Reducing the renal uptake of radiolabeled antibody fragments and peptides for diagnosis and therapy: present status, future prospects and limitations

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Abstract. Elevated renal uptake and prolonged retention of radiolabeled antibody fragments and peptides is a problem in the therapeutic application of such agents. Over recent years, one of the focuses of research has therefore been to develop suitable methods to reduce this renal uptake, and to evaluate whether the resulting methodology will benefit therapy with antibody fragments and peptides. In these studies it has been shown that the kidney uptake of antibody fragments in animals can be reduced in a dose-dependent manner by almost one order of magnitude by the systemic administration of cationic amino acids and their derivatives, whereas the uptake in all other organs, as well as the tumor, remains unaffected. A similar reduction in renal retention is achieved for all intracellularly retained radionuclides (e.g., radiometals) or radioiodinated immunoconjugates, as well as for smaller peptides. Lysine is usually the preferred agent, and its D- and L-isomers are equally effective whether given intraperitoneally or orally. Amino sugars are effective, but their N-acetyl derivatives, lacking the positive charge, are not. Basic polypeptides are also effective, and their potency increases with increasing molecular weight (i.e., the amount of positive charges per molecule). Urine analysis of treated individuals shows the excretion of unmetabolized, intact fragments or peptides, in contrast to mostly low-molecular-weight metabolites in untreated controls. In therapy studies using radiometal-conjugated Fab fragments, the kidney is the first dose-limiting organ. Administration of cationic amino acids results in a substantial increase in the maximum tolerated dose of such Fab fragments. No biochemical or histological evidence of renal damage has been observed under these conditions. As was the case in animal studies, in pilot clinical trials the renal uptake in patients injected with Fab′ fragments and given amino acids could be decreased significantly, whereas the uptake by all other organs remained unaffected. These recent studies indicate that a variety of basic compounds are capable of inhibiting the tubular reabsorption of peptides and proteins, thus significantly lowering the renal uptake of antibody fragments or peptides in both animals and patients. On a molecular basis, the effect seems to rely essentially on the presence of a positively charged amino group. Thus, radiation nephrotoxicity of antibody fragments and peptides can be overcome successfully; this may provide new prospects for cancer therapy with radiolabeled antibody fragments and peptides.

Key words: Renal uptake – Radiation nephrotoxicity – Cationic amino acids – Lysine – Antibody fragments – Peptides

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Introduction

Antibody fragments and peptides have shown advantages over whole IgG with respect to tumor-to-nontumor ratios [1]. Lower-molecular-weight agents generally provide better target-to-nontarget ratios, due to their rapid background clearance [1, 2]. However, although such agents are suitable for diagnostic purposes, the renal uptake of proteins and peptides poses a severe problem for their therapeutic use. This is expecially true below the renally filterable size (i.e., approximately 60 kDa), and when intracellularly retained nuclides are used [3, 4]. Even bivalent antibody fragments [e.g., $F(ab)_2$], with their molecular weight of approximately 100 kDa, exhibit a significant renal uptake [5]. Hence, therapy with antibody fragments or peptides conjugated to intracellularly retained radiometals may be limited by radionephrotoxicity [5]. This is probably one of the reasons why very few therapeutic trials have been performed with monovalent Fab fragments [6]; furthermore, nephrotoxicity is a serious concern in the few therapeutic peptide trials to have been conducted so far [7, 8]. Therefore, over recent years we and other groups have focused on establishing methodologies that allow the renal uptake of small proteins or peptides to be reduced. The aim of this review is to discuss the major aspects of this work.

Fundamentals of renal physiology of protein and peptide metabolism

The kidney is well known as a major site in the catabolism of low-molecular-weight proteins [9–11]. The handling of proteins in the kidney is a rather complex process which involves several distinct and essentially independent mechanisms, occurring at different microanatomical sites within the kidney [10]. Renal uptake of peptides and small proteins is a consequence of their glomerular filtration followed by tubular reabsorption and subsequent lysosomal degradation [9–11]. Plasma proteins reach the glomerular capillaries in the kidney through the renal arteries and their branches. The glomerulus filters proteins in a molecular-weight-dependent manner. If their molecular weight exceeds a molecular weight of approximately 60 kDa, these proteins are too large to be filtered through the intact glomerular basement membrane. Larger molecules, such as complete IgG with its molecular weight of 150 kDa, will therefore pass the glomerulus without appearing in the primary urine, whereas smaller molecules are able to pass this basement membrane (the lower the molecular weight, the easier it is for them to do so). Furthermore, the basement membrane of the glomerulus is negatively charged. Cationic peptides and proteins attach to the basement membrane and can pass more easily into the primary urine. In contrast, filtration of anionic proteins and peptides is less efficient, due to electrostatic repulsion from the basement membrane.

Filtered proteins will pass through the proximal tubule. Under physiological conditions, all proteins and peptides are reabsorbed almost quantitatively by the cells of the proximal tubule by means of pinocytosis (Fig. 1). For this purpose, the proteins or peptides bind to negatively charged "receptors" on the cell surface of the proximal tubule cells via electrostatic interaction. Therefore again, cationic proteins are preferably reabsorbed in comparison with anionic ones. Once taken up in the tubule cells, peptides are transferred into lysosomes and digested by proteolytic enzymes. Resulting breakdown products [i.e., amino acids and their (radiolabeled) derivatives] are mainly transferred back into the bloodstream, but are also excreted to some extent into the urine. Iodinated tyrosine, which is the major metabolic product of conventionally radioiodinated proteins, is quickly released from the tubule cells. By contrast, radiometal-chelated amino acids (such as ^{111}In-, $^{88/90}$Y- or ^{161}Tb-DTPA-lysine) or carbohydrate-linked amino acid derivatives (e.g., $^{125/131}$I-labeled dilactitol-tyramine), as a form of residualizing radioiodine label, cannot leave the lysosomes and will, therefore, remain trapped in the proximal tubule cells [12–15].

