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In-vivo Evaluation of Indium-111-Diethylenetriaminepentaacetic Acid-labelling for Determining the Sites and Rates of Protein Catabolism in Mice

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Abstract

Pharmacokinetic analyses of protein pharmaceuticals are of prime importance for their clinical application. Because many proteins have pharmacological activity at low concentrations, radiolabelling of proteins is widely used to identify the sites and determine the rates of protein catabolism in-vivo due to the high sensitivity of detection of radioactivity. Recently, a metallic radionuclide, ^{111}In, has been used to trace the pharmacokinetics of proteins of interest after conjugation of the proteins with diethylenetriaminepentaacetic acid (DTPA). In this study, galactosyl-neoglycoalbumin (NGA) was reacted with the cyclic dianhydride of DTPA and labelled with ^{111}In to estimate the validity of this radiolabelling procedure for pharmacokinetic analyses. For comparison, we also evaluated direct radioiodination, because directly-radioiodinated proteins are widely used to assess the pharmacokinetics of proteins of interest.

The hepatic radioactivity profile after intravenous injection of [^{131}I]NGA or [^{111}In]DTPA–NGA into mice was analysed pharmacokinetically, and the first-order rate constant representing the elimination of the respective radiometabolite from hepatic parenchymal cells was determined.

The results indicated that direct radioiodination is inappropriate for pursuing the pharmacokinetics of the proteins, because of rapid elimination of the radioactivity from the sites of protein catabolism. These findings also implied that the [^{111}In]DTPA label could be used to identify the catabolic sites and determine the rates of catabolism of proteins with relatively short biological half-lives, although characterization of radiolabelled species at the sites of accumulation would be required for accurate determination of the catabolic sites of proteins.

With advances in molecular genetics, pharmaceutical applications of recombinant proteins have attracted much attention (Koths 1995). Chemical modifications of proteins are extensively investigated for targeted delivery or for prolonging retention in the blood circulation (Takakura et al 1996). Pharmacokinetic analyses such as the estimation of catabolic sites and rates of newly developed or chemically modified proteins are of prime importance for assessing their pharmaceutical applications. Because many proteins are pharmacologically active at very low concentrations, pharmacokinetic analyses should be performed at low protein concentrations. Radiolabelling of proteins has been widely used for this purpose, because of the high sensitivity of detection of radioactivity (femtomolar concentrations). In addition, single-photon emission computed tomography (SPECT) has recently become available for pursuing the real-time localization of the radioactivity in living small laboratory animals (Weber et al 1994; Ishizu et al 1995). This procedure enables real-time tracing of the in-vivo behaviour of proteins in an animal by external measurement of the radioactivity.

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For accurate identification of the sites and estimation of the rates of protein catabolism in-vivo, long residence times of the radioactivity at the catabolic sites are required. Recent metabolic studies of radiolabelled proteins have indicated that the in-vivo behaviour of radiometabolites generated after lysosomal proteolysis is of critical importance in determining the levels of radioactivity in tissues in which proteins are catabolized (Duncan & Welch 1993; Arano et al 1994a, b, 1995; Rogers et al 1995, 1996; Duncan et al 1997; Mukai et al 1998). Indeed, several radiolabels with saccharide units have been developed for these purposes because of the generation of radiometabolites with limited ability to diffuse through the lysosomal (or cell) membrane (Thorpe et al 1993). These radiolabelling reagents are called residualizing labels. Recently, a metallic radionuclide, indium-111 (^{111}In), has been used as a residualizing label after reaction of proteins with cyclic diethylenetriaminepentaacetic dianhydride (cDTPA) (Fujita et al 1992; Nishikawa et al 1992). ^{111}In emits γ-rays of adequate energy (173 and 247 keV) both for measuring the radioactivity with a γ-counter and for external imaging using SPECT for small animals. The physical half-life (2·8 days) of this radionuclide is sufficient for pursuing the pharmacokinetics of proteins with long plasma half-life but not too long for the disposal of radioactive materials. In addition, ^{111}In-labelled proteins are prepared with high radiochemical yields by a simple addition of ^{111}In solution to the conjugate solution, because of a rapid complexation reaction between DTPA-conjugated proteins and ^{111}In ions (Hnatowich et al 1983). As a result, [^{111}In]DTPA-labelled proteins suitable for subsequent pharmacokinetic studies are made available without further purification, which constitutes another advantage of this procedure. To apply [^{111}In]DTPA-labelling to pharmacokinetic analysis of a variety of proteins, assessments of the residence time of the radiometabolite derived from [^{111}In]DTPA-labels at the catabolic sites are required.

