedback mechanism that accelerates endocytosis in response to an increase in the amount of filtered proteins. After binding of ligands to endocytic receptors, the plasma membrane invaginates, forming clathrin-coated pits and early endosomes. Early endosomes fuse with late endosomes, in which the acidic pH causes dissociation of ligands from receptors. The receptors concentrate on 1 side of the endosomal membrane, which buds off and recycles to the cell membrane. The late endosomes fuse with lysosomes, in which the ligands are catabolized by proteolytic enzymes. The cytoskeleton plays an important role in the intracellular trafficking of these vesicles (*6*).

Megalin and cubilin, large endocytic receptors, play an important role in the proximal tubular reabsorption of various structurally different proteins, peptides, drugs and other molecules, including albumin, apolipoproteins, hemoglobin, gentamycin, and many others (*8*). Megalin and cubilin are expressed exclusively on the apical membrane of proximal tubular cells (*8*). The cubilin receptor does not possess an intracellular signaling domain, and evidence suggests that expression and endocytosis of the cubilin receptor are dependent on interactions with megalin and amnionless, another transmembrane protein (*9,10*). Several ligands have been reported to bind both to megalin and to cubilin (*8*). In vitro studies have indicated that radiolabeled somatostatin analogs are actively reabsorbed in proximal tubular cells by receptor-mediated endocytosis, probably involving megalin or cubilin (*11*). Autoradiography studies of rodent kidneys showed that various radiolabeled peptides accumulate mainly in the renal cortex (*12*). De Jong et al. reported that the renal uptake of ^{111}In-octreotide was significantly decreased in kidney-specific megalin-deficient mice (*13*), and our group recently found that renal uptake of ^{111}In-labeled octreotate, minigastrin, exendin, and neurotensin was also decreased by 40%−70% in these megalin-deficient mice (*14*), implying that megalin or megalin-dependent receptors such as cubilin are indeed involved in the proximal tubular reabsorption of these radiolabeled peptides.

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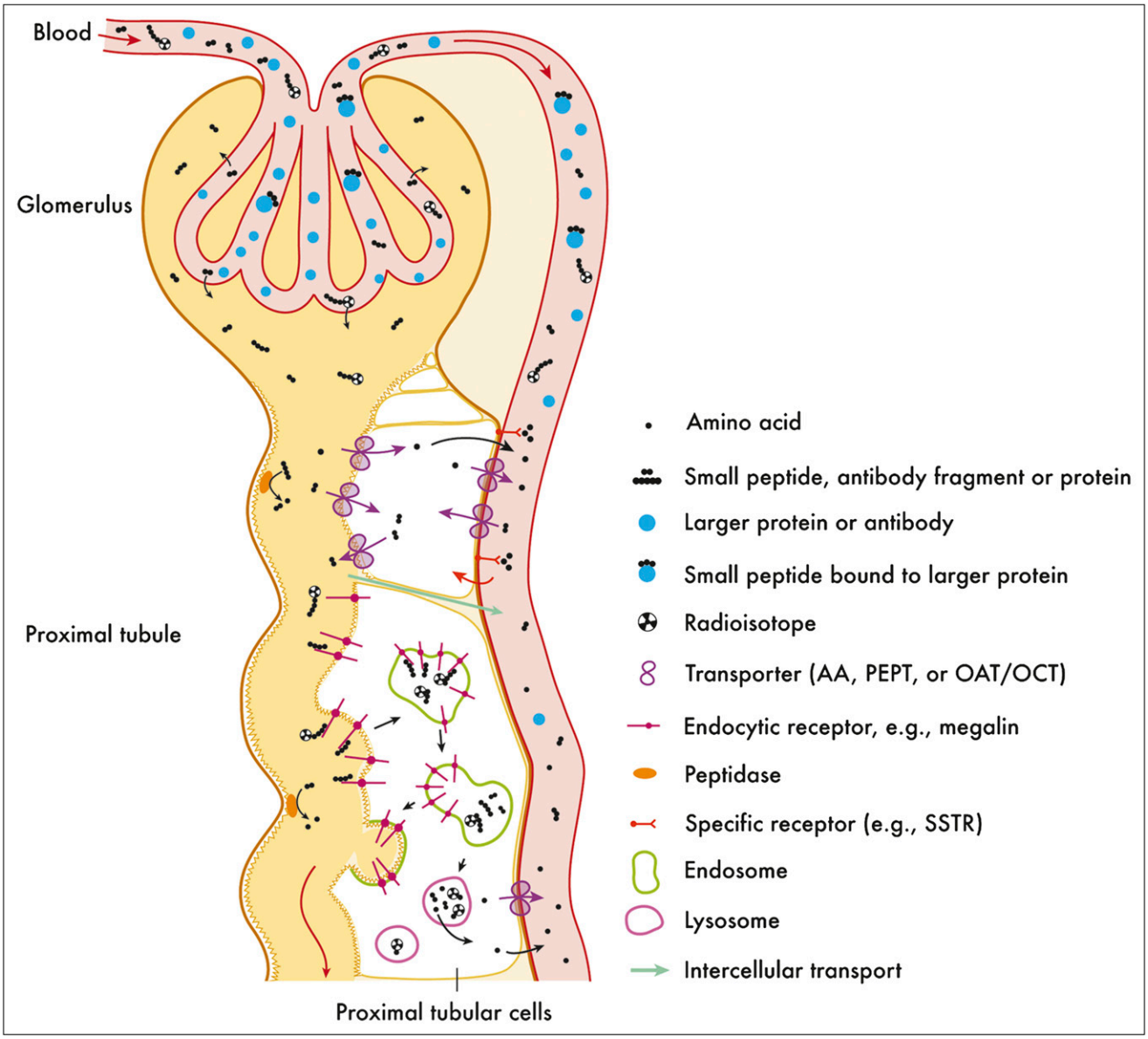


FIGURE 1. Overview of glomerular and proximal tubular handling of proteins and peptides. AA = amino acid transporter; OCT = organic cation transporter; PEPT = oligopeptide transporter.

Cationic peptides are generally reabsorbed more efficiently by the proximal tubules than are neutral and anionic peptides. This difference is thought to be caused by preferential binding of cationic groups to anionic binding sites on the brush border membrane. However, the highly anionic peptide minigastrin also expresses high renal uptake (*3*), and megalin ligands can be cationic or anionic. This indicates that other ligand properties, such as structure and charge distribution, also determine reabsorption.