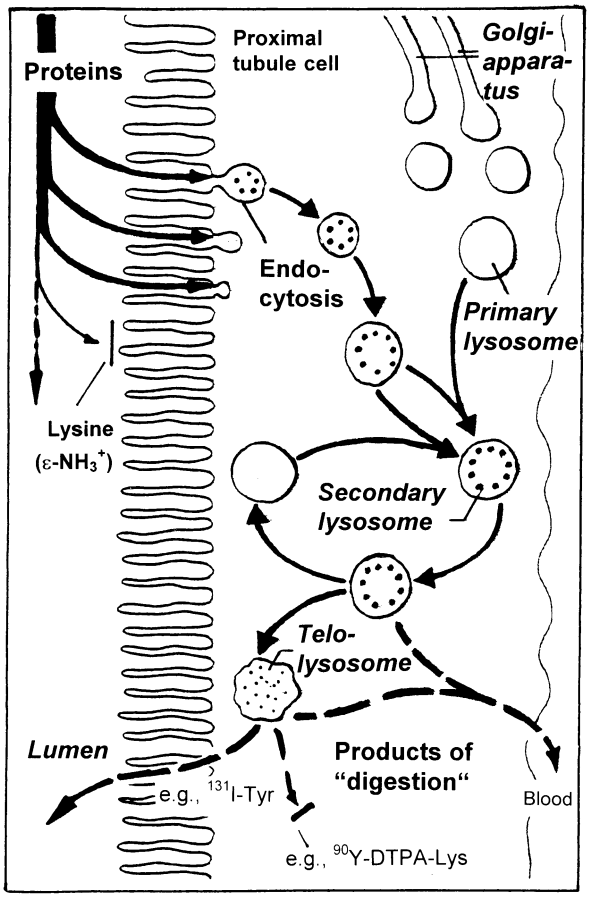


Fig. 1. Schematic representation of the physiology of protein reabsorption and metabolism in the renal proximal tubule cells. Under physiological conditions, glomerular-filtered proteins and peptides are almost quantitatively reabsorbed and lysosomally digested. Whereas the resulting catabolic products of radioiodinated proteins and peptides (mainly monoiodotyrosine) are rapidly excreted, radiometal chelates (e.g., ^{111}In- or ^{90}Y-DTPA-lysine) remain trapped within lysosomes. Pharmacological amounts of cationic amino acids and their derivatives can cause tubular proteinuria by neutralization of negative charges on the luminal cell surface of tubule cells which are thought to be essential for the binding of proteins and peptides to their respective receptors. (Modified from [41])

Early attempts to decrease the renal uptake of radiolabeled proteins and peptides

In an early attempt to decrease the renal uptake of renally filterable proteins, use of heavy metal salts (especially uranyl nitrate) has been proposed. Such heavy metal salts can induce, in a dose-dependent fashion, acute necrosis of the tubule which is reversible within a few days [16]. For obvious reasons [very narrow margins between pharmacological and irreversible toxic effects, as well as the long-lived radioactivity of uranium salts (mainly ^{238}U) and their decay products], these attempts have not found broader application.

Subsequently, two independent studies suggested that L-lysine may be effective in decreasing the renal uptake

of radiolabeled peptides. Hammond and co-workers [17] studied the effect of an intravenous nutritive amino acid solution on the renal uptake of the ^{111}In-labeled somatostatin analogue, octreotide, in patients. Although a substantial reduction in the renal uptake of octreotide was clearly demonstrated, the analysis performed by these authors was only semiquantitative. Independently, Pimm and Gribben [18] reported on the effect of repeated intraperitoneal injections of high doses of L-lysine on the renal uptake of ^{111}In-labeled monoclonal antibody Fab′ fragments in BALB/c mice. A highly significant reduction in renal uptake was demonstrated in lysine-treated animals as compared to untreated controls, and a clear dose-effect relationship was found.

However, several questions remained open. These studies did not evaluate the effectiveness of radiolabels other than ^{111}In, nor did they address the molecular characteristics of the substance that enabled it to reduce the renal uptake, its (patho-)physiological mechanisms, or the effectiveness of different immunoglobulin subclasses. Furthermore, no consideration was given to the uptake of larger protein molecules, e.g., $F(ab')_2$, which is of particular therapeutic interest. Since its molecular weight is above the renally filterable size [10], the mechanism of renal uptake of $F(ab')_2$ is still poorly understood [19]. In addition, no data were reported on the effect of such methodology on tumor uptake, which is, of course, the most crucial point with respect to its clinical utility.

In earlier studies, attempts were made to reduce the kidney uptake of Fab′ fragments by modification of the antibody itself, e.g., by shielding its positive charges through N-acetylation of free amino groups [20]. This approach was modeled on the findings that positive charges favor renal filtration and tubular uptake of polypeptides and proteins (see above [10]). The success of this method, however, was limited and the immunoreactivity of the antibody fragments was compromised [20], probably by chemically modifying lysine residues in the complementarity-defining region, which may be involved in antibody-antigen interaction. Accordingly, moderately reduced renal uptake was accompanied by significantly decreased tumor-to-nontumor ratios.

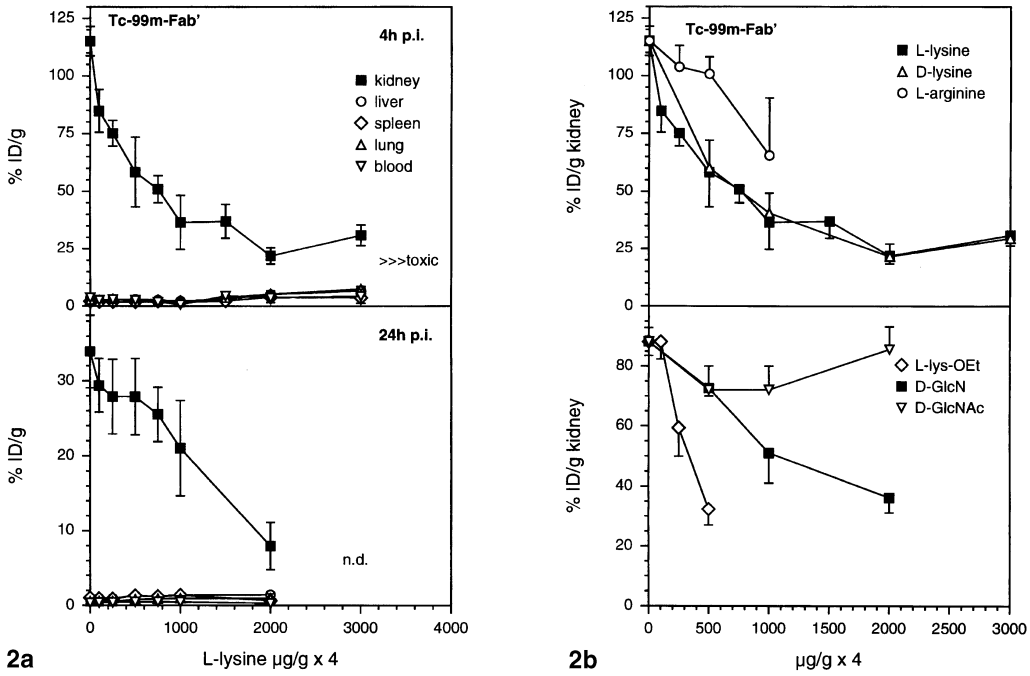
Based on these previous studies, we embarked upon establishing safe and effective procedures to reduce the renal uptake of a variety of radiometals, as well as iodine, bound to Fab or $F(ab)_2$ fragments [21]. We analyzed the possible physiological mechanisms of such a reduction in uptake, and found common molecular characteristics of the effective agents [21, 22]. Furthermore, we evaluated the application of this methodology to cancer therapy with ^{90}Y- and ^{188}Re-labeled immunoconjugates (fragments as well as IgG) [22–24]. Finally, pilot clinical trials were undertaken to examine whether the established methodology would be successful in reducing the renal uptake of antibody fragments and peptides in patients [23].