Previous studies indicated that galactosyl-neoglycoalbumin (NGA) is a useful polypeptide for estimation of the behaviour of radiometabolites generated after lysosomal proteolysis in hepatic parenchymal cells (Arano et al 1994a, b). Thus, NGA was radiolabelled with ^{111}In after reaction with cDTPA to evaluate the [^{111}In]DTPA-labelling procedure for pharmacokinetic analyses of proteins. The biodistribution of radioactivity after intravenous injection of [^{111}In]DTPA-NGA into mice was analysed, and the disappearance rate constant of the radiometabolite from the mouse liver was determined. Because the direct iodination of proteins is used for pharmacokinetic analyses of proteins (Schmer et al 1978; Xi et al 1996), the rate of disappearance of the radiometabolite from the liver after injection of NGA labelled with iodine-131 (^{131}I) was determined. Validity and caution in using the two radiolabelling procedures for assessment of the sites and rates of protein catabolism in-vivo are discussed.

Materials and Methods

Reagents

$^{111}InCl_3$ (74 MBq mL^{-1} in 0·02 N HCl) was kindly supplied by Nihon Medi-Physics (Takarazuka, Japan). Serum albumin from man (HSA: A-3782) and cDTPA were purchased from Sigma (St Louis, MO) and Dojindo Laboratories (Kumamoto, Japan), respectively. Other reagents were reagent-grade products obtained commercially.

Animals

Animal studies were performed on male ddY mice, 27–30 g. Mice were maintained with standard mouse food and water freely available. Animal studies were performed in compliance with generally accepted guidelines governing such work.

Synthesis of NGA

NGA was prepared by reaction of HSA with cyanomethyl-2,3,4,6-tetra-*O*-acetyl-thio-β-D-galactopyranoside, according to the procedures of Stowell & Lee (1980). The numbers of sugars incorporated into HSA were determined by the phenol-sulphuric acid method (Dubois et al 1956) to be 44.

Preparation of [^{111}In]DTPA-NGA

DTPA-NGA was prepared as described previously (Arano et al 1994a, b). Briefly, a 5 molar excess of cDTPA in dimethylsulphoxide (2·5 mg mL^{-1}) was added to a solution of NGA (10 mg mL^{-1}) in borate-buffered saline (0·05 M, pH 8·5). After stirring gently for 30 min, the conjugate was purified by Sephadex G-50 (Pharmacia Biotech, Tokyo, Japan) column chromatography (1·8 × 40 cm), equilibrated and eluted with 0·1 M citrate buffer (pH 6·0). The conjugate fractions were collected and subsequently concentrated to 5 mg mL^{-1} with the Diaflow system (8 MC model, Amicon Grace, Tokyo, Japan). DTPA-NGA was labelled with ^{111}In by adding $^{111}InCl_3$ (25 μL) to conjugate (100 μL) in the citrate buffer. After incubation for 1 h at room temperature, 144 μL ethylenediaminetetraacetic

acid (10 mM) in acetate buffer (0·1 M, pH 6·0) was added, and the reaction mixture was gently agitated for 1 h at room temperature. The [^{111}In]DTPA-NGA was purified by a centrifuged column procedure (Meares et al 1984) using Sephadex G-50 equilibrated with 0·1 M acetate buffer (pH 6·0). The radiochemical purity of the [^{111}In]DTPA-NGA was determined by cellulose acetate electrophoresis (CAE) and size-exclusion high-performance liquid chromatography (HPLC). CAE was performed at an electrostatic field strength of 0·8 mA cm^{-1} for 45 min in veronal buffer (I = 0·05, pH 8·6); size-exclusion HPLC (7·5 mm × 600 mm Cosmosil 5Diol-120 column; Nacalai Tesque, Kyoto, Japan) was performed with phosphate buffer (0·1 M, pH 6·8) as mobile phase at a flow rate of 1 mL min^{-1}.

Biodistribution of [^{111}In]DTPA-NGA

The protein concentration of [^{111}In]DTPA-NGA was adjusted to 90 μg mL^{-1} with 0·1 M phosphate-buffered saline (pH 6·0). Radiolabelled NGA (9 μg, 15 kBq) was injected into the tail vein of mice. The mice were housed in metabolic cages and urine and faeces were collected for 24 h. At 10 and 30 min and 1, 3, 6 and 24 h postinjection, groups of five mice were killed by decapitation. The organs of interest were removed and weighed and the radioactivity in blood, urine, faeces and organs was determined with a well counter (ARC 2000, Aloka, Tokyo, Japan) and expressed as a percentage of the injected dose.