Role of Ligand-Specific Receptors. Particular peptides may also be taken up by ligand-specific receptors, such as somatostatin receptors (*15*). Abundant expression of all somatostatin receptor subtypes has been described in the human and murine kidney (*16,17*), and subtypes 3 and 4 were found in the rat kidney (*18*). Rolleman et al. retrospectively studied ^{111}In-octreotide scans of patients who were scanned before and during treatment with unlabeled octreotide. A mean 18% reduction in kidney uptake during octreotide treatment was found, suggesting some involvement of somatostatin receptors in the renal uptake of ^{111}In-octreotide (*19*). However, an excess of unlabeled octreotide may also interfere with binding of ^{111}In-octreotide to other receptors such as megalin. Expression of cholecystokinin 2 and glucagonlike peptide 1 receptors on proximal tubular cells was described (*20,21*), and many other ligand-specific receptors may be expressed in the kidneys. The importance of these receptors for the renal uptake of radiolabeled peptides remains to be determined.

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Other Pathways for Reabsorption. Fluid-phase endocytosis (pinocytosis) is likely to play a role in the proximal tubular reabsorption of proteins and peptides. However, the capacity of this pathway is reported to be much lower than the capacity of receptor-mediated endocytosis (*22*). For hydrophobic molecules, passive diffusion across the luminal membrane may play an additional role.

Part of the transport of water and electrolytes occurs via the intercellular pathway, through pores in the tight junctions between cells and through the interstitium. It is unknown whether small peptides may be reabsorbed through this route. Recently, a transcellular pathway for reabsorption of albumin has been proposed, consisting of large endocytic vesicles that are transported rapidly from the apical to the basolateral membrane of proximal tubular cells (*7*). This route may also play a role in the reabsorption of radiolabeled peptides, but its existence remains controversial (*23*). In any case, peptides and proteins transported via these routes are thought to be returned to the circulation intact and are not retained in the kidneys. Therefore, these pathways are unlikely to contribute to nephrotoxicity significantly.

Peritubular Absorption and Tubular Secretion. Many endogenous and exogenous substances, such as hormones and drugs, are actively secreted from the blood into the lumen of the proximal tubules by the processes of peritubular absorption and tubular secretion. Several transporters involved in these energy-requiring processes have been identified, such as the organic anion transporters (OATs) and organic cation transporter families, oligopeptide transporters, and the multidrug resistance proteins (*6*). Different transporters are expressed on the basolateral and on the apical membranes of the proximal tubular cells, and some are also involved in the reabsorption of substances from the tubular lumen. The substrates transported by these pathways are relatively small. The tripeptide ^{99m}Tc-mercaptoacetyltriglycine is excreted mainly by tubular secretion, and Trejtnar et al. reported that three ^{99m}Tc-labeled tetrapeptides were secreted in the proximal tubules (*24*). A study suggests that OATs may be involved in the tubular uptake of ^{111}In-octreotide, either from the lumen or from the plasma (*25*).

Intracellular Catabolism and Retention. Peptides and proteins that undergo endocytosis in the proximal tubules are subsequently catabolized into amino acids in lysosomes. These amino acids are transported or diffuse out of the lysosomes into the cytoplasm and return to the circulation. Monoiodotyrosine, the primary radiolabeled catabolite from iodinated antibodies, is indeed released quickly from cells (*26*). Other radiolabeled catabolites, such as lysine–chelate conjugates, N-terminal amino acid chelate conjugates (*27*), and specifically engineered iodinated products (*28*), cannot cross the lysosomal membrane. These products, which are trapped in the lysosomes, are referred to as residualizing radiolabels. Both for scintigraphy and for PRRT, the entrapment of radiolabels is a desirable effect in target cells, enhancing the tumor-to-background ratio. However, this entrapment also contributes to the relatively high retention of radioactivity in the kidneys.

Oligopeptides that are taken up by transporters such as OATs or organic cation transporters can leave the cell via similar transporters or by diffusion or can be processed further in the cytosol or in lysosomes (*6*). The contribution of these transporters to the renal retention of radiolabeled peptides remains to be investigated.

KIDNEY TOXICITY

The bone marrow is the dose-limiting organ in most types of radionuclide therapy, since its rapidly dividing cells are sensitive to radiation. Acute myelodepression can occur in the first days to weeks after treatment and can be managed by supportive measures or by bone marrow transplantation in the case of myeloablative therapy. Up to years after therapy, late myelotoxicity such as myelodysplastic syndrome and leukemia can develop, which may also be treated with bone marrow transplantation (*29–31*). However, the kidneys are sensitive to radiation as well, and given the relatively high renal retention of many radiolabeled peptides, the kidneys are often the dose-limiting organs in PRRT (*5,32,33*).

Nephropathy After External-Beam Radiation Therapy (EBRT)

Radiation nephropathy caused by EBRT has been described extensively (*34,35*). The only signs of radiation nephropathy may be solitary hypertension or asymptomatic proteinuria. Acute radiation nephropathy is defined as signs of kidney failure (proteinuria, hypertension, heart failure, azotemia, and anemia) developing within 6–12 mo after irradiation. Pathologic kidney changes include atrophy, tubulointerstitial scarring, mesangiolysis, and, in severe cases, thrombotic microangiopathy. Acute radiation nephropathy can be reversible or progress to chronic nephropathy. Chronic radiation nephropathy can also develop up to many years after irradiation and is characterized by loss of kidney mass and function (*36*). In animal models, early kidney damage developing in the first days to weeks after irradiation has been described, including damage to glomeruli, tubuli, and vasculature (*35*). The initial radiation-induced damage is DNA damage, which is caused mostly indirectly through the formation of reactive oxygen species and can lead to cell death. Subsequent tissue reactions to this damage, mediated in part by the renin-angiotensin-aldosterone system, are involved in the development of chronic damage (*36*).

Nephropathy After Radionuclide Therapy

Data about nephrotoxicity resulting from radionuclide therapy are limited. In mice, acute radiation nephritis with uremia and pronounced tubular damage developing in the first weeks after administration of ^{90}Y-labeled anti–carcinoembryonic antigen Fab fragments (9–15 MBq) and ^{225}Ac-labeled antibodies (13 kBq) has been described

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(*37,38*). Renal damage in rats was described 3–9 mo after injection of 278–555 MBq of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate (^{177}Lu-DOTATATE) (*39,40*). In patients, acute radiation nephropathy developing 0.5–6 mo after treatment with more than 7.4 GBq of [^{90}Y-DOTA^{0}-Tyr^{3}]octreotide (^{90}Y-DOTATOC) per square meter has been described (*1,41*), as well as chronic radiation nephropathy after PRRT with ^{90}Y-labeled somatostatin or gastrin analogs (*32,42*).