Basic amino acids and their derivatives are able to reduce the renal uptake of antibody fragments and peptides in animals

In our aforementioned studies [21, 22], BALB/c mice or nude mice bearing the human GW-39 colon carcinoma xenograft were given intravenous or intraperitoneal injections of various basic amino acids, or a range of different cationic amino acid derivatives, amino sugars, and basic oligo- and polypeptides. The effect of these agents on the biodistribution of Fab and $F(ab)_2$ fragments of different monoclonal antibody isotypes (IgG_1, IgG_{2a}), radiolabeled with ^{99m}Tc, ^{188}Re, ^{111}In, $^{88/90}$Y or $^{125/131}$I, was studied.

The kidney uptake of Fab′ fragments in animals was reduced by cationic amino acids and their derivatives in a dose-dependent manner by almost one order of magnitude as compared to untreated controls (Fig. 2). The uptake in all other organs, as well as the tumor, was unaffected. A similar reduction in renal retention was seen for all other intracellularly retained isotopes, as well as for $F(ab)_2$ fragments. D- and L-isomers of lysine were equally effective, whether given i.p. or orally. D-Glucosamine was effective, but its N-acetylated derivative, lacking the positive charge, was not (Fig. 2b). Basic polypeptides, e.g., poly-L-lysine, were also effective, their potency increasing with increasing molecular weight (and thus, an increasing amount of positive charges per molecule). Thus, the molecular characteristics that enable a compound to inhibit protein uptake seem to be very variable, the prerequisite apparently being that the substance carries a positive charge through an amino group. The potency of the substances seems to increase with the amount of positive charges per molecule (Fig. 2b). For example, lysine ethyl ester, with its shielded negative carboxyl charge, was more potent than lysine itself, and the potency of polypeptides with lysyl residues rises with the molecular weight. The fact that glucosamine was as effective as lysine supports the concept that the effectivity of a compound essentially relies on the presence of a positively charged amino group. Accordingly, its N-acetylated derivative, lacking the positive charge, was not able to reduce the renal uptake of Fab′ fragments.

High-performance liquid chromatography (HPLC) analysis of the urine taken from treated animals showed the excretion of intact Fab′, in contrast to mostly low-molecular-weight metabolites in the control group (Fig. 2d). This finding supports the theory of Morgenson and co-workers [6] that the major principle is inhibition of tubular reabsorption of primarily glomerular-filtered peptides (Fig. 1). Also the fact that L- and D-isomers are equally effective in reducing renal retention supports the view that simple neutralization of negative charges of the luminal tubular cell surface by positively charged molecules hinders the reabsorption of protein molecules, given that there is no luminal carrier known for D-lysine in the mammalian tubule cells [10] which would take up



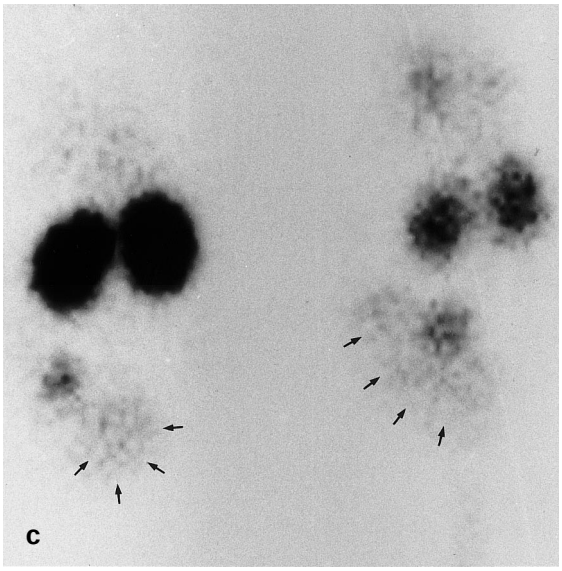
the D-isomer to the intracellular compartment. The finding that D-lysine was also effective when administered orally seemed at first to be surprising, because one might expect there to be no effective intestinal transporters for the resorption of D-amino acids. However, in the high concentrations used, passive diffusion along a tremendous concentration gradient may be possible.

Nevertheless, the physiological mechanism that regulates the reduction of renal uptake for larger molecules, such as $F(ab)_2$ fragments, remains a matter of speculation. With its 100-kDa molecular weight, $F(ab)_2$ is certainly too large to be filtered through the intact glomerular basement membrane [2, 5, 10]. In lysine-treated mice, no intact $F(ab)_2$ was found in the urine, but only substances of lower molecular weight, which is in contrast to the observations with Fab′. Therefore, we postulated that the catabolism of $F(ab)_2$ takes place elsewhere (e.g., the liver), and that the smaller metabolized products would be filtered and excreted via the kidneys [21, 22].

Table 1 shows the radiation dosimetry for a variety of antibodies, degrees of fragmentation [Fab and $F(ab)_2$], and radiolabels, comparing radiation doses with and without reduction of renal uptake by cationic amino acids. These data clearly demonstrate that the effect of lysine is not restricted to In-labeled compounds, but extends to all isotopes and antibody isoforms tested. Thus, the effect is largely independent of the immunoglobulin subclass or other protein characteristics (e.g., the pK_a). As expected, we found the effect to be more pronounced with intracellularly retained isotopes (radiometals) than with released ones (e.g., iodine).

Subsequent studies by other investigators [25–29] essentially have confirmed and corroborated these fundamental findings. They have shown that the basic amino acid technology is applicable to ^{177}Lu-labeled fragments [26] as well as smaller proteins (such as Fv fragments [25, 27]) or peptides (such as octreotide [28, 29]). Furthermore, they have investigated in more detail the biokinetics and metabolism of the cationic amino acids used for this purpose [26]. Interestingly, as we had predicted [21–24, 30], D-lysine was confirmed as the optimal agent for reduction of renal uptake in all of these subsequent studies. In sharp contrast to all studies using immunoconjugates, in which L- and D-lysine selectively inhibited the renal uptake without affecting the uptake in tumor or other normal organs, L-lysine was found to compromise the tumor uptake of ^{111}In- and ^{161}Tb-labeled octreotide in neuroendocrine cancers whereas its D-stereoisomer did not (M. DeJong, personal communication). Since an L-lysyl moiety is involved in the receptor-binding properties of octreotide, we speculate that L-lysine in high concentrations may competitively hinder this ligand-receptor interaction. D-Lysine in contrast, has never been shown in any system tested so far to compromise tumor uptake of immunoconjugates or peptides.

