EDITORIAL

Potential Use of Amino Acid Cocktails to Reduce Renal Tubular Reabsorption of Radioconjugates

An explosion of knowledge in modern biology has led to new possibilities for detection of cancer by radionuclide scintigraphy as well as exciting approaches for delivery of systemic, tumor-targeted radiotherapy. To realize this potential, rapid excretion of non-tumor bound radioactivity is necessary. Small, radioactive oncophilic molecules such as monoclonal antibody (MAb) fragments or peptides provide rapid tumor targeting. If these molecules are labeled with a radiometal, renal uptake of the radiometal ranges from 6% to 25% of the injected dose (*1–5*), thereby compromising the potential for cancer detection in the kidney region and limiting the therapeutic potential. Renal accumulation of radiometal results from glomerular filtration of molecules less than 60 kD, intraluminal degradation followed by tubular reabsorption and intracellular lysosomal degradation (*6,7*). The kidney is a major site for catabolism of low molecular weight proteins and, whereas radioiodine is quickly released from the cells, radiometals are retained and likely transferred to intracellular metalloproteins (*7,8,9*).

PRELIMINARY STUDIES OF RENAL UPTAKE REDUCTION

Studies of renal physiology starting as early as 1806 show that small proteins and amino acids are filtered by the kidney and greater than 99% of amino acids are normally reabsorbed by the tubular cell (*6,7,10*). Tubular reabsorption is accomplished by active transport mechanisms that are saturable. In 1977, Morganson and Sölling (*11*) demonstrated that an infusion of lysine and arginine blocked renal tubular re-absorption of peptides. Recent studies performed in mice showed that lysine and other basic amino acids decreased renal uptake of co-injected radiometal labeled peptides and MAb fragments (*12–14*). In 1993, Hammond et al. published a study of 16 patients in which an infusion of lysine and arginine was utilized to reduce renal uptake of indium-labeled pentetreotide (*15*). The renal uptake, compared to the controls, was thought to be reduced at 4 hr in this study although the analysis was not performed in the usual quantitative manner.

A preliminary clinical study evaluating the reduction of renal uptake of ^{99m}Tc-Fab′ fragments by co-infusion of an amino acid and electrolyte solution, Periamin X™, is reported in this issue of *JNM* (*16*). At 24 hr postinjection, the renal uptake of radioactivity was 11.1 ± 2.0% of the injected dose, whereas previously studied patients that received an equivalent volume of normal saline had a renal uptake of 17.7 ± 7.0%. This difference between the renal uptake in the two groups had a level of significance of $p < 0.05$, although no statistically significant differences were seen at the earlier time point. The clearances of ^{99m}Tc from organs other than the kidney, and from the whole body, were similar for the two patient groups.

Chromatography of the urine from the amino acid treated group suggested that the radioactivity was largely associated with intact Fab′, whereas data for urine from the control group suggested that the radioactivity was associated with degraded peptides and smaller protein fragments. The authors provided an important conclusion that "the mechanism of the reduction of the renal uptake of radiolabeled fragments seems, therefore, to rely on an inhibition of the tubular reabsorption of glomerularly-filtered proteins, so that they appear directly in the urine without prior lysosomal degradation to low-molecular-weight compounds in the proximal tubule cells." This agreed with the results from studies in mice that they had recently reported (*14*).

NORMAL RENAL PHYSIOLOGY

In considering these data, it is useful to review general aspects of renal physiology (*6,7,9*). Renal excretion of a substance depends principally on: (a) the filtered load, i.e., the product of glomerular filtration rate and the concentration of the substance in the filtrate, (b) metabolic degradation (hydrolysis) of the substance within the lumen of the tubule and (c) tubular reabsorption and/or secretion of the substance. Tubular mechanisms that have been described for handling peptides, amino acids and small proteins include: (a) carrier/receptor mediated resorption including resorption of amino acids after luminal protein/peptide hydrolysis, (b) resorption by endocytosis and subsequent lysosomal degradation and (c) peritubular uptake. Many oligopeptides can be hydrolyzed so quickly that the metabolites can be absorbed within the proximal nephron. The total concentration of free plasma amino acids in humans is 2.5 m*M* and amino acids enter the tubular cells not only from the lumen, but from the blood as well. Urinary excretion of amino acids is very small, especially when compared to the filtered load. In fact, only 1% of the filtered load of amino acids is excreted by normal human kidneys, and the most essential amino acids are reabsorbed to the greatest degree. The transport mechanisms, however, for amino acids into tubular cells are saturable for most amino acids, so that a substantial increase in the filtered load of amino acids leads to aminoaciduria. Experiments wherein amino acid was infused have increased the glomerular filtration rate especially when a high load of sodium and water was co-infused, thus increasing urine flow rates.