In EBRT, kidney dose limits of 15–17 Gy in 2-Gy fractions are considered safe (*43*). Doses of 23 Gy cause chronic kidney failure in approximately 5% of patients within 5 y (*44*). However, the reaction of tissue to radiation does not depend only on the total absorbed dose but also on dose rate, fractionation, microdistribution of dose within the organ, and radiation type (α-, β-, γ-, or x-radiation) and energy (*29,33,43*). Typically, in PRRT, dose rates are much lower than in EBRT, distribution within the organ is more inhomogeneous, and the radiation has a shorter range of penetration. Therefore, maximum tolerable dose limits for PRRT may differ significantly from those for EBRT. Use of the biological effective dose, which takes the effects of dose fractionation and sensitivity of the organ to radiation into account, may be more appropriate for setting dose limits than is the absorbed dose. Barone et al. described a group of 18 patients treated with ^{90}Y-DOTATOC and found that a kidney biological effective dose of more than 45 Gy increased the risk of rapid deterioration of renal function after PRRT (*45*). Bodei et al. studied dosimetry in 28 patients receiving PRRT with ^{90}Y-DOTATOC or ^{177}Lu-DOTATATE and found that a biological effective dose of less than 40 Gy was safe in patients without preexisting risk factors for renal disease, such as hypertension, diabetes, or old age (n = 16), and that a lower biological effective dose threshold of less than 28 Gy was safe for patients who did have these risk factors (n = 12) (*42*).

METHODS TO REDUCE NEPHROTOXICITY

Several different approaches to reduce the risk of nephrotoxicity in PRRT have been studied. Most of these methods aim at decreasing the radiation dose to the kidneys, such as by reducing the proximal tubular reabsorption of peptides. Other methods focus on reducing the harmful effects of the radiation. An overview of these methods is presented in Table 1.

Reduction of Renal Uptake

Structural Modification of Radiolabeled Peptides. Various attempts have been made to change the elimination characteristics of radiolabeled peptides in order to reduce their renal retention. Structural change of a peptide may result in reduced glomerular filtration, such as by increasing size or protein binding, or in reduced tubular reabsorption, such as by reducing the affinity of the peptide for renal transporters. Obviously, affinity of the radiolabeled peptides for their specific receptors, which is important for accumulation in target tissues, should remain intact.

Several studies have shown that the introduction or substitution of positively or negatively charged groups can influence the renal uptake of peptides and antibody fragments. Ekblad et al. studied renal uptake of ^{99m}Tc-labeled

TABLE 1. Processes and Factors in Renal Handling and Retention of Radiolabeled Peptides and Strategies to Interfere

Site	Process	Methods to reduce nephrotoxicity	Examples
Glomerulus	Ultrafiltration	Increase binding to albumin or other proteins	Albumin binding sequence
		Increase size	PEGylation
Proximal tubule	Degradation by brush border enzymes	Insert cleavable linker	Glycyl-lysine linker
	Reabsorption by endocytosis or transporters	Saturate with competitive inhibitors	Coinfusion of lysine/arginine/ succinylated gelatin
		Decrease affinity by modification of structure or charge of the peptide	Substitution, addition or removal of charged groups; PEGylation
		Decrease endocytosis (i.e., by reduction of energy supply or interference with formation or trafficking of endosomes)	Maleate, colchicine
	Degradation and retention in lysosomes	Use nonresidualizing radiolabels	Conventionally iodinated peptides
	Peritubular absorption	Block transporters	Probenecid
Whole kidney	Radiation damage and repair	Use short-range radionuclides to limit radiation dose to the glomeruli	^{177}Lu instead of ^{90}Y
		Scavenging of free radicals using radioprotectors	Amifostine
		Increase time to repair sublethal damage	Dose fractionation
		Mitigate damaging processes	Spironolactone, L-158,809

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HER2 Affibody molecules ($Z_{HER2:342}$) with different chelators. They reported that the substitution of a negatively charged glutamic acid residue by a neutral serine residue in the chelator sequence mercaptoacetyl-Glu-Glu-Glu significantly reduced renal uptake without reducing tumor uptake (*46*). Miao et al. found that the renal uptake of ^{90}Y- and ^{177}Lu-labeled α-melanocyte–stimulating hormone analogs was decreased by adding a glutamic acid residue to the peptide. Although the uptake in melanoma was significantly decreased as well, an improved tumor-to-kidney uptake ratio was achieved (*47*). Removal of the anionic N-terminal pentaglutamic acid sequence of minigastrin analogs, or replacement by a histidine-histidine-glutamic acid sequence, resulted in a marked decrease in kidney uptake. Again this was accompanied by a decrease in tumor uptake but an increased tumor-to-kidney ratio (*48*). Replacing minigastrin's pentaglutamic acid sequence by a neutral pentaglutamine sequence reduced uptake in tumors and organs positive for the cholecystokinin 2 receptor more than it reduced renal uptake (*3*). ^{99m}Tc-labeled bombesin analogs were shown to express higher renal uptake when 2 glutamic acid residues were introduced, compared with 1 (*49*).

Akizawa et al. studied the renal uptake of ^{111}In-diethylenetriaminepentaacetic acid (DTPA)-L-Phe^1-octreotide analogs in which the neutral phenylalanine residue was substituted by a neutral methionine, a positively charged lysine, or a negatively charged aspartic acid residue. They observed that the renal uptake of ^{111}In-DTPA-L-Lys^1-octreotide was significantly higher than that of ^{111}In-DTPA-L-Phe^1-octreotide, whereas the renal uptake of ^{111}In-DTPA-L-Asp^1-octreotide was lower. However, the effect of these substitutions on the uptake in somatostatin receptor–positive organs was not reported (*50*). Dimeric cyclic RGD peptides also showed increased renal accumulation when a lysine residue was introduced in the spacer linking both RGD moieties (*51*).

Apart from the substitution, addition, or deletion of amino acids, other molecules can also be coupled to peptides to alter their pharmacokinetics. Examples include poly(ethylene glycol) (PEG) groups or albumin binding sequences. Addition of PEG groups (which can range in molecular weight from ~2 to >200 kDa) is referred to as PEGylation (*52,53*). PEGylation can alter pharmacokinetics by increasing the size of the molecule, increasing hydrophilicity, masking charges, and decreasing antigenicity. Insertion of 2- to 4-kDa PEG groups was reported to reduce the renal uptake and increase the tumor-to-kidney ratio of ^{125}I- and ^{18}F-labeled RGD peptides (*52,54*). Addition of larger PEG groups or albumin binding sequences can reduce glomerular filtration and increase the circulatory half-life of peptides (*53,55*). Coupling of albumin binding sequences to radiolabeled Fab fragments and Affibody molecules resulted in an increased circulatory half-life, a reduced kidney uptake, and good tumor targeting, compared with the native constructs (*55,56*). However, an increased circulatory half-life also increases the bone marrow radiation dose.

