EDITORIAL

Improved Prospects for Cancer Therapy with Radiolabeled Antibody Fragments and Peptides?

The development and first clinical application of radiolabeled antibodies against defined human tumor-associated antigens in the late 1970s and early 1980s (*1–4*) has now matured to a multitude of agents that not only can reveal occult cancer sites, but also have applications in the detection of infection, inflammation, infarction, atherosclerotic plaques and thrombi (*5,6*). Such selective targeting of disease has stimulated the extension of these approaches to the therapeutic modalities involving immunoconjugates of antibodies linked to drugs, toxins, radionuclides and cytokines, in the expectation that normal tissues will be spared while malignant cells will be killed selectively (*5,7*). This prospect has been hindered by a number of obstacles, including a very low accretion of the immunoconjugate in tumor, dose-limiting myelotoxicity for radioimmunoconjugates, and a host immune response when foreign, animal (particularly rodent) antibodies are employed, especially when given more than once (*5,8*). Finally, drug immunoconjugates suffer from delivering too few drug molecules to the tumor, thus requiring large quantities of antibody to be administered in order to approach therapeutic doses or the use of extremely toxic drugs that can be given in minute quantities (*5*). Also, most immunoconjugates studied to date have involved the use of whole immunoglobulins, particularly IgG. However, the advent of smaller antibody fragments and new classes of receptor-binding peptides has shown improved target-to-background tissue ratios, which is due to an enhanced and rapid background clearance (*9*). Thus, excellent detection rates have been achieved with antibody fragments (*5,6,9*) and peptides, such as somatostatin or vasoactive intestinal peptide analogs (*10,11*). However, therapy with these smaller and rapidly targeting molecules has been precluded by their high renal accretion (*8–10*), which is anticipated to result in severe nephrotoxicity at therapeutic doses.

The kidney is well-known as a major site in the catabolism of low-molecular-weight proteins (*12*). This renal accretion of peptides and small proteins is a result of the glomerular filtration of molecules smaller than 60 kD, followed by their tubular reabsorption and lysosomal degradation (*13*). Whereas iodine is released quickly from cells, radiometals would be retained, probably trapped in lysosomes, and bound to ubiquitous intracellular metal-binding proteins (*13*).

Based on two previous studies suggesting that basic amino acids may be capable of decreasing this renal accretion of radiolabeled peptides (*14,15*), we recently reported our preclinical results on reducing the renal uptake of a variety of radiometals, as well as iodine, bound to Fab′ or $F(ab')_2$ fragments (*16*). Our pilot clinical study is published in this issue of *JNM* (*17*). Since these clinical data are preliminary, we are grateful to the editor-in-chief of *JNM* for inviting us to summarize the preclinical results that led to our pilot clinical study.

Figure 1 summarizes some of the most important findings of this preclinical work, using the example of ^{99m}Tc-labeled Fab′ fragments. BALB/c or tumor-bearing nude mice were injected intraperitoneally four times, in hourly intervals, with different cationic amino acids, amino sugars or their derivatives, such as L-lysine, L-lysyl ethyl ester, L-arginine, D-lysine or D-glucosamine. Whereas no significant effect on the uptake and retention in the tumor or any other normal organ became apparent, the renal accretion of radiolabeled Fab′ fragments was decreased, in a strong dose-