Pharmacokinetic analysis

Experimental data for [^{131}I]NGA have been reported elsewhere (Arano et al 1994c). A hepatic radioactivity profile after injection of each dose of radiolabelled NGA was fitted to equation 1 by non-linear least-squares analysis (Yamaoka et al 1981):

$$X_1 = De^{-K_1(t-\tau)} \quad (1)$$

(where X_1 is the amount of radiometabolites in the liver, D (%) and τ (h) are the maximum level of radioactivity accumulated in the liver and the post-injection time to reach the maximum level of radioactivity in the liver, respectively, and K_1 is the first-order rate constant for elimination from hepatic parenchymal cells). The first-order rate constant (K_1) was determined and the elimination half-life ($t_{1/2}$) was calculated by dividing 0·693 by K_1.

Results and Discussion

The radiolabelling of DTPA-NGA with ^{111}In was performed by addition of $^{111}InCl_3$ to the conjugate solution. To remove the ^{111}In-species loosely bound to the protein molecule, [^{111}In]DTPA-NGA was purified by a centrifuged column procedure after incubation in the presence of a large amount of ethylenediaminetetraacetic acid. Size-exclusion HPLC and CAE analysis indicated that 99·0% of the radioactivity was in the protein fractions. Because cDTPA has two anhydride groups as the binding sites for proteins, inter- and intramolecular cross-linking is unavoidable during conjugation of cDTPA with polypeptides (Maisano et al 1992). Previous studies also indicated that DTPA molecules that formed intramolecular cross-links acted as chelating sites for ^{111}In and such ^{111}In-species were difficult to remove by gel-permeation chromatography (Arano et al 1996). Although high radiochemical purity of [^{111}In]DTPA-NGA was registered by size-exclusion HPLC and CAE analysis, [^{111}In]DTPA-NGA showed slightly lower radioactivity levels in the liver at an early post-injection time (Table 1) than NGAs labelled with ^{111}In by use of other chelating agents (Arano et al 1994b, 1995). This suggested the presence of small amounts of ^{111}In-species which were attached to the chelating sites produced by intramolecular cross-linking. However, because all the radiolabelled species were present as [^{111}In]DTPA-lysine in the liver 1 h after injection of [^{111}In]DTPA-NGA (data not shown), redistribution of radiolabelled species other than [^{111}In]DTPA-lysine to the liver was negligible. Therefore, the pharmacokinetic estimation of the radiometabolite derived from [^{111}In]DTPA-labels was performed on the basis of the results in Table 1. More than 88% of the injected radioactivity accumulated in the liver 10 min after injection (Table 1). At 24 h postinjection, 51% of the injected radioactivity remained in the liver with 18% and 12% of the radioactivity being excreted in the urine and faeces, respectively.

The radioactivity profile in the liver after intravenous injection of [^{111}In]DTPA-NGA or [^{131}I]NGA was fitted to equation 1, and the first-order rate constant was determined. As shown in Figure 1, the fitting curves were in good agreement with the experimental data. The pharmacokinetic parameters obtained are listed in Table 2. The K_1 value for [^{111}In]DTPA-NGA was identical with that calculated by the compartment model reported previously (Mukai et al 1998) (data not shown). The calculated $t_{1/2}$ value for [^{131}I]NGA was very close to the half-life (10–25 min) of the disappearance of the radioactivity from the rat liver after injection of [^{125}I]asialofetuin (Dunn et al 1979).

Pharmacokinetic analysis of [^{131}I]NGA indicated that the radioactivity was eliminated from the

Table 1. Biodistribution of radioactivity after intravenous injection of [^{111}In]diethylenetriaminepentaacetic acid-galactosyl-neoglycoalbumin into mice.