Use of Different Radiolabels, Linkers, or Chelators. As described above, after endocytosis and catabolism in the proximal tubules, iodinated tyrosine can diffuse freely out of the lysosomes and the tubular cells, leading to relatively low renal retention of conventionally radioiodinated products. However, many peptides and antibodies are also internalized in the target organs or tumors, from which iodine is also rapidly cleared. This leads to low target-to-background ratios and renders conventionally ^{131}I-labeled products less useful for labeling of internalizing antibodies or peptides (*57*).

Radiometal-chelated products are retained longer in targeted tissue but also in the kidneys. A strategy to reduce the kidney retention of radiolabeled peptides involves the insertion of metabolizable linkers between the radiometal–chelator complex and the peptide. These linkers must be stable in plasma. Specific linkers were designed to be cleaved in the kidneys by hydrolases at the proximal tubular brush border membrane, releasing the radiometal–chelator complex from the peptide before it undergoes endocytosis. The radiometal will then be excreted in the urine. Uehara et al. used a glycyl-lysine bond which can be cleaved by renal brush border enzymes. A radiolabeled construct of Fab fragments with these linkers, ^{188}Re-tricarbonyl-(cyclopentadienyl)-glycyl-lysine-Fab, expressed significantly lower kidney uptake than the native construct. Tumor uptake was not affected (*58*).

Other linkers that have been studied are cleaved by lysosomal enzymes, releasing the radiometal–chelator complex from the cells after endocytosis. Tibben et al. found that a cleavable ester bond between a chelator and a Fab′ fragment reduced the kidney uptake of Fab′ fragments without affecting tumor uptake (*59*). Smith-Jones et al. studied the biodistribution of ^{111}In-DTPA–labeled somatostatin analogs with several ester and diester linkers. None of the metabolizable linkers led to higher tumor-to-kidney ratios, because tumor uptake decreased more than kidney uptake (*60*).

Competitive Inhibition of Proximal Tubular Reabsorption. The interaction of peptides with receptors or transporters on the apical membrane of the proximal tubular cells may be disturbed by other ligands of these transporters. An excess of ligands in the ultrafiltrate during the elimination phase of the radiolabeled peptides causes competitive inhibition of the renal transport mechanisms, leading to a reduction in the uptake of the radiolabeled peptides. Again, the used substances should not interfere with the specific binding of the peptides to their native receptors, because this would also decrease uptake in target lesions.

The inhibition of tubular reabsorption of proteins by positively charged (basic) amino was first described in the 1970s. Several years later, coadministration of the basic amino acids lysine and arginine was found to reduce the renal uptake of radiolabeled antibody fragments and somatostatin analogs (*61,62*). Behr et al. reported that poly-L-

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lysine was more potent than L-lysine or D-lysine in reducing the renal retention of radiolabeled Fab′ fragments in rats, but poly-L-lysine was also more toxic. No difference between L-lysine and D-lysine was found (*63*). Rolleman et al. reported a significant decrease in parameters of kidney damage after coinfusion of lysine in rats receiving ^{177}Lu-DOTATATE therapy (*40*). The same group studied the effects and side effects of coinfusion of an amino acid mixture, L-lysine, or a combination of L-lysine and L-arginine during PRRT with [^{111}In-DTPA^{0}]octreotide in patients. The combination of 25 g of lysine and 25 g of arginine was more effective than 50 g of lysine and caused relatively few side effects. The highest dose—75 g of lysine—was most effective in reducing renal uptake but frequently caused side effects, such as vomiting (*64*). Therefore, further increasing the lysine dose to improve kidney protection is not recommended. For somatostatin analogs, a maximum reduction of the kidney radiation dose of approximately 50% has been achieved (*64,65*). Coinfusion of basic amino acids is currently the standard renoprotective regimen in clinical PRRT.

The renal uptake of minigastrin, a peptide that lacks positively charged amino acid residues, is not reduced by coinfusion of lysine (*4,66*). Béhé et al. found that negatively charged polyglutamic acid chains of at least 5 glutamic acid residues did reduce renal uptake of minigastrin significantly (*66*).

Our group found that the plasma expander succinylated gelatin (Gelofusine; Braun) efficiently reduced the renal reabsorption of ^{111}In-octreotide in humans (*67*) and of ^{111}In-labeled octreotide, minigastrin, and exendin in rats (*68,69*). Succinylated gelatin consists of peptides with an average molecular weight of 30 kDa and a net negative charge. The group of Baum et al. used succinylated gelatin combined with the above-mentioned lysine/arginine mixture for kidney protection in 902 courses of PRRT with ^{90}Y-DOTATOC (mean activity, 14 ± 4.8 GBq in 4 cycles) or ^{177}Lu-DOTATATE (mean activity, 28 ± 6 GBq in 4 cycles). Since this regimen has been in use, no serious kidney toxicity has occurred. Mild to moderate allergic side effects (common toxicity criteria grade 1 or 2) of the renoprotective regimen were recorded in 1.4% of courses, and grade 3 reactions occurred in 0.6% (Richard P. Baum, written communication, November 2009). Extensive experience with the administration of succinylated gelatin to patients in acute care or during surgical procedures exists, and serious side effects are rare. Severe anaphylactoid reactions are described in approximately 0.04% of patients (*70*).

We recently described that coadministration of fractionated albumin or an albumin-derived peptide efficiently reduced renal uptake of radiolabeled peptides in rats. Five milligrams of the albumin-derived peptide was equally effective as 20 mg succinylated gelatin in reducing renal uptake of ^{111}In-octreotide, ^{111}In-minigastrin, and ^{111}In-exendin (*71*). This peptide consists of the 36 C-terminal amino acid residues of albumin and contains 3 positively and 6 negatively charged residues.

The wide variety of substances able to reduce renal reabsorption of radiolabeled peptides may well reflect the nonselective nature of renal transport systems such as the megalin receptor. The selectivity of certain competitive inhibitors, such as lysine for octreotide and polyglutamic acid for minigastrin, may reflect their binding to different sites on megalin, or the involvement of other receptors or transporters.

Alternative Ways to Interfere with Proximal Tubular Reabsorption. Apart from competitive inhibition, the process of endocytosis may be disturbed at various other stages. Albumin endocytosis was reported to be decreased by extracellular hypertonicity, which interferes with the formation of clathrin lattice, thus inhibiting the formation of clathrin-coated pits (*72*). Gekle et al. also reported that alkalinization of endosomes by the addition of NH_4Cl or bafilomycin A_1 to opossum kidney cells in vitro reduced the receptor-mediated endocytosis of albumin but did not affect fluid-phase endocytosis of dextran (*73*). The same group described reduced endocytosis of albumin by cytochalasin D, which disrupts the actin cytoskeleton, and by nocodazole, which interferes with microtubule formation (*72*). However, toxicity of these agents may preclude their clinical use. Disruption of microtubule formation by pretreatment of rats with colchicine also significantly reduced renal uptake of ^{111}In-octreotide (*74*).