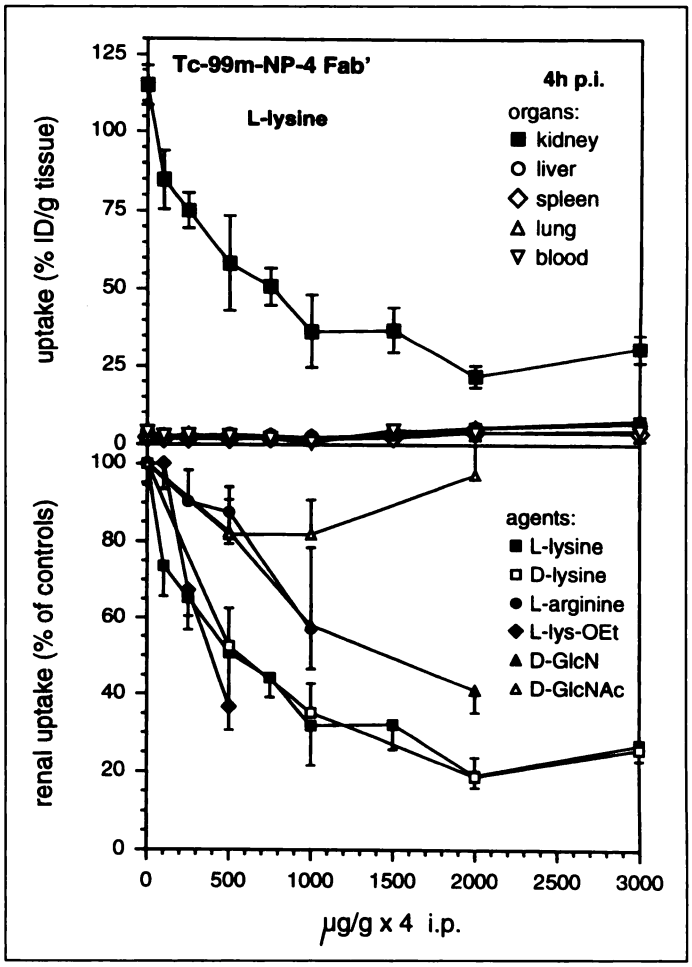

FIGURE 1. The upper panel shows the dose-effect relationship between L-lysine hydrochloride, administered intraperitoneally at hourly intervals, on the uptake of ^{99m}Tc-NP-4 Fab′-fragments in the kidneys and other organs in BALB/c mice at 4 hr postinjection. The dose-effect relationship of L/D-lysine, L-arginine, L-lysyl ethyl ester, D-glucosamine and N-acetyl D-glucosamine on the renal accretion of ^{99m}Tc-Fab′.

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TABLE 1

Dosimetry of ^{90}Y-MN-14 Fab and F(ab)'$_2$ (anti-CEA), ^{188}Re-Mu-9 Fab' (anti-CSAp) and ^{131}I-NP-4 Fab' and F(ab')$_2$ (anti-CEA) with and without Lysine Administration (4 × 2 mg/g body weight) in Nude Mice*

	^{90}Y-Fab' (cGy/mCi)			^{188}Re-Fab' (cGy/mCi)			^{131}I-Fab' (cGy/mCi)		
	Contr.	Lysine	Ratio lys/contr.	Contr.	Lysine	Ratio lys/contr.	Contr.	Lysine	Ratio lys/contr.
GW-39	4954	5751	1.16	620	763	1.23	n/d	n/d	n/d
Liver	1340	1652	1.23	611	588	0.96	82	77	0.94
Spleen	627	499	0.80	306	289	0.94	35	42	1.20
Kidney	33057	6231	0.18	7819	3085	0.39	230	116	0.50
Lung	386	431	1.12	538	410	0.76	126	120	0.95
Blood	594	664	0.95	256	203	0.79	118	95	0.81
Bone	493	588	1.19	121	107	0.89	n/d	n/d	n/d

	^{90}Y-F(ab')$_2$ (cGy/mCi)			^{131}I-F(ab')$_2$ (cGy/mCi)		
	Contr.	Lysine	Ratio) lys/contr.	Contr.	Lysine	Ratio lys/contr.
GW-39	5876	6500	1.11	3160	3379	1.07
Liver	2334	3974	1.70	265	256	0.97
Spleen	738	849	1.15	263	243	0.82
Kidney	14893	3974	0.27	1351	633	0.47
Lung	978	992	1.01	279	238	0.85
Blood	2795	2317	0.83	644	726	1.13
Bone	570	787	1.38	n/d	n/d	n/d

*The dosimetry was calculated from biodistribution data according to Sharkey et al. (*24*).

dependent manner, up to sixfold (i.e., by over 80%). Accordingly, renal radiation doses were decreased by approximately five- to six-fold (Table 1), while doses to the tumor and other organs remained unaffected. L- and D-enantiomers were equally potent, as were also natural or synthetic cationic polymers (e.g., protamine or poly-lysines). On a molecular basis, this effect was dependent solely upon the presence of a positively-charged amino group. For example, glucosamine was almost as effective as lysine, but its N-acetylated derivative, lacking the positive charge, was not (Fig. 1), as were also all neutral or anionic amino acids tested. Interestingly, a *single oral* administration of lysine (surprisingly also here both stereoisomers) was as effective as the repeated intraperitoneal injection scheme, which is probably due to more stable blood concentrations through a slower active resorption from the intestine than would occur by passive diffusion along the concentration gradient from the peritoneal cavity. Similar or equal effectiveness was seen in the inhibition of renal accretion of Fab' fragments labeled with all the radiometals tested (i.e., ^{99m}Tc, ^{188}Re, ^{111}In, ^{88}Y), residualizing forms of iodine (e.g., iodinated dilactitol-tyramine (*18*), as well as F(ab')$_2$ fragments. Because of the slower clearance kinetics of the latter, a bihourly administration schedule was used for them. Although the renal retention of conventionally radioiodinated Fab' or F(ab')$_2$ is lower by more than one order of magnitude, as compared to the respective radiometal labels, also here a 50% reduction of renal accretion, thus radiation dose, could be achieved (Table 1).

As we showed in our clinical study in this issue of *JNM* (*17*), the mechanism of action of these compounds was identified as an inhibition of the tubular reabsorption of glomerularly-filtered proteins, most likely due to a simple neutralization of negative charges on the cell surface of the proximal tubular cells by these cationic agents. High-performance liquid chromatography (HPLC) analysis of the urine from untreated control mice showed the majority of radiolabel bound to small-molecular-weight metabolites, whereas lysine-treated animals excreted essentially unchanged Fab' fragments that retained their immunoreactivity. Interestingly, HPLC analysis of the urine from F(ab')$_2$-treated mice failed to show intact F(ab')$_2$ or Fab' fragments. Only substances of low molecular weight were found, indicating that the physiological mechanism of the F(ab')$_2$ catabolism is different from that of Fab'. With its 100-kD molecular weight, F(ab')$_2$ is certainly too large to be filtered through the glomerular basement membrane. Presumably, the catabolism of such larger proteins takes place elsewhere (e.g., the liver), and the smaller metabolic products would be filtered and excreted via the kidneys. Although the precise mechanism needs to be investigated further, the fact that the dose to the kidneys could be reduced by 75% offers new prospects for radioimmunotherapy with radiometal-conjugated bivalent fragments. This could be one of the most immediate clinical applications of this technology in clinical radioimmunotherapy.