CLINICAL STUDY OF RENAL UPTAKE REDUCTION

Certain aspects of the data and design of the clinical trial of Behr et al. (*16*), preclude definitive declaration as to the potential worth of amino acid infusions as a mode of decreasing renal parenchymal uptake of radioconjugates in patients. The size of the experimental group was small, renal function, e.g., creatinine clearance and urinary protein were not determined, and the nature and amounts of the aminoaciduria was not characterized. Additionally, the control group received a saline infusion, whereas the experimental group received a "cocktail" of amino acids, salts and xylitol. The amount of xylitol (100 g) could have been sufficient to induce an osmotic diuresis and alter urinary pH, perhaps increasing filtration of radioimmunoconjugate and/or affecting intraluminal hydrolysis of peptides and small proteins. This initial clinical trial (*16*), however, has important implications, and the authors acknowledged that optimization of the kind and amount of amino acids needed further study in a larger patient population.

To evaluate the potential for further reducing tubular reabsorption of radiometal labeled proteins and peptides and their subsequent lysosomal digestion and associated radiometal transchelation, more infor-

Received Oct. 16, 1995; accepted Oct. 18, 1995.
For correspondence or reprints contact: Sally J. DeNardo, MD, University of California, Davis Molecular Cancer Institute, Radiodiagnosis/Therapy, 1508 Alhambra Blvd., Rm. 214, Sacramento, CA 95816.

mation on the pharmacokinetic changes caused by a particular amino acid regimen is needed. Renal uptake of radiometals from radioconjugates could be influenced, at least to some extent, by the glomerular filtration rate, urinary flow rate, urinary pH and solute and protein/amino acid concentrations. A significant degree of aminoaciduria should occur in the amino acid treated patients if treatment with amino acids saturates the absorptive mechanisms, either by blocking the negative tubular membrane charges or by blocking specific receptors (or both). Thus, at a "blocking" dose, the urine should demonstrate increase in excretion of both radioconjugate and non-radioactive infused amino acids. Urinary amino acid profiles could, therefore, be correlated with inhibition of tubular absorption as well as decreased intracellular metabolism of filtered radioconjugates.

CONCLUSION

The multiplicity of variables make it difficult to predict the effect of the experimental manipulation a priori. The hypothesis and preliminary work in animals and patients suggesting that an amino acid infusion mixture results in decreased tubular absorption of peptides and small radioimmunoconjugate proteins is certainly worthy of further study. Only a modest reduction in renal uptake was found in this initial clinical study (*16*), but the results are interesting and the implications of potentially great importance. Additional clinical trials with documentation of renal function, and quantitative amino acid and protein excretion profiles for radioactive and nonradioactive species in the baseline and postinfusion urine, are warranted. Knowledge of ways to decrease renal uptake of radiometals would be invaluable for tumor scintigraphy and targeted radiotherapy.

Sally J. DeNardo
Robert T. O'Donnell
Gerald L. DeNardo
University of California
Sacramento, California
Veterans Administration Northern California
Healthcare System
Sacramento, California