Because the process of endocytosis requires energy, disrupting the energy supply also reduces endocytosis. De Jong et al. showed that maleate, an inhibitor of the citric acid cycle, significantly reduced renal uptake of ^{111}In-octreotide in rats. However, because of the reported renal toxicity of maleate, its use in PRRT was not studied further. Acidification of urine by administration of NH_4Cl significantly reduced renal uptake of ^{111}In-octreotide as well, possibly through neutralization of negatively charged membrane binding sites (*65*).

Stahl et al. reported that probenecid, an inhibitor of OATs and multidrug resistance proteins, reduced renal uptake of ^{111}In-octreotide in rats by 30%. Octreotide is an organic cation, but OATs have been shown to transport neutral and weak cations as well as anions (*25*).

The influence of increasing urinary flow on the excretion of radiolabeled peptides has been studied in rats as well, either by increasing fluid intake or by administering furosemide. However, increasing fluid intake had no effect on renal uptake of ^{111}In-octreotide (*65*), and furosemide even caused a 44% increase in uptake (*25*).

Reduction of Nephrotoxicity at a Given Radiation Dose

Other approaches to preventing nephrotoxicity aim at reducing the damage induced by a given absorbed radiation dose.

Use of Radioprotectors. Radioprotectors reduce oxidative stress by scavenging free radicals. Because the formation of free radicals depends on the presence of oxygen, healthy, well-oxygenated tissues are protected to a larger extent than tumors, which are usually hypoxic. Further-

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more, amifostine, the prototype radioprotector, is converted to its active metabolite by alkaline phosphatase. In healthy tissue, including the renal brush border membrane, this enzyme is expressed at much higher levels than in tumor capillaries, leading to 10- to 100-fold higher concentrations of the active metabolite in healthy tissue than in tumor cells (*75*). Amifostine is approved for use in EBRT and has been studied in PRRT. Rolleman et al. coadministered this radioprotector to rats during administration of ^{177}Lu-DOTATATE and for 7 d after PRRT. They found that amifostine was equally as effective as lysine in preventing nephrotoxicity (*76*).

Use of Mitigating Agents. Mitigating agents aim at reducing the risk of nephrotoxicity by inhibiting counterproductive tissue reactions to radiation-induced damage (*77*). From research in EBRT it is known that inhibition of the renin-angiotensin-aldosterone system after irradiation of the kidneys can prevent nephropathy (*78*). Activation of the renin-angiotensin-aldosterone system can be inhibited by angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, or aldosterone receptor blockers. Jaggi et al. studied the effects of renin-angiotensin-aldosterone system inhibition in mice that were injected with α-emitting ^{225}Ac-labeled untargeted antibodies. Starting 10 d after injection, the mice were treated for 39 wk with spironolactone (an aldosterone receptor antagonist), captopril (an angiotensin-converting-enzyme inhibitor), L-158,809 (an angiotensin II receptor 1 blocker), or placebo. Animals receiving placebo developed significant kidney damage (tubulolysis, thickening of basement membranes, and elevated blood urea nitrogen), which was efficiently reduced by administration of spironolactone and to a lesser extent by L-158,809, but not by captopril (*77*).

Dose Fractionation. Dose fractionation is used in EBRT to use the potential of normal tissue to repair sublethal damage between irradiation cycles. In radionuclide therapy, dose fractionation is difficult because of the relatively low dose rates, long residence times, and long half-lives of the radionuclides. In PRRT, comparisons between different dose fractionation schemes are scarce. Barone et al. analyzed 18 patients who were treated with ^{90}Y-DOTATOC and found that patients receiving large radiation doses with little fractionation had the highest risk of developing nephrotoxicity (*45*). Rolleman et al. studied fractionation of ^{177}Lu-DOTATATE therapy in rats. Dividing the total dose over 2 fractions significantly reduced kidney damage, and 3 fractions caused significantly less kidney damage when given at weekly or monthly intervals than when given at daily intervals. No significant differences in tumor response were found between these regimens (*40*).

Influence of Radionuclide Characteristics. Renal toxicity appears to occur less frequently after therapy with ^{177}Lu-DOTATATE than after therapy with ^{90}Y-DOTATOC (*42*). Of 504 patients receiving up to 30 GBq of ^{177}Lu-DOTATATE (predicted kidney dose, <23 Gy), only 2 (0.4%) developed nephrotoxicity, which was probably unrelated to PRRT (*31*). In another series of 60 patients receiving up to 15 GBq of ^{90}Y-DOTATOC (again, predicted kidney dose was <23 Gy), 7 (12%) developed kidney toxicity (*5*). The difference may be due to the lower β-energy and the consequently lower range of the β-emission of ^{177}Lu, which results in a relatively lower radiation dose to the radiosensitive glomeruli.

Combination Strategies

Several groups have studied combinations of renoprotective strategies for PRRT in rats. Combining lysine and succinylated gelatin showed additive reduction of the kidney uptake of ^{111}In-DTPA-octreotide and ^{111}In-DOTA-octreotate, whereas tumor uptake was not affected (*4,79*). Kidney uptake of ^{111}In-exendin was reduced most potently by a combination of polyglutamic acid and succinylated gelatin (*4*). Combining lysine with colchicine also additively reduced the renal uptake of ^{111}In-DTPA-octreotide (*74*). All above-mentioned strategies used combinations of agents that interfere with proximal tubular endocytosis. Combinations of these with other strategies, such as radiation protection or mitigation, are likely to further reduce the risk of nephropathy.