Sodium maleate has also been proposed as an effective agent to decrease the renal uptake of antibody fragments and peptides [28, 29]. This compound seems to have two independent effects [28, 29]. First, it decreases the glomerular filtration rate, leading to longer residence times in the plasma and, consequently, to higher normal organ uptake [28]. Secondly, it has an independent effect on tubular protein reabsorption [28]. However, since at least in animals irreversible kidney failure has been reported in the absence of any obvious dose-effect relationship, sodium maleate does not seem to be a good candidate for human studies [28]. Furthermore, the increased normal organ uptake may lead to significantly decreased tumor-to-nontumor dose ratios [28].



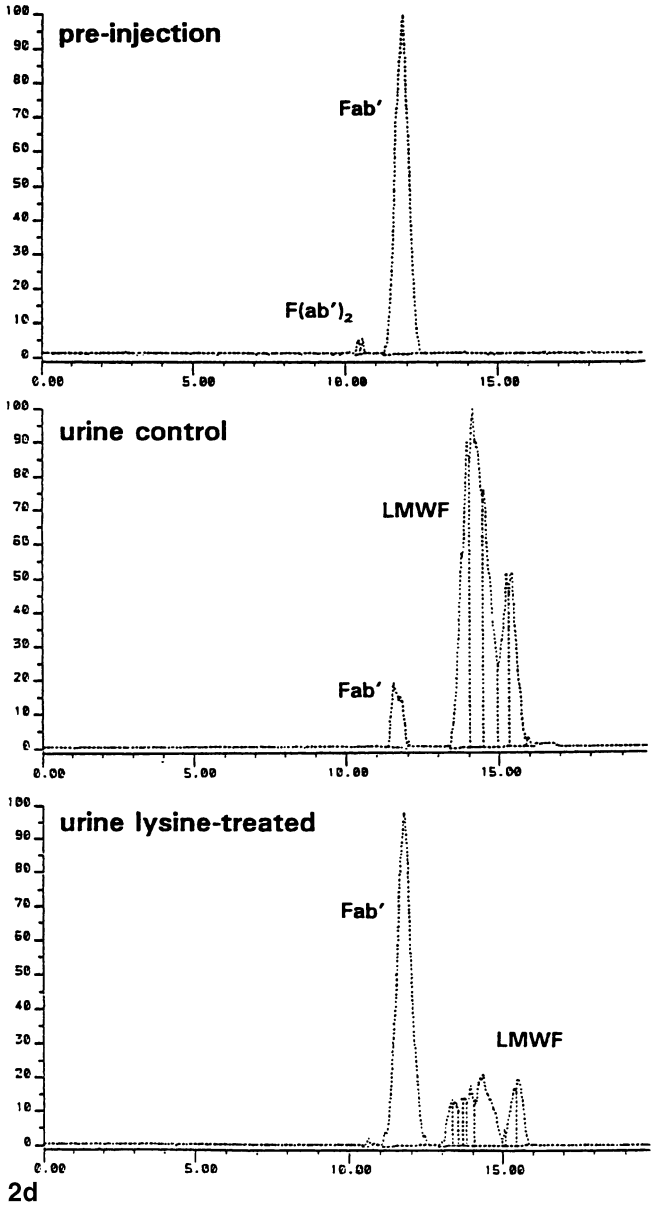


Fig. 2a–d. Reduction of the renal uptake of antibody fragments in animals. **a, b** Dose-effect relationships in respect of the renal uptake of the ^{99m}Tc-labeled Fab′ fragment of the anti-CEA antibody NP-4: **a** for L-lysine hydrochloride, administered i.p. at hourly intervals, and **b** for L- and D-lysine, L-lysine ethyl ester, L-arginine, D-glucosamine and its N-acetylated derivative, administered i.p. at hourly intervals. **c** External scintigraphy of human GW-39 colon carcinoma-bearing nude mice 4 h after injection of ^{99m}Tc-NP-4 Fab′. The animal on the *left* was untreated, the animal on the right received lysine i.p. (*arrows* indicate the tumor. (Modified from [21]). **d** Size-exclusion HPLC profiles of the urine of BALB/c mice treated with lysine in comparison to controls. *Upper panel*: HPLC profile of the pre-injection solution (^{99m}Tc-Fab′ NP-4), containing 98% of the total activity bound to Fab′, and 2% bound to residual F(ab′)$_2$. *Middle panel*: In the urine of the controls, more than 95% of excreted ^{99m}Tc is bound to low-molecular-weight metabolites (*LMWF*). *Lower panel*: In the urine of the lysine-treated mice, however, 65% of the activity is bound to intact Fab′

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 in nude mice (adopted from [22]). The dosimetry was calculated from biodistribution data according to [21, 22]

	^{90}Y-Fab Gy/mCi		^{188}Re-Fab′ Gy/mCi		^{131}I-Fab′ Gy/mCi		^{90}Y-F(ab)$_2$ Gy/mCi		^{131}I-F(ab′)$_2$ Gy/mCi	
	Control	Lysine	Control	Lysine	Control	Lysine	Control	Lysine	Control	Lysine
GW-39	49.5	57.5	6.2	7.6	3.0	3.1	58.8	65.0	31.6	33.8
Liver	13.4	16.5	6.1	5.9	0.8	0.7	23.3	39.7	2.7	2.6
Spleen	6.3	5.0	3.1	2.9	0.4	0.4	7.4	8.5	2.6	2.4
Kidney	*330.6*	*62.3*	*78.2*	*30.9*	*2.3*	*1.2*	*148.9*	*39.7*	*13.5*	*6.3*
Lung	3.9	4.3	5.4	4.1	1.3	1.2	9.8	9.9	2.8	2.4
Blood	5.9	6.6	2.6	2.0	1.2	1.0	28.0	23.2	6.4	7.3
Bone	4.9	5.9	1.2	1.1	ND	ND	5.7	7.8	ND	ND

ND, Not determined

Although at this point strong evidence exists that basic amino acids may be capable of reducing the renal uptake of small proteins or peptides and thus of alleviating the potential radiation nephrotoxicity of such agents, a major concern is the toxicity and side-effects of the compounds used for this purpose. There are contradictory opinions on the toxicity of amino acids given in high amounts [31]. The situation is made especially difficult by the fact that the toxicity of L-lysine seems to be species dependent. Zager et al. [32] found, in rats, that high doses of L-lysine can cause acute renal failure, whereas clinical studies by Abel et al. [33] suggested that intravenously administered amino acid solutions might even have a protective effect for the renal function of patients with acute tubular necrosis. It is of interest that, unlike other inborn errors of amino acid metabolism, the known genetic defect of familial hyperlysinemia is not associated with any known symptoms [34]. However, large quantities of L-lysine may be expected to disturb the balance of the physiological amino acid metabolism. Since the toxicity of lysine seems to be restricted to its L-isomer [10], D-lysine should be metabolically inert and applicable without endangering the metabolic balance between the different amino acids and their metabolites [10]. This is especially so given that no transmembrane transporters capable of taking up D-lysine are known in humans. Indeed, the maximum tolerated dose of D-lysine in mice is approximately 1.4-fold higher than the maximum tolerated dose of its L-isomer [20]. The efficacy of orally administered lysine is encouraging because this would obviate the need for prolonged i.v. infusion and would thus represent a much more convenient method for clinical use. The toxicity of the other agents, such as polylysines, is still poorly understood. The fact that polylysines are used in cell culture as cell-adhesion-mediating agents may suggest that severe toxicity could occur. Furthermore, in vivo polylysines are known to exert protamine-like heparin-antagonistic effects.