Organ/tissue*	Time after injection					
	10 min	30 min	1 h	3 h	6 h	24 h
Liver	88·53 ± 4·11	86·89 ± 6·62	86·46 ± 4·96	80·10 ± 3·11	79·10 ± 4·51	51·16 ± 4·01
Intestine	0·69 ± 0·14	2·62 ± 0·59	3·28 ± 0·47	3·79 ± 0·78	3·04 ± 0·93	6·59 ± 0·58
Kidney	0·27 ± 0·03	0·29 ± 0·05	0·59 ± 0·12	1·95 ± 0·26	1·68 ± 0·34	4·36 ± 0·72
Spleen	0·08 ± 0·00	0·05 ± 0·01	0·06 ± 0·02	0·08 ± 0·02	0·06 ± 0·03	0·03 ± 0·02
Stomach	0·14 ± 0·09	0·21 ± 0·07	0·09 ± 0·06	0·14 ± 0·06	0·22 ± 0·15	0·34 ± 0·14
Blood†	1·04 ± 0·23	0·66 ± 0·15	0·76 ± 0·14	0·71 ± 0·60	0·26 ± 0·11	0·27 ± 0·02
Urine	–	–	–	–	–	17·54 ± 4·74
Faeces	–	–	–	–	–	12·02 ± 2·80

* Expressed as a percentage of injected dose (mean ± standard deviation of results from five animals). † Calculated assuming a mouse blood volume of 2·5 mL.

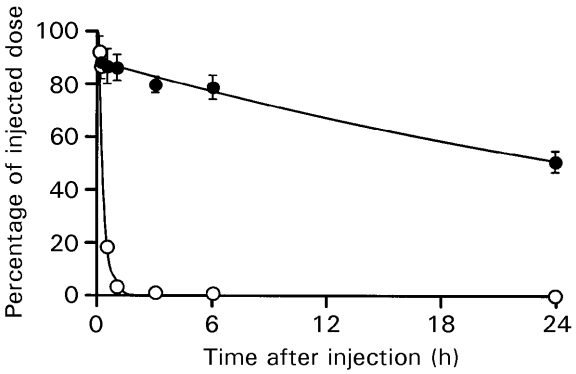

Figure 1. Radioactivity profiles in the liver after intravenous injection of [^{111}In]DTPA-NGA (●) or [^{131}I]NGA (○) into mice. Each point was expressed as the percentage of injected dose (mean ± standard deviation of results from five experiments). The experimental data for [^{131}I]NGA have been reported previously (Arano et al 1994c). Fitting curves were drawn using the values listed in Table 2.

catabolic site very quickly ($t_{1/2}$ = 0·23 h). This rapid elimination was a result of rapid metabolism of radioiodinated proteins to mono- and diiodotyrosine in the lysosome and subsequent conversion of these radiometabolites to free radioiodine by a cytoplasmic enzyme (LaBadie et al 1975). Thus, the direct radioiodination procedure was inappropriate for identification of the sites and estimation of the rates of protein catabolism.

The $t_{1/2}$ of [^{111}In]DTPA-NGA was 130 times larger than that of [^{131}I]NGA (Table 2), suggesting that the [^{111}In]DTPA label could be used for pharmacokinetic analysis of proteins with relatively short biological half-lives although the elimination of radioactivity from the liver might still be too rapid to be applicable to proteins with long biological half-lives (Thorpe et al 1993). The relatively long residence time of the radioactivity after injection of [^{111}In]DTPA-NGA was attributable to the slow rate of elimination of the radiometabolite, [^{111}In]DTPA-lysine, from the lysosomal compartment in hepatic parenchymal cells (Duncan & Welch 1993; Arano et al 1994a; Franano et al 1994). Recent studies also indicated that when [^{111}In]DTPA was attached to the *N*-terminal aspartic acid or phenylalanine residues of proteins, [^{111}In]DTPA-aspartic acid or [^{111}In]DTPA-phenylalanine, respectively, was generated as the final radiometabolite and the residence times of both

Table 2. Pharmacokinetic parameters for elimination of radioactivity from the liver after intravenous injection of radiolabelled galactosyl-neoglycoalbumin into mice.

	[^{111}In]DTPA-NGA	[^{131}I]NGA
Experimental data		
Maximum level of hepatic radioactivity (D,%)*	88·53	92·49
Time after injection when the level of radioactivity in the liver is maximum (τ,h)	0·17	0·083
Calculated data		
First-order rate constant for elimination from hepatic parenchymal cells (K_1,h^{-1})	0·023	3·0
Elimination half-life ($t_{1/2}$,h)	30	0·23

* Percentage of the injected dose. DTPA-NGA, diethylenetriaminepentaacetic acid-galactosyl-neoglycoalbumin; NGA, galactosyl-neoglycoalbumin.

metabolites at the sites of protein catabolism were long (Rogers et al 1995; Bass et al 1998). Thus, the long residence time of the radiometabolites at the catabolic site when [^{111}In]DTPA is attached to the cationic (lysine), anionic (aspartic acid) or aromatic (phenylalanine) amino acid residues of the proteins might be because of the inherent (hydrophilic and anionic) characteristics of [^{111}In]DTPA chelate.