CONCLUSION AND FUTURE DIRECTIONS

High kidney uptake of radiolabeled peptides can cause dose-limiting nephrotoxicity after PRRT. Reduction of this uptake can enable the administration of higher activities, which improves the effectiveness of therapy. Although administration of higher activities also increases the risk of bone marrow toxicity, the high radiation dose to the kidneys remains a problem in current somatostatin analog PRRT with renoprotective regimens, especially when ^{90}Y-labeled analogs are used. For other peptides such as minigastrin and exendin, which express significantly higher kidney uptake than somatostatin analogs, efficient reduction of kidney uptake will be even more important to enable safe PRRT. Evidence indicates that the most important pathway for the renal uptake of most radiolabeled peptides (including antibody fragments) consists of glomerular filtration followed by receptor-mediated endocytosis and retention in the proximal tubular cells. Many strategies have been designed to improve the target-to-kidney ratio of radiolabeled peptides, several of which show promising results. Subtle changes in the amino acid sequence of a peptide, thereby changing its structure or charge distribution, can affect renal uptake considerably. Several peptide analogs have been designed that exhibit lower renal retention with unaltered target uptake. Another approach focuses on enhancing the clearance of radiolabeled products from the kidneys. Linkers that are selectively cleaved in the kidneys may prevent the accumulation of radiolabels in the kidneys without loss of retention in targeted tissues. In clinical PRRT, an established method to reduce the absorbed kidney dose is the coinfusion of competitive inhibitors of proximal tubular reabsorption. These have been shown to significantly reduce

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the renal uptake of various peptides. However, a baseline renal uptake of radiolabeled somatostatin analogs seems to persist, possibly reflecting nonsaturatable fluid phase endocytosis. Different methods to interfere with endocytosis, such as disruption of microtubule formation, may also reduce fluid-phase endocytosis. To prevent nephrotoxicity at a given radiation dose, dose fractionation and treatment with radioprotectors or mitigating agents have shown promising results.

Because most of these strategies interfere with different mechanisms of kidney uptake and damage, the combination of 2 or more strategies is likely to reduce kidney damage progressively. Combining 2 agents, both of which reduce endocytosis, has already been shown to further reduce the kidney uptake of various radiolabeled peptides.

Most strategies for the reduction of kidney damage in PRRT and RIT have, as yet, not been tested on patients, and more research is needed to study their potential for clinical application. If we can further reduce the risk of renal damage caused by radiolabeled peptides, higher-activity doses can be administered safely, and highly effective targeted tumor therapy comes within reach.

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REFERENCES

1. Otte A, Herrmann R, Heppeler A, et al. Yttrium-90 DOTATOC: first clinical results. *Eur J Nucl Med.* 1999;26:1439–1447.
2. Van Essen M, Krenning EP, Kam BL, De Jong M, Valkema R, Kwekkeboom DJ. Peptide-receptor radionuclide therapy for endocrine tumors. *Nat Rev Endocrinol.* 2009;5:382–393.
3. Behr TM, Behe M, Angerstein C, et al. Cholecystokinin-B/gastrin receptor binding peptides: preclinical development and evaluation of their diagnostic and therapeutic potential. *Clin Cancer Res.* 1999;5:3124s–3138s.
4. Gotthardt M, Van Eerd-Vismale J, Oyen WJ, et al. Indication for different mechanisms of kidney uptake of radiolabeled peptides. *J Nucl Med.* 2007;48:596–601.
5. Cwikla JB, Sankowski A, Seklecka N, et al. Efficacy of radionuclide treatment DOTATATE Y-90 in patients with progressive metastatic gastroenteropancreatic neuroendocrine carcinomas (GEP-NETs): a phase II study. *Ann Oncol.* 2010;21:787–794.
6. Brenner BM. *Brenner & Rector's The Kidney.* Philadelphia, PA: Saunders Elsevier; 2008:sections I.2–I.5, I.7.
7. Russo LM, Sandoval RM, McKee M, et al. The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells: retrieval is disrupted in nephrotic states. *Kidney Int.* 2007;71:504–513.
8. Christensen EI, Verroust PJ. Megalin and cubilin, role in proximal tubule function and during development. *Pediatr Nephrol.* 2002;17:993–999.
9. Ahuja R, Yammani R, Bauer JA, Kalra S, Seetharam S, Seetharam B. Interactions of cubilin with megalin and the product of the amnionless gene (AMN): effect on its stability. *Biochem J.* 2008;410:301–308.
10. Coudroy G, Gburek J, Kozyraki R, et al. Contribution of cubilin and amnionless to processing and membrane targeting of cubilin-amnionless complex. *J Am Soc Nephrol.* 2005;16:2330–2337.