Basic amino acids are also able to reduce the renal uptake of antibody fragments and peptides in patients

It is logical that the development of a relatively simple approach to reduce renal retention of labeled antibody fragments or peptides might provide greater opportunities for diagnostic and therapeutic applications of these agents. Therefore, we undertook preliminary clinical studies to investigate whether this methodology will work in patients as well. In a pilot clinical trial [23], five patients were infused with a commercially available nutritive amino acid solution, while 75 control patients received the same volume of saline. The renal uptake of ^{99m}Tc-Fab′ in the amino acid-treated group was significantly lower than that in the control group (11.1%±2.0% injected dose vs 17.7%±7.0% injected dose at 24 h p.i.; $P<0.05$), whereas the uptake of all other organs re-

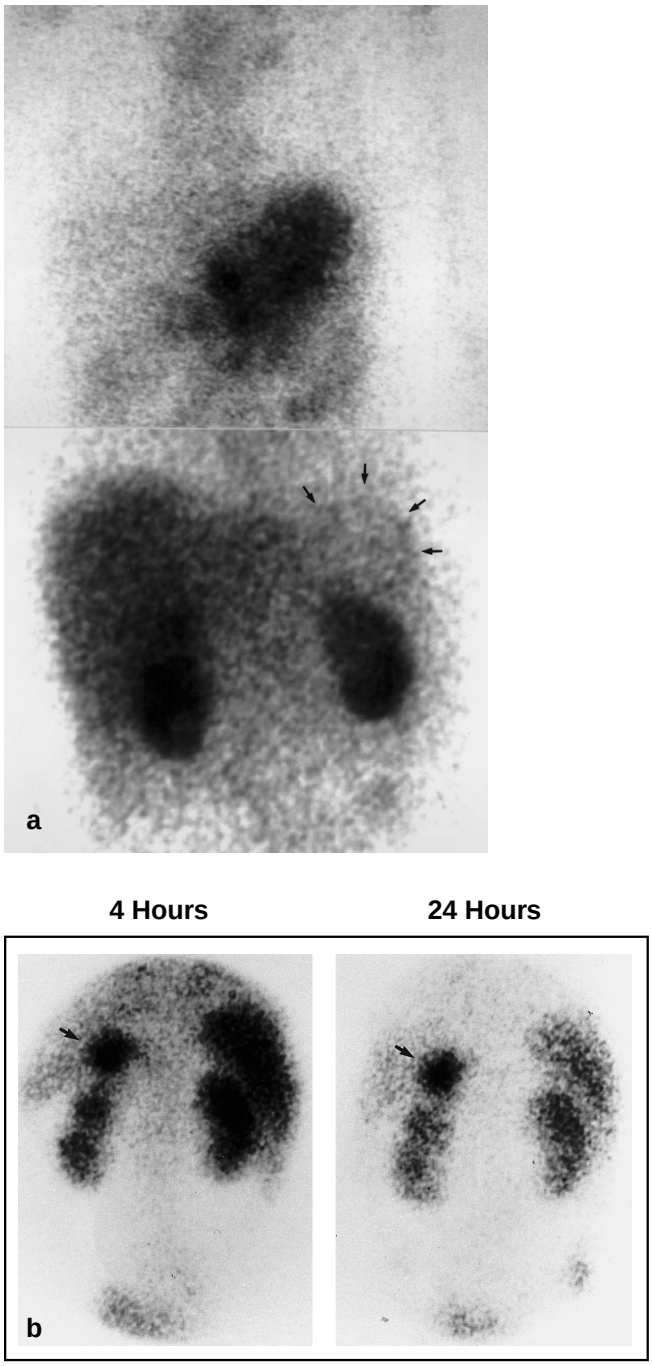


Fig. 3a, b. Consequences of the renal uptake of ^{99m}Tc-labeled Fab′ fragments for clinical diagnostic accuracy. **a** Intraindividual comparison of ^{67}Ga citrate and the ^{99m}Tc-labeled anti-CD22 antibody, LL2, in correctly diagnosing a primary gastric MALT lymphoma. Whereas diffuse lymphoma infiltration of the stomach wall is clearly seen with ^{67}Ga (*upper panel*), the high renal uptake of Fab′ fragments makes the diagnosis of gastric involvement more difficult (*arrowheads*, *lower panel*). **b** In contrast, in a patient infused with a commercially-available amino acid solution, the renal uptake of ^{99m}Tc-anti-CEA Fab′ fragments (clone F023C5) is significantly reduced, facilitating the diagnosis of a gastric cancer primary (*arrow* (modified from [23]). In comparing **a** (*lower panel*) and **b**, note that the kidney/liver ratio is significantly reduced by amino acid treatment

mained unaffected (Fig. 3). Size exclusion chromatography of urine from the amino acid-treated patients showed that a significantly higher amount of excreted activity was bound to intact Fab′ than in the control group [23]. We concluded that the renal uptake of monoclonal antibody fragments in patients can be reduced significantly by amino acid infusion, even at considerably lower doses than were found to be safe and effective in animals [21–23]. As in animals [21, 22], the mechanism seems to rely on inhibition of the reabsorption of tubular-filtered proteins by the proximal tubule cells. These results encourage further clinical trials, but thorough toxicity studies in primates are warranted, especially since the toxicity of L-lysine seems to be species-related [31–33] (see above).

Recently, Carrasquillo and co-workers [25] repeated these clinical studies in other primate species and found a similar dose-effect relationship between the amount of amino acids infused intravenously as commercially available nutritive solution and the reduction in the renal uptake of dsFv fragments in baboons [23, 25]. Unfortunately, no further toxicological studies, using varying amounts of these amino acids, were performed in the course of these primate studies.