In conclusion, pharmacokinetic analysis of radiometabolite elimination from hepatic parenchymal cells was performed to estimate the applicability of direct radioiodination and [^{111}In]DTPA-labelling procedures for identification of the sites and assessment of the rates of protein catabolism in-vivo. The rate of elimination of the radiometabolite of [^{131}I]NGA was too high to be applicable to the pharmacokinetic analysis of proteins. The residence time of radioactivity in the liver was much longer after administration of [^{111}In]DTPA-NGA, suggesting that [^{111}In]DTPA-labelling might be applicable for identification of the sites and estimation of the rates of catabolism of proteins with relatively short biological half-lives. However, because the formation of intramolecular cross-links is unavoidable when DTPA molecules are attached to proteins via currently available procedures such as reaction with cDTPA, characterization of radiolabelled species in the catabolic sites would be required for accurate estimation of protein pharmacokinetics. Thus, it might be necessary to develop chelating agents that enable DTPA-conjugated proteins to be prepared without inducing intramolecular cross-linking.

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References

Arano, Y., Mukai, T., Uezono, T., Motonari, H., Wakisaka, K., Yokoyama, A. (1994a) Biological comparison of DTPA and SCN-benzyl-EDTA as chelating agents for indium labeling of antibodies. J. Labelled Compd. Radiopharm. 35: 381–383

Arano, Y., Mukai, T., Uezono, T., Wakisaka, K., Motonari, H., Akizawa, H., Taoka, Y., Yokoyama, A. (1994b) A biological method to evaluate bifunctional chelating agents to label antibodies with metallic radionuclides. J. Nucl. Med. 35: 890–898

Arano, Y., Wakisaka, K., Ohmomo, Y., Uezono, T., Mukai, T., Motonari, H., Shiono, H., Sakahara, H., Konishi, J., Tanaka, C., Yokoyama, A. (1994c) Maleimidoethyl 3-(tri-*n*-butylstannyl)hippurate: a useful radioiodination reagent for protein radiopharmaceuticals to enhance target selective radioactivity localization. J. Med. Chem. 37: 2609–2618

Arano, Y., Mukai, T., Akizawa, H., Uezono, T., Motonari, H., Wakisaka, K., Kairiyama, C., Yokoyama, A. (1995) Radiolabeled metabolites of proteins play a critical role in radioactivity elimination from the liver. Nucl. Med. Biol. 22: 555–564

Arano, Y., Uezono, T., Akizawa, H., Ono, M., Wakisaka, K., Nakayama, M., Sakahara, H., Konishi, J., Yokoyama, A. (1996) Reassessment of diethylenetriaminepentaacetic acid (DTPA) as a chelating agent for indium-111 labeling of polypeptides using a newly synthesized monoreactive DTPA derivative. J. Med. Chem. 39: 3451–3460

Bass, L. A., Lanahan, M. V., Duncan, J. R., Erion, J. L., Srinivasan, A., Schmidt, M. A., Anderson, C. J. (1998) Identification of the soluble in vivo metabolites of indium-111-diethylenetriaminepentaacetic acid-D-Phe^1-octreotide. Bioconjugate Chem. 9: 192–200

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956) Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356

Duncan, J. R., Welch, M. J. (1993) Intracellular metabolism of indium-111-DTPA-labeled receptor targeted proteins. J. Nucl. Med. 34: 1728–1738

Duncan, J. R., Stephenson, M. T., Wu, H. P., Anderson, C. J. (1997) Indium-111-diethylenetriaminepentaacetic acid-octreotide is delivered in vivo to pancreatic, tumor cell, renal, and hepatocyte lysosomes. Cancer Res. 57: 659–671

Dunn, W. A., LaBadie, J. H., Aronson Jr, N. N. (1979) Inhibition of ^{125}I-asialofetuin catabolism by leupeptin in the perfused rat liver and in vivo. J. Biol. Chem. 254: 4191–4196

Franano, F. N., Edwards, W. B., Welch, M. J., Duncan, J. R. (1994) Metabolism of receptor targeted ^{111}In-DTPA-glycoproteins: identification of ^{111}In-DTPA-ε-lysine as the primary metabolic and excretory product. Nucl. Med. Biol. 21: 1023–1034