11. Trejtnar F, Novy Z, Petrik M, et al. In vitro comparison of renal handling and uptake of two somatostatin receptor-specific peptides labeled with indium-111. *Ann Nucl Med.* 2008;22:859–867.
12. Melis M, Krenning EP, Bernard BF, De Visser M, Rolleman E, De Jong M. Renal uptake and retention of radiolabeled somatostatin, bombesin, neurotensin, minigastrin and CCK analogues: species and gender differences. *Nucl Med Biol.* 2007;34:633–641.
13. De Jong M, Barone R, Krenning E, et al. Megalin is essential for renal proximal tubule reabsorption of ^{111}In-DTPA-octreotide. *J Nucl Med.* 2005;46:1696–1700.
14. Vegt E, Melis M, Eek A, et al. Megalin plays an important role in the renal uptake of various radiolabelled peptides: studies in megalin-deficient mice [abstract]. *Eur J Nucl Med Mol Imaging.* 2009;36(suppl 2):231.
15. Waser B, Tamma ML, Cescato R, Maecke HR, Reubi JC. Highly efficient in vivo agonist-induced internalization of sst2 receptors in somatostatin target tissues. *J Nucl Med.* 2009;50:936–941.
16. Bates CM, Kegg H, Petrevski C, Grady S. Expression of somatostatin receptors 3, 4, and 5 in mouse kidney proximal tubules. *Kidney Int.* 2003;63:53–63.
17. Bhandari S, Watson N, Long E, et al. Expression of somatostatin and somatostatin receptor subtypes 1-5 in human normal and diseased kidney. *J Histochem Cytochem.* 2008;56:733–743.
18. Bruno JF, Xu Y, Song J, Berelowitz M. Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. *Endocrinology.* 1993;133:2561–2567.
19. Rolleman EJ, Kooij PP, De Herder WW, Valkema R, Krenning EP, De Jong M. Somatostatin receptor subtype 2-mediated uptake of radiolabelled somatostatin analogues in the human kidney. *Eur J Nucl Med Mol Imaging.* 2007;34:1854–1860.
20. de Weerth A, Jonas L, Schade R, et al. Gastrin/cholecystokinin type B receptors in the kidney: molecular, pharmacological, functional characterization, and localization. *Eur J Clin Invest.* 1998;28:592–601.
21. Schlatter P, Beglinger C, Drewe J, Gutmann H. Glucagon-like peptide 1 receptor expression in primary porcine proximal tubular cells. *Regul Pept.* 2007;141:120–128.
22. Schwegler JS, Heppelmann B, Mildenberger S, Silbernagl S. Receptor-mediated endocytosis of albumin in cultured opossum kidney cells: a model for proximal tubular protein reabsorption. *Pflugers Arch.* 1991;418:383–392.
23. Christensen EI, Birn H, Rippe B, Maunsbach AB. Controversies in nephrology: renal albumin handling, facts, and artifacts! *Kidney Int.* 2007;72:1192–1194.
24. Trejtnar F, Laznicek M, Laznickova A, Mather SJ. Pharmacokinetics and renal handling of ^{99m}Tc-labeled peptides. *J Nucl Med.* 2000;41:177–182.
25. Stahl AR, Wagner B, Poethko T, et al. Renal accumulation of [^{111}In]DOTATOC in rats: influence of inhibitors of the organic ion transport and diuretics. *Eur J Nucl Med Mol Imaging.* 2007;34:2129–2134.
26. Press OW, Shan D, Howell-Clark J, et al. Comparative metabolism and retention of iodine-125, yttrium-90, and indium-111 radioimmunoconjugates by cancer cells. *Cancer Res.* 1996;56:2123–2129.
27. Duncan JR, Behr TM, DeNardo SJ. Intracellular fate of radiometals [letter]. *J Nucl Med.* 1997;38:829.
28. Shankar S, Vaidyanathan G, Kuan CT, Bigner DD, Zalutsky MR. Anti-epidermal growth factor variant III scFv fragment: effect of radioiodination method on tumor targeting and normal tissue clearance. *Nucl Med Biol.* 2006;33:101–110.
29. Behr TM, Behe M, Sgouros G. Correlation of red marrow radiation dosimetry with myelotoxicity: empirical factors influencing the radiation-induced myelotoxicity of radiolabeled antibodies, fragments and peptides in pre-clinical and clinical settings. *Cancer Biother Radiopharm.* 2002;17:445–464.
30. Press OW, Eary JF, Appelbaum FR, et al. Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N Engl J Med.* 1993;329:1219–1224.
31. Kwekkeboom DJ, De Herder WW, Kam BL, et al. Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. *J Clin Oncol.* 2008;26:2124–2130.
32. Behr TM, Behe MP. Cholecystokinin-B/gastrin receptor-targeting peptides for staging and therapy of medullary thyroid cancer and other cholecystokinin-B receptor-expressing malignancies. *Semin Nucl Med.* 2002;32:97–109.
33. Wessels BW, Konijnenberg MW, Dale RG, et al. MIRD pamphlet no. 20: the effect of model assumptions on kidney dosimetry and response—implications for radionuclide therapy. *J Nucl Med.* 2008;49:1884–1899.
34. Krochak RJ, Baker DG. Radiation nephritis: clinical manifestations and pathophysiologic mechanisms. *Urology.* 1986;27:389–393.
35. Cassady JR. Clinical radiation nephropathy. *Int J Radiat Oncol Biol Phys.* 1995;31:1249–1256.
36. Cohen EP, Robbins ME. Radiation nephropathy. *Semin Nephrol.* 2003;23:486–499.
37. Behr TM, Sharkey RM, Sgouros G, et al. Overcoming the nephrotoxicity of radiometal-labeled immunoconjugates: improved cancer therapy administered to a nude mouse model in relation to the internal radiation dosimetry. *Cancer.* 1997;80:2591–2610.