Therapeutic application of the established methodology of kidney uptake reduction

Based on our preclinical and preliminary clinical results, we embarked on additional studies to determine what impact this methodology of inhibition of the renal uptake of antibody fragments might have on radionuclide therapy for cancer [24]. First, studies were carried out with ^{188}Re-labeled Fab′ fragments of the monoclonal antibody Mu-9, which is directed against colon-specific antigen-p (CSAp) [22]. CSAp is a mucin antigen present in more than 80% of human colon cancers. Surprisingly, at an injected activity of 1.25 mCi ^{188}Re-Fab′ (which would deliver close to 100 Gy to mouse kidneys) no acute nephrotoxicity was observed, as indicated by persisting normal serum blood urea nitrogen (BUN) and creatinine levels. However, after approximately ten 10–12 weeks, rising serum BUN levels were observed in non-lysine-treated animals, at which time the first deaths occurred. Renal histology showed focal glomerulosclerosis, tubular atrophy, and fibrinoid necrosis, which are well known to be typical for chronic radiation nephropathy [22]. It is especially noteworthy in this context that, as is known from external beam radiotherapy of patients, radiation nephrotoxicity can occur months or even years after the radiation treatment, potentially without any prior acute prodromal symptoms. In none of the lysine-protected ^{188}Re-Fab′-treated animals, however, were any histological changes or BUN abnormalities observed [22].

Due to the clearly superior tumor dosimetry of ^{90}Y-labeled Fab fragments of the high-affinity anti-CEA antibody, MN-14 (cf. Table 1), this agent was chosen for a further detailed evaluation of our amino acid methodology in radioimmunotherapy with radiometal-labeled immunoconjugates [24]. Studies were performed in order (a) to assess whether this methodology will benefit therapy with ^{90}Y-labeled antibody fragments [Fab, $F(ab)_2$], (b) to establish the relationship between radiation dosimetry and observed biological effects, and (c) to compare the antitumor efficacy of antibody fragments with whole IgG [24, 30]. The maximum tolerated dose (MTD) and the dose-limiting organ toxicity of ^{90}Y-labeled anti-CEA MN-14 monoclonal antibodies [Fab, $F(ab)_2$, and IgG] were determined in GW-39 human colon cancer xenograft-bearing nude mice. Mice were studied with or without kidney protection by administration of D-lysine, and with or without bone marrow transplantation (BMT), as well as with combinations thereof. Blood counts, kidney and liver function parameters, and tumor growth were monitored at weekly intervals after therapy. Dosimetry was calculated from biodistribution studies using ^{88}Y-labeled antibody, using actual mouse anatomy as represented by nuclear magnetic resonance imaging (MRI) with a three-dimensional internal dosimetry package (3D-ID) developed by Sgouros et al. [30]. This mouse-specific approach seemed to be particularly important since cross-fire between organs is an important issue with high-energy β-emitters, such as ^{90}Y, which have a path length of several millimeters in small animals such as nude mice (Fig. 4).

The kidney was the first dose-limiting organ with the use of Fab fragments [24, 30]. Acute radiation nephritis occurred at injected activities ≥325 μCi (corresponding to a renal dose of approximately 100 Gy), and chronic nephrosis at doses ≥250 μCi (corresponding to a renal dose of approximately 70 Gy; Figs. 5, 6) [24, 30]. Activities of 200 μCi were tolerated by 100% of the animals (i.e., this represented the MTD). Administration of lysine decreased the renal dose by approximately fivefold, enabling an increase in the MTD by 25% (to 250 μCi). At this point myelotoxicity became dose limiting, despite red marrow doses of less than 5 Gy. By using BMT and lysine, the MTD could be doubled from 200 to 400 μCi, at which dose no biochemical or histological evidence of renal damage was observed (kidney dose (40 Gy; cf. Figs. 5, 6). At injected activities ≥325 μCi without kidney protection, and with a hepatic self-to-self dose of only 4 Gy, rising liver enzymes were observed. This could be explained only by cross-organ radiation from radioactivity in the kidneys (up to >150 Gy in the immediate neighborhood of the right kidney; cf. Fig. 4). The MTD of $F(ab)_2$ fragments could be elevated only by the combination of BMT and lysine, probably due to cross-fire from the kidneys to the bone marrow of the spine and to the spleen (Fig. 4). With IgG the bone marrow alone was dose limiting. The dose intensification which was made possible by lysine administration led to improved antitumor efficacy (Fig. 7). Tumor dosimetry correlated well with antitumor effects (≥10 Gy led to growth inhibition for 5 weeks, and ≥20 Gy did so for

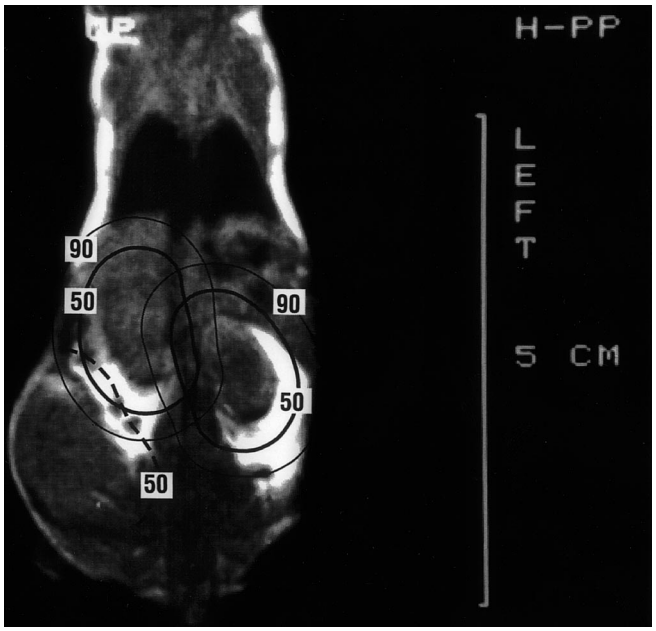


Fig. 4. Coronal MRI slice through a s.c. tumor-bearing nude mouse in the plane of the kidneys (Siemens Magnetom 1.5 T, Siemens, Erlangen, Germany). The *solid lines* around both kidneys indicate the region in which 50% and 90%, respectively, of the β-particles of ^{90}Y originating from the kidneys will be absorbed; the *dashed lines* indicate the 50% region for β-particles originating from the tumor. Due to the long path length of ^{90}Y′s β-particles, cross-fire radiation between the kidneys and liver, the spleen and bone marrow (spine), and the tumor and bone marrow (pelvis) may well play an important role

≥13 weeks). Fab was more effective than $F(ab)_2$, consistent with its more favorable dosimetry. Fab may also be more effective than IgG due to a higher dose rate and more homogeneous distribution [24, 30].