Although no long-term toxicity of any of the compounds used for renal uptake reduction has been observed in our animal studies, toxicity in humans is a major concern that needs to be considered before undertaking large-scale clinical dose-escalation trials with these nephroprotective agents. This is the reason why only comparatively low doses of amino acids were used in the pilot clinical trial. Although our clinical results are encouraging, it should be possible to reduce renal accretion further, especially if therapy with such radiometal-conjugated agents is considered. Thus, higher doses will be necessary. This small cohort of patients represents a preliminary feasibility study to demonstrate the anticipated effect. However, thorough toxicity studies in primates are warranted, especially because the toxicity of L-lysine seems to be species-related (*16*). Since toxicity was found to be restricted to its L-isomer, D-lysine seems to be the preferred can-

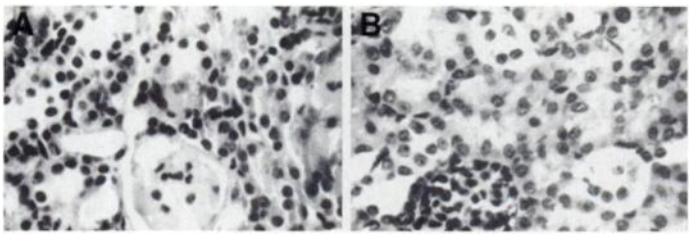

FIGURE 2. Renal histology (250-fold magnification) 12 wk after the injection of 1.25 mCi ^{188}Re-Mu-9 Fab′ in nude mice (A) without and (B) with renal protection through lysine. Whereas the unprotected kidneys are characterized by focal glomerular sclerosis, tubular atrophy and fibrinoid necrosis, no major histological changes are noticeable in lysine-treated animals.

didate, because it should not interfere with the natural amino acid metabolic balance. Indeed, the maximum tolerated dose (MTD) of D-lysine in mice is approximately 1.4-fold higher than the MTD of its L-isomer (*16*). The efficacy of orally-administered lysine (surprisingly, of both stereoisomers) is of particular value, because it would avoid the need for a long intravenous infusion, and would thus present a much more convenient method for clinical use.

Based on our preclinical as well as clinical results, we have embarked on additional preclinical studies to determine what impact this methodology of inhibition of the renal accretion of antibody fragments may have on the radioisotopic therapy of cancer. Preliminary studies have been carried out with ^{188}Re-labeled Fab′ fragments of the monoclonal antibody Mu-9, which is directed against colon-specific antigen-p (CSAp) (*19*). CSAp is a mucin antigen present in over 60% of human colon cancers (*19*). At injected activities of 1.25 mCi ^{188}Re-Fab′, which would deliver close to 100 Gy to the mouse kidneys, surprisingly no acute nephrotoxicity was observed, as indicated by persisting normal serum BUN and creatinine levels. However, after approximately 10 to 12 wk, rising serum BUN levels were observed in non-lysine treated animals, when the first deaths occurred. Renal histology showed focal glomerulosclerosis, tubular atrophy and fibrinoid necrosis, which are typical for chronic radiation nephropathy (Fig. 2A). In none of the lysine-protected ^{188}Re-Fab′-treated animals, however, were any histological changes or BUN abnormalities observed (Fig. 2B). It is especially noteworthy in this context that, as is known from external beam radiation (*20–22*), radiation nephrotoxicity can occur months, or in humans even years, after the radiation, potentially without any prior acute prodromal symptoms.

Due to the clearly superior dosimetry of ^{90}Y-labeled Fab fragments of the high-affinity (K_a = 10^9 1/mol) anti-CEA antibody, MN-14 (Table 1), this agent was chosen for a detailed evaluation of our amino acid methodology in radioimmunotherapy with radiometal-labeled immunoconjugates. Studies were performed in GW-39 human colon cancer xenograft-bearing nude mice, using subcutaneous tumors as well as a metastatic model (*23*), in order to determine the dose-limiting, radiation-induced, organ toxicities, as well as the feasibility of the amino acid methodology to overcome the observed nephrotoxicity. Furthermore, the relationship between the observed biological effects and the internal radiation dosimetry was established for the respective ^{90}Y-labeled IgG, F(ab)$_2$′ and Fab′ immunoconjugates. The results of these experiments are being prepared for publication and indeed suggest a potential role of such renal radioprotective agents for improved radioimmunotherapy with antibody fragments (Behr TM, unpublished data). Thus, we believe that this approach of nephro-radioprotection may provide a new prospect for cancer therapy with radiolabeled antibody fragments and peptides.

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