Fujita, T., Nishikawa, M., Tamaki, C., Takakura, Y., Hashida, M., Sezaki, H. (1992) Targeted delivery of human recombinant superoxide dismutase by chemical modification with mono- and polysaccharide derivatives. J. Pharmacol. Exp. Ther. 263: 971–978

Hnatowich, D. J., Layne, W. W., Childs, R. L., Lanteigne, D., Davis, M. A., Griffin, T. W., Doherty, P. W. (1983) Radioactive labeling of antibody: a simple and efficient method. Science 220: 613–615

Ishizu, K., Mukai, T., Yonekura, Y., Pagani, M., Fujita, T., Magata, Y., Nishizawa, S., Tamaki, N., Shibasaki, H., Konishi, J. (1995) Ultra-high resolution SPECT system using four pinhole collimators for small-animal studies. J. Nucl. Med. 36: 2282–2287

Koths, K. (1995) Recombinant proteins for medical use: the attractions and challenges. Curr. Opin. Biotechnol. 6: 681–687

LaBadie, J. H., Chapman, K. P., Aronson Jr, N. N. (1975) Glycoprotein catabolism in rat liver: lysosomal digestion of iodinated asialo-fetuin. Biochem. J. 152: 271–279

Maisano, F., Gozzini, L., de Haën, C. (1992) Coupling of DTPA to proteins: a critical analysis of the cyclic dianhydride method in the case of insulin modification. Bioconjugate Chem. 3: 212–217

Meares, C. F., McCall, M. J., Reardan, D. T., Goodwin, D. A., Diamanti, C. I., McTigue, M. (1984) Conjugation of antibodies with bifunctional chelating agents: isothiocyanate and bromoacetamide reagents, methods of analysis, and subsequent addition of metal ions. Anal. Biochem. 142: 68–78

Mukai, T., Arano, Y., Nishida, K., Sasaki, H., Nakamura, J., Saji, H., Yokoyama, A. (1998) Pharmacokinetic models to evaluate radiolabeling reagents for protein radiopharmaceuticals. Nucl. Med. Biol. 25: 31–36

Nishikawa, M., Ohtsubo, Y., Ohno, J., Fujita, T., Koyama, Y., Yamashita, F., Hashida, M., Sezaki, H. (1992) Pharmacokinetics of receptor-mediated hepatic uptake of glycosylated albumin in mice. Int. J. Pharm. 85: 75–85

Rogers, B. E., Franano, F. N., Duncan, J. R., Edwards, W. B., Anderson, C. J., Connett, J. M., Welch, M. J. (1995) Identification of metabolites of ^{111}In-diethylenetriaminepentaacetic acid-monoclonal antibodies and antibody fragments in vivo. Cancer Res. 55: 5714s–5720s

Rogers, B. E., Anderson, C. J., Connett, J. M., Guo, L. W., Edwards, W. B., Sherman, E. L. C., Zinn, K. R., Welch, M. J. (1996) Comparison of four bifunctional chelates for radiolabeling monoclonal antibodies with copper radioisotopes: biodistribution and metabolism. Bioconjugate Chem. 7: 511–522

Schmer, G., Holcenberg, J. S., Roberts, J. (1978) Kinetics of uptake and activity in mouse liver of glutaminase coupled to desialated orosomucoid. Biochim. Biophys. Acta 538: 397–405

Stowell, C. P., Lee, Y. C. (1980) Preparation of some new neoglycoproteins by amidination of bovine serum albumin using 2-imino-2-methoxyethyl 1-thioglycosides. Biochemistry 19: 4899–4904

Takakura, Y., Mahato, R. I., Nishikawa, M., Hashida, M. (1996) Control of pharmacokinetic profiles of drug-macromolecule conjugates. Adv. Drug Del. Rev. 19: 377–399

Thorpe, S. R., Baynes, J. W., Chroneos, Z. C. (1993) The design and application of residualizing labels for studies of protein catabolism. FASEB J. 7: 399–405

Weber, D. A., Ivanovic, M., Franceschi, D., Strand, S.-E., Erlandsson, K., Franceschi, M., Atkins, H. L., Coderre, J. A., Susskind, H., Button, T., Ljunggren, K. (1994) Pinhole SPECT: an approach to in vivo high resolution SPECT imaging in small laboratory animals. J. Nucl. Med. 35: 342–348

Xi, K., Tabata, Y., Uno, K., Yoshimoto, M., Kishida, T., Sokawa, Y., Ikada, Y. (1996) Liver targeting of interferon through pullulan conjugation. Pharm. Res. 13: 1846–1850

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobiodyn. 4: 879–885