Interestingly, two histologically distinct pathologies were generated in kidneys of animals given ^{90}Y-labeled fragments without lysine protection (cf. Fig. 5). At high-dose therapy (renal doses above approximately 100–140 Gy), an acute radiation nephritis-like picture with uremia and pronounced tubular damage but only slight glomerular change was observed in the initial weeks after radiation (Fig. 5a). The proximal tubules were shown by microautoradiography to be the predominant site of reabsorption and storage of the labeled antibody fragments. The second category occurred after more than 5 weeks at considerably lower renal doses (80–100 Gy), and was characterized by pronounced glomerular and vascular damage consistent with chronic radiation nephrosis (Fig. 5b, [24]). Interestingly, in this group there were no acute biochemical or histological signs in the early weeks after radiation to indicate radiation damage to the kidneys. It is also noteworthy that, at the MTD with lysine and BMT, no histological or biochemical evidence of any kidney damage occurred at a renal dose of approximately 20 Gy, which is also considered safe in external beam radiotherapy (Fig. 5c, [24]).

These studies clearly indicate that two potentially dose-limiting organ systems have to be taken into consideration, when using radiolabeled antibody fragments or peptides with therapeutic intent. The first is the bone marrow, which is well known to be the most radiosensitive mammalian tissue, and thus the dose-limiting organ most frequently encountered in external beam whole-body irradiation or the therapeutic application of internal emitters. Doses causing potentially life-threatening leuko- and thrombocytopenia have been shown to vary between species [35, 36] and to be strongly dose rate dependent [24, 37]. Depending on these parameters, the maximum tolerated red marrow doses usually range between 2.5 and 15 Gy [24, 35, 37]. The second dose-limiting organ to be considered is the kidney. Insufficient data are available on the influence of species or dose rate with regard to the kidney. However, renal doses below 20 Gy are generally considered safe, whereas above this threshold chronic or, at even higher doses, acute radiation nephritis may result [24, 38–40]. The application of amino acid technology may allow the dose-limiting effect of the kidney to be overcome, as has been achieved for dose-limiting myelotoxicity by means of bone marrow or stem cell transplantation [36].

Summarizing, inhibition of the renal uptake of radiolabeled proteins and peptides can be achieved by the administration of cationic amino acids or their derivatives. This technology reduces the renal dose so that the bone marrow, rather than the kidney, becomes dose limiting. This should allow a considerable dose escalation of the radioconjugate or peptide. The antitumor effects observed with Fab fragments in several preclinical models have been quite promising and indicate their potential superiority to complete IgG [24, 37].

Future prospects and limitations of the therapeutic application of radiolabeled antibody fragments and peptides

Encouraging results have been achieved in reducing the renal uptake of antibody fragments and peptides. Nevertheless, careful evaluation of the toxicity of larger amounts of the basic amino acids, especially D-lysine, is warranted in humans before the application of this technology on a larger scale in clinical trials. In such trials the issues of radiation nephrotoxicity of the radiotherapeutic agent and potentially toxic side-effects of the compounds used for renal uptake reduction will need to be carefully addressed. Clinical signs of radiation toxicology that might occur may exceed those appreciable in a mouse model, such as renal failure and uremia. Other effects, such as (malignant) hypertension, are known to be related to renal irradiation in humans. Obviously, as is known from experience with external beam radiotherapy, the occurrence of chronic radiation nephrosis does not necessarily require a phase of acute

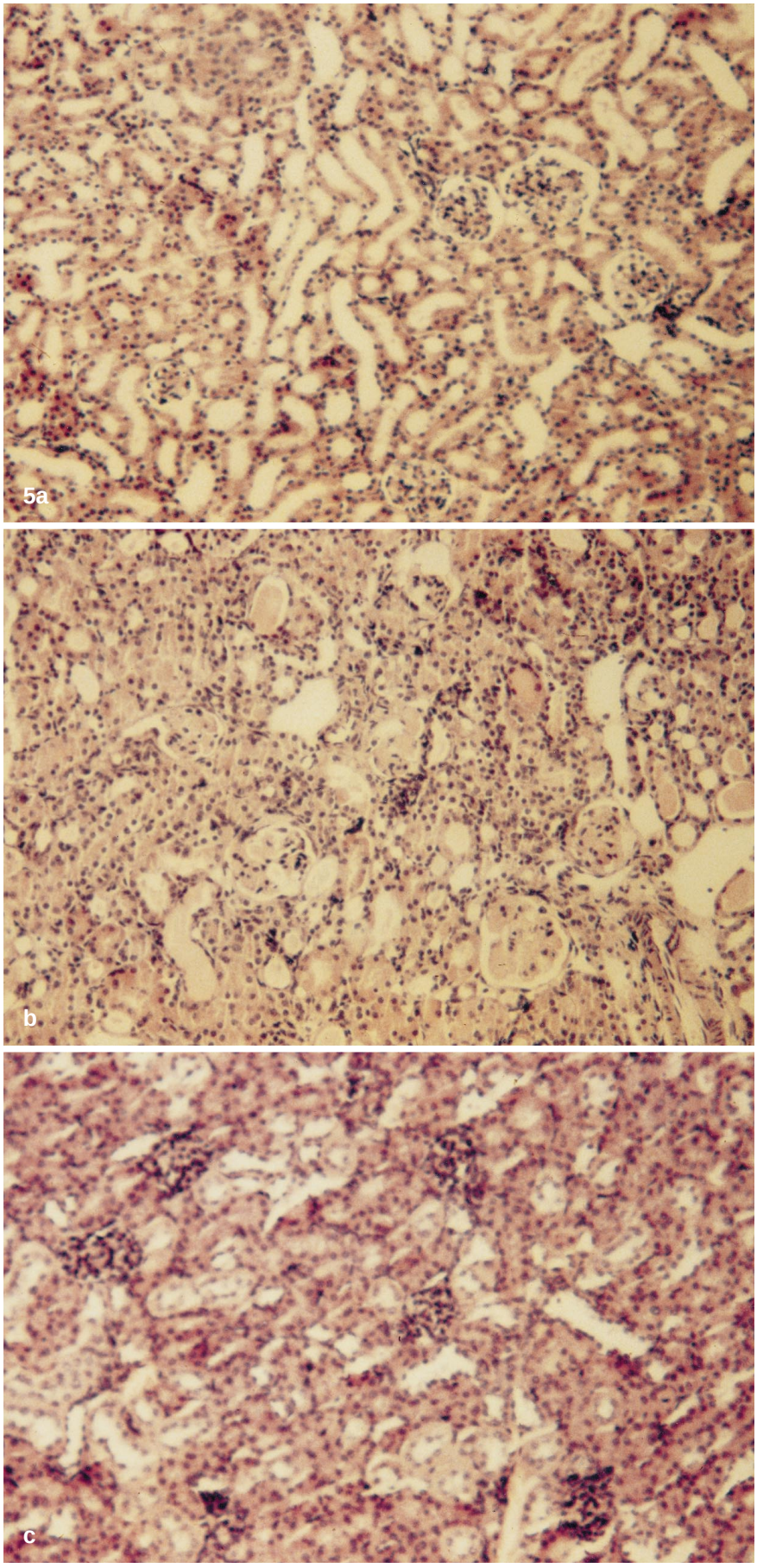


Fig. 5a–c. Histological changes in the kidneys following therapy with ^{90}Y-Fab. **a** Acute radiation nephritis 2 1/2 weeks after the injection of 400 µCi without kidney protection by lysine: marked tubular dilatation and atrophy are the major histological findings (renal dose approximately 100 Gy). **b** Chronic radiation nephropathy 10 weeks after 250 µCi without lysine is characterized by severe glomerular necrosis, tubular atrophy, arteriolar intimal thickening, and fibrinoid necrosis (renal dose approximately 70 Gy). **c** The kidneys of an animal treated with 400 µCi and BMT under lysine protection do not show any major histopathological changes (renal dose below 20 Gy) at 15 weeks following radioantibody injection. (modified from [24])