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38. Jaggi JS, Seshan SV, McDevitt MR, LaPerle K, Sgouros G, Scheinberg DA. Renal tubulointerstitial changes after internal irradiation with alpha-particle-emitting actinium daughters. *J Am Soc Nephrol.* 2005;16:2677–2689.
39. Forrer F, Rolleman E, Bijster M, et al. From outside to inside? Dose-dependent renal tubular damage after high-dose peptide receptor radionuclide therapy in rats measured with in vivo ^{99m}Tc-DMSA-SPECT and molecular imaging. *Cancer Biother Radiopharm.* 2007;22:40–49.
40. Rolleman EJ, Krenning EP, Bernard BF, et al. Long-term toxicity of [^{177}Lu-DOTA (0),Tyr (3)]octreotate in rats. *Eur J Nucl Med Mol Imaging.* 2007;34:219–227.
41. Moll S, Nickeleit V, Mueller-Brand J, Brunner FP, Maecke HR, Mihatsch MJ. A new cause of renal thrombotic microangiopathy: yttrium 90-DOTATOC internal radiotherapy. *Am J Kidney Dis.* 2001;37:847–851.
42. Bodei L, Cremonesi M, Ferrari M, et al. Long-term evaluation of renal toxicity after peptide receptor radionuclide therapy with ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE: the role of associated risk factors. *Eur J Nucl Med Mol Imaging.* 2008;35:1847–1856.
43. O'Donoghue J. Relevance of external beam dose-response relationships to kidney toxicity associated with radionuclide therapy. *Cancer Biother Radiopharm.* 2004;19:378–387.
44. Emami B, Lyman J, Brown A, et al. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys.* 1991;21:109–122.
45. Barone R, Borson-Chazot F, Valkema R, et al. Patient-specific dosimetry in predicting renal toxicity with ^{90}Y-DOTATOC: relevance of kidney volume and dose rate in finding a dose-effect relationship. *J Nucl Med.* 2005;46(suppl 1):99S–106S.
46. Ekblad T, Tran T, Orlova A, et al. Development and preclinical characterisation of ^{99m}Tc-labelled Affibody molecules with reduced renal uptake. *Eur J Nucl Med Mol Imaging.* 2008;35:2245–2255.
47. Miao Y, Fisher DR, Quinn TP. Reducing renal uptake of ^{90}Y- and ^{177}Lu-labeled alpha-melanocyte stimulating hormone peptide analogues. *Nucl Med Biol.* 2006; 33:723–733.
48. Mather SJ, McKenzie AJ, Sosabowski JK, Morris TM, Ellison D, Watson SA. Selection of radiolabeled gastrin analogs for peptide receptor-targeted radionuclide therapy. *J Nucl Med.* 2007;48:615–622.
49. García Garayoa E, Schweinsberg C, Maes V, et al. Influence of the molecular charge on the biodistribution of bombesin analogues labeled with the [^{99m}Tc(CO)$_3$]-core. *Bioconjug Chem.* 2008;19:2409–2416.
50. Akizawa H, Arano Y, Mifune M, et al. Effect of molecular charges on renal uptake of ^{111}In-DTPA-conjugated peptides. *Nucl Med Biol.* 2001;28:761–768.
51. Dijkgraaf I, Liu S, Kruijtzer JA, et al. Effects of linker variation on the in vitro and in vivo characteristics of an ^{111}In-labeled RGD peptide. *Nucl Med Biol.* 2007;34:29–35.
52. Chen X, Park R, Shahinian AH, Bading JR, Conti PS. Pharmacokinetics and tumor retention of ^{125}I-labeled RGD peptide are improved by PEGylation. *Nucl Med Biol.* 2004;31:11–19.
53. Caliceti P, Veronese FM. Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates. *Adv Drug Deliv Rev.* 2003;55:1261–1277.
54. Wu Z, Li ZB, Cai W, et al. ^{18}F-labeled mini-PEG spacered RGD dimer (^{18}F-FPRGD2): synthesis and microPET imaging of alphavbeta3 integrin expression. *Eur J Nucl Med Mol Imaging.* 2007;34:1823–1831.
55. Dennis MS, Jin H, Dugger D, et al. Imaging tumors with an albumin-binding Fab, a novel tumor-targeting agent. *Cancer Res.* 2007;67:254–261.
56. Tolmachev V, Orlova A, Pehrson R, et al. Radionuclide therapy of HER2-positive microxenografts using a ^{177}Lu-labeled HER2-specific Affibody molecule. *Cancer Res.* 2007;67:2773–2782.
57. Sharkey RM, Behr TM, Mattes MJ, et al. Advantage of residualizing radiolabels for an internalizing antibody against the B-cell lymphoma antigen, CD22. *Cancer Immunol Immunother.* 1997;44:179–188.
58. Uehara T, Koike M, Nakata H, et al. Design, synthesis, and evaluation of [^{188}Re]organorhenium-labeled antibody fragments with renal enzyme-cleavable linkage for low renal radioactivity levels. *Bioconjug Chem.* 2007;18:190–198.
59. Tibben JG, Massuger LF, Boerman OC, et al. Decreased kidney uptake of technetium-99m-labelled Fab′ fragments in ovarian carcinoma bearing nude mice using a cleavable chelator. *Nucl Med Biol.* 1994;21:17–24.
60. Smith-Jones PM, Stolz B, Albert R, Knecht H, Bruns C. Synthesis, biodistribution and renal handling of various chelate-somatostatin conjugates with metabolizable linking groups. *Nucl Med Biol.* 1997;24:761–769.
61. Hammond PJ, Wade AF, Gwilliam ME, et al. Amino acid infusion blocks renal tubular uptake of an indium-labelled somatostatin analogue. *Br J Cancer.* 1993; 67:1437–1439.
62. Pimm MV, Gribben SJ. Prevention of renal tubule re-absorption of radiometal (indium-111) labelled Fab fragment of a monoclonal antibody in mice by systemic administration of lysine. *Eur J Nucl Med.* 1994;21:663–665.
63. Behr TM, Sharkey RM, Juweid ME, et al. Reduction of the renal uptake of radiolabeled monoclonal antibody fragments by cationic amino acids and their derivatives. *Cancer Res.* 1995;55:3825–3834.
64. Rolleman EJ, Valkema R, De Jong M, Kooij PP, Krenning EP. Safe and effective inhibition of renal uptake of radiolabelled octreotide by a combination of lysine and arginine. *Eur J Nucl Med Mol Imaging.* 2003;30:9–15.
65. De Jong M, Rolleman EJ, Bernard BF, et al. Inhibition of renal uptake of indium-111-DTPA-octreotide in vivo. *J Nucl Med.* 1996;37:1388–1392.
66. Béhé M, Kluge G, Becker W, Gotthardt M, Behr TM. Use of polyglutamic acids to reduce uptake of radiometal-labeled minigastrin in the kidneys. *J Nucl Med.* 2005;46:1012–1015.
67. Vegt E, Wetzels JF, Russel FG, et al. Renal uptake of radiolabeled octreotide in human subjects is efficiently inhibited by succinylated gelatin. *J Nucl Med.* 2006;47:432–436.
68. van Eerd JE, Vegt E, Wetzels JF, et al. Gelatin-based plasma expander effectively reduces renal uptake of ^{111}In-octreotide in mice and rats. *J Nucl Med.* 2006;47: 528–533.
69. Vegt E, Van Eerd JE, Eek A, et al. Reducing renal uptake of radiolabeled peptides using albumin fragments. *J Nucl Med.* 2008;49:1506–1511.
70. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet.* 1977;1:466–469.
71. Vegt E, Eek A, Oyen WJ, De Jong M, Gotthardt M, Boerman OC. Albumin-derived peptides efficiently reduce renal uptake of radiolabelled peptides. *Eur J Nucl Med Mol Imaging.* 2010;37:226–234.
72. Gekle M, Mildenberger S, Freudinger R, Schwerdt G, Silbernagl S. Albumin endocytosis in OK cells: dependence on actin and microtubules and regulation by protein kinases. *Am J Physiol.* 1997;272:F668–F677.
73. Gekle M, Mildenberger S, Freudinger R, Silbernagl S. Endosomal alkalinization reduces Jmax and Km of albumin receptor-mediated endocytosis in OK cells. *Am J Physiol.* 1995;268:F899–F906.
74. Rolleman EJ, Krenning EP, Van Gameren A, Bernard BF, De Jong M. Uptake of [^{111}In-DTPA0]octreotide in the rat kidney is inhibited by colchicine and not by fructose. *J Nucl Med.* 2004;45:709–713.
75. Capizzi RL. The preclinical basis for broad-spectrum selective cytoprotection of normal tissues from cytotoxic therapies by amifostine (Ethyol). *Eur J Cancer.* 1996;32A(suppl 4):S5–S16.
76. Rolleman EJ, Forrer F, Bernard B, et al. Amifostine protects rat kidneys during peptide receptor radionuclide therapy with [^{177}Lu-DOTA0,Tyr3]octreotate. *Eur J Nucl Med Mol Imaging.* 2007;34:763–771.
77. Jaggi JS, Seshan SV, McDevitt MR, Sgouros G, Hyjek E, Scheinberg DA. Mitigation of radiation nephropathy after internal alpha-particle irradiation of kidneys. *Int J Radiat Oncol Biol Phys.* 2006;64:1503–1512.
78. Moulder JE, Fish BL, Cohen EP. Impact of angiotensin II type 2 receptor blockade on experimental radiation nephropathy. *Radiat Res.* 2004;161: 312–317.
79. Melis M, Bijster M, De Visser M et al. Dose-response effect of Gelofusine on renal uptake and retention of radiolabelled octreotate in rats with CA20948 tumours. *Eur J Nucl Med Mol Imaging.* July 8, 2009 [Epub ahead of print].

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Renal Toxicity of Radiolabeled Peptides and Antibody Fragments: Mechanisms, Impact on Radionuclide Therapy, and Strategies for Prevention

Erik Vegt, Marion de Jong, Jack F.M. Wetzels, Rosalinde Masereeuw, Marleen Melis, Wim J.G. Oyen, Martin Gotthardt and Otto C. Boerman

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