REFERENCES

1. Behr TM, Becker WS, Bair HJ, et al. Comparison of complete versus fragmented technitium-99m-labeled anti-CEA monoclonal antibodies for immunoscintigraphy in colorectal cancer. *J Nucl Med* 1995;36:430–441.
2. Baum RP, Niesen A, Hertel A, et al. Initial clinical results with technetium-99m-labeled LL2 monoclonal antibody fragment in the radioimmunodetection of B-cell lymphomas. *Cancer* 1994;73:896–899.
3. Krenning EP, Bakker WH, Kooij PP, et al. Somatostatin receptor scintigraphy with indium-111-DTPA-D-Phe-1-octreotide in man: metabolism, dosimetry and comparison with iodine-123-tyr-3-octreotide. *J Nucl Med* 1992;33:652–658.
4. Behr T, Becker W, Hannappel E, Goldenberg DM, Wolf F. Targeting of liver metastases of colorectal cancer with IgG $F(ab')_2$, and FAB' anti-CEA antibodies labeled with ^{99m}Tc: the role of metabolism and kinetics. *Cancer Res* (suppl) 1995:5777S–5785S.
5. Buijs WCAM, Massuger LFAG, Claessens RAMJ, Kenemans P, Corstens FHM. Dosimetric evaluation of immunoscintigraphy using indium-111-labeled monoclonal antibody fragments in patients with ovarian cancer. *J Nucl Med* 1992;33:1113–1120.
6. Silbernagl S. The renal handling of amino acids and oligopeptides. *Physiol Rev* 1988;68:912–986.
7. Maack T, Johnson V, Kan ST, Figueiredo J, Sigulem D. Renal filtration, transport and metabolism of low molecular weight proteins: a review. *Kidney Int* 1979; 16:251–270.
8. Duncan JR, Welch MJ. Intracellular metabolism of indium-111-DTPA-labeled receptor targeted proteins. *J Nucl Med* 1993;34:1728–1738.
9. Motta-Hennessy C, Sharkey RM, Goldenberg DM. Metabolism of indium-111-labeled murine monoclonal antibody in tumor and normal tissue of the athymic mouse. *J Nucl Med* 1990;31:1510–1519.
10. Kirk E. Studies on the amino acid clearance. *Acta Med Scand* 1936;89:450–453.
11. Morgenson CE, Sölling K. Studies on renal tubular protein re-absorption: partial and near complete inhibition by certain amino acids. *Scand J Clin Lab Invest* 1977;37:477–486.
12. Pimm MV, Gribben SJ. Prevention of renal tubule re-absorption of radiometal (indium-111) labeled Fab fragment of a monoclonal antibody in mice by systemic administration of lysine. *Eur J Nucl Med* 1994; 21:663–665.
13. Behr TM, Sharkey RM, Juweid ME, Aninipot R, Goldenberg DM. Reduction of the kidney uptake of Fab' fragments of monoclonal antibodies: animal experimental and initial clinical results [Abstract]. *Proc Am Assoc Cancer Res* 1995;86:617.
14. 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.
15. Hammond PJ, Wade AF, Gwilliam ME, et al. Amino acid infusion blocks renal tubular uptake of indium-labelled somatostatin analogue. *Br J Cancer* 1993;67: 1437–1439.
16. Behr TM, Becker WS, Sharkey RM, et al. Reduction of the renal uptake of monoclonal antibody fragments by amino acid infusion: initial clinical results. *J Nucl Med* 1996;37:829–833.

Exercise Renography in Untreated Subjects with Essential Hypertension

Eugene J. Fine, M. Donald Blaufox, Jon D. Blumenfeld, John H. Laragh, Kwang J. Chun, Sherman L. Heller and Josephine A. Bongiovanni
Department of Nuclear Medicine, Montefiore Medical Center, Moses Division, The Albert Einstein College of Medicine; and Department of Medicine, Cornell University Medical Center, Ithaca, New York

Exercise induced renal dysfunction is reported to occur in treated hypertensive patients but not seen normotensive subjects. It is unclear if this phenomenon is related to the disease or to treatment. **Methods:** Four normal volunteers and 15 hypertensive subjects (antihypertensive medications were discontinued for more than 4 wk) were studied with upright radionuclide renography at rest and during bicycle exercise. The amount of exercise was sufficient to increase the heart rate at least 20 bpm above the resting value. All subjects were healthy, without evidence of left ventricular hypertrophy renal disease or hypertensive retinal disease. BUN, serum creatinine concentration and urinalysis were normal in all subjects. Renograms were performed for 12–15 min after injection of either 1 mCi [^{123}I]orthoiodohippurate (OIH) or 2–7 mCi ^{99m}Tc-mercaptoacetyltriglycine (MAG3). Visual analysis and mean transit time calculation were performed on the rest and exercise studies. **Results:** Seven of 14 hypertensive subjects and none of the normal volunteers demonstrated abnormal prolongation in renal transit during exercise which was not seen on the resting renogram. Four of these seven subjects had a history of hypertension for 2 yr or less. **Conclusion:** About 50% of individuals with mild-to-moderate hypertension and normal renal function may have abnormal renal transit of renal excretion agents during exercise, although their baseline studies are normal. This finding is unassociated with therapy and appears to be related directly to the pathophysiology of essential hypertension.

Key Words: renal hypertension; iodine-123-OIH; technetium-99m-MAG3; renal circulation

J Nucl Med 1996; 37:838–842

Received Mar. 10, 1995; revision accepted Oct. 8, 1995.
For correspondence or reprints contact: Eugene J. Fine, MD, Bronx Municipal Hospital Center, Nuclear Medicine Department, Jacobi Hospital BN13, Pelham Pkwy. and Eastchester Rd., Bronx, NY 10461.