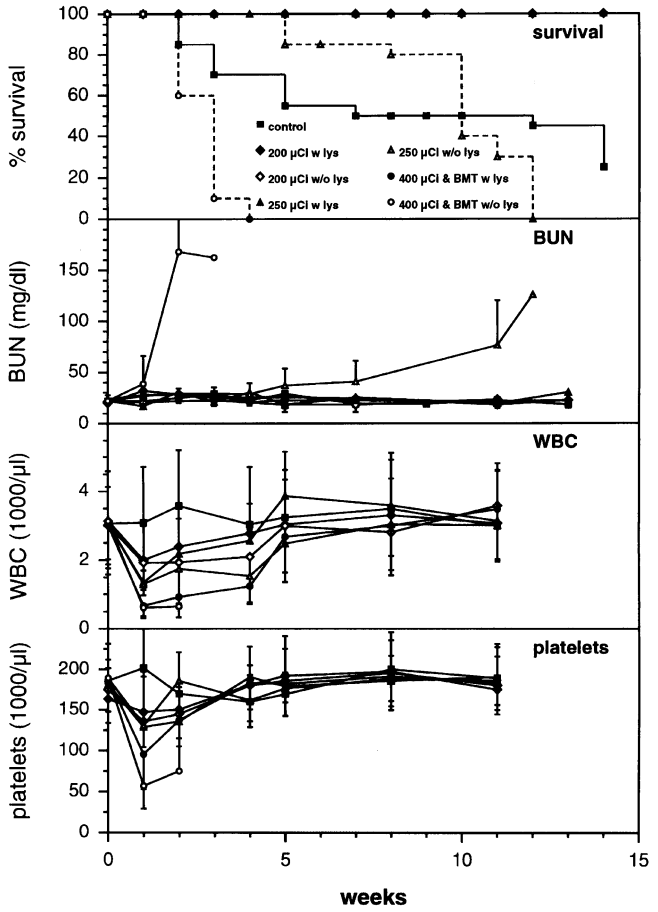


Fig. 6. Toxicity of ^{90}Y-labeled Fab fragments of the high-affinity anti-CEA antibody MN-14 at escalating administered activities. Two hundred µCi is the MTD without artificial support, merely causing modest and transient myelotoxicity. At activities >200 µCi, chronic radiation nephrosis becomes the dose-limiting organ toxicity, as represented by rising BUN levels several weeks after therapy. With lysine, dose escalation to 250 µCi is possible, with the bone marrow becoming the next dose-limiting organ. The combination of lysine and BMT permits dose intensification up to 400 µCi without dose-limiting nephrotoxicity. In contrast, 400 µCi without lysine leads to acute nephritis, as represented by steeply rising BUN levels (cf. Fig. 5). (modified from [24])

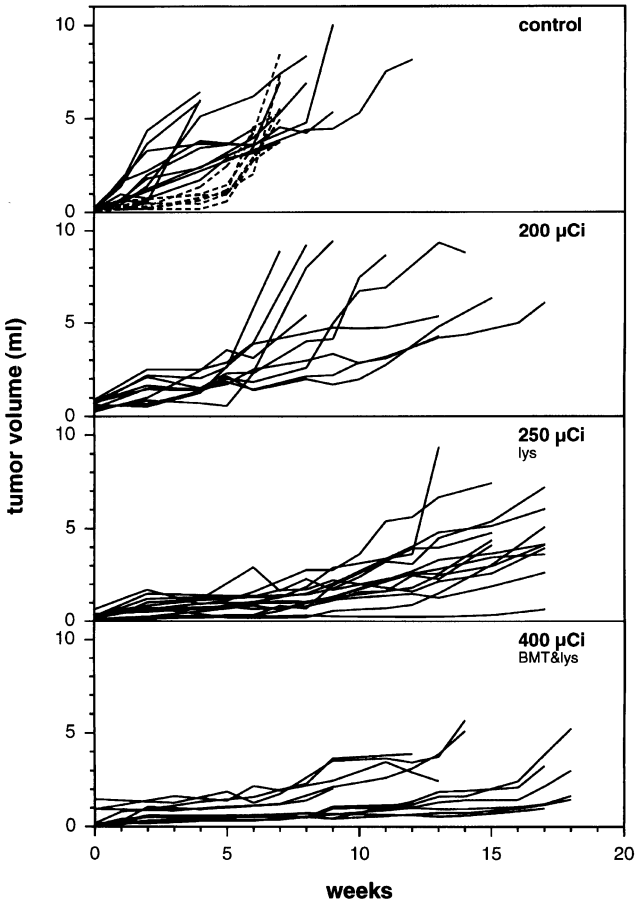


Fig. 7. Comparative anti tumor effects in the subcutaneous GW-39 model with ^{90}Y-MN-14 Fab at the respective MTDs (200 µCi without artificial support, 250 µCi with lysine, and 400 µCi with lysine and BMT), in comparison to controls which were either untreated (*solid lines*) or treated with 400 µCi irrelevant Fab′ (LL2 anti-CD22 Fab′, *dashed lines*). The figure clearly shows that dose intensification made possible by overcoming the nephrotoxicity leads to improved antitumor efficacy

radiation nephritis [38–40]. This fact has to be carefully considered in all clinical dose-escalation trials involving mainly terminally ill patients, since it is known from external beam radiation data that such chronic nephrosis can occur as late as 5 years or more after irradiation [38–40]. The observation time in the usual phase I patient studies may be much too short to address this issue adequately.

We nevertheless believe that such studies are urgently needed, since this methodology may be crucial for the future clinical application of therapeutic agents based on proteins or receptor-binding peptides. The above-cited studies on the physiology of the renal handling of peptides have improved our comprehension of the molecular characteristics that facilitate (e.g., positive charges in the form of amino groups) or hinder the renal uptake of such radiopharmaceuticals. Future studies will show whether appropriately designed peptides may exhibit lower renal uptake in their own right, without the need for additional pharmacological intervention.

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