Renal Disposition of Recombinant Human Interleukin-11 in the Isolated Perfused Rat Kidney

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Purpose. To clarify the mechanism of the renal clearance of recombinant human interleukin-11 (rhIL-11), we investigated the renal disposition characteristics of rhIL-11 in the perfused rat kidney.

Methods. The disposition characteristics of ^{111}In-labeled rhIL-11 were analyzed using a single-pass indicator dilution technique and statistical moment analysis in the perfused rat kidney under filtering and nonfiltering conditions.

Results. Steady-state distribution volume (V_d) calculated from the venous outflow patterns of rhIL-11 at the doses of 0.3 to 10 μg/kidney was between 0.35 and 0.40 ml/g kidney. However, V_d at the highest dose decreased to a value almost identical to that of bovine serum albumin, suggesting that there is a reversible and saturable interaction between the capillary wall and rhIL-11 molecule. In filtering kidney, a remarkable accumulation of rhIL-11 was observed while its urinary excretion was highly restricted at all doses. In nonfiltering kidney, rhIL-11 showed a decreased but still significant renal uptake. Taken together, the marked renal uptake of rhIL-11 may be explained by both efficient tubular reabsorption and significant uptake from the capillary side. These processes were not saturable within the tested dose range. These characteristics of rhIL-11 are likely based on non-specific electrostatic interaction with the tissues due to its cationic charge in the cytokine.

Conclusions. The renal disposition processes of rhIL-11 were clarified at organ level in a quantitative manner. These findings agree well with previous observations in an *in vivo* disposition study in mice.

KEY WORDS: rhIL-11; rat kidney perfusion; glomerular and postglomerular permselectivity; tubular reabsorption.

INTRODUCTION

Cytokines are assuming increasing importance as therapeutic agents. However, the clinical use of cytokines is often restricted by their rapid clearance after systemic administration. The kidney plays an important role in the disposition of protein drugs since proteins with a molecular mass of less than 30 kDa are susceptible to filtration through the glomeruli (1) and many proteins are also metabolically degraded in this organ (2). Like most cytokines, rhIL-11 has a molecular mass within this range.

In a previous study (3), we demonstrated that rhIL-11 rapidly disappeared from the circulation after intravenous injection and that large tissue uptake clearance was obtained for the kidney. However, urinary excretion was very small, suggesting that rhIL-11 was rapidly cleared by glomerular filtration and then subject to efficient tubular reabsorption.

The disposition of proteins in the kidney involves several processes such as glomerular filtration, tubular reabsorption, and interaction at the capillary side. The development of strategies for the clinical use and design of protein-macromolecule conjugates and/or drug delivery dosage forms therefore requires the quantitation of these processes. In the present study, we quantitatively analyzed the disposition characteristics of rhIL-11 using a single-pass indicator dilution technique and statistical moment analysis in the perfused rat kidney under filtering and nonfiltering conditions (4).

MATERIALS AND METHODS

Chemicals

rhIL-11 (MW = 19047, pI = 11.7) was kindly supplied by Genetics Institute, Inc., Massachusetts, USA. [^{111}In]Cl_3 (74 MBq/ml) was a gift from Nihon Mediphysics Co., Takarazuka, Japan. All other chemicals were of reagent grade and obtained commercially.

Radiolabeling of rhIL-11

rhIL-11 was radiolabeled with ^{111}In using the bifunctional chelating agent diethylenetriaminepenta-acetic acid (DTPA) anhydride (Dojindo Labs., Kumamoto, Japan) according to the method of Hnatowich *et al.* (5). The biological activity of rhIL-11 was maintained after radiolabeling with ^{111}In using DTPA anhydride. It has been confirmed that the pI value of rhIL-11 was not greatly changed by radiolabeling and the pharmacokinetic behavior of ^{111}In-labeled rhIL-11 was same as that of intact molecule (3).

Perfusate

In filtering and nonfiltering kidney perfusion experiments, the perfusate consisted of Krebs-Henseleit bicarbonate buffer, pH 7.45, containing glucose (5 mM) and BSA (filtering kidney:5%, nonfiltering kidney:10%). The perfusate was normally oxygenated with 95% O_2/5% CO_2.

Isolated Rat Kidney Perfusion

The kidneys of Wistar strain male rats weighing 215–255 g were isolated according to the method of Nishiitsutsuji-Uwo *et al.* (6). All procedures were done in compliance with "Principles of Laboratory Animal Care". Briefly, rats were anesthetized with pentobarbital (40 mg/kg, i.p.) and the femoral vein was exposed, into which 100 mg (1 ml) of mannitol was injected. The right kidney was exposed by midline incision and the ureter was cannulated for urine collection with polyethylene (PE-10) tubing. A venous catheter (o.d. 2.33 mm, i.d. 1.33 mm) was placed in the vena cava and the renal artery was cannulated using a 20 G needle via the mesenteric artery to avoid interrupting the renal blood flow. The whole operation took about 15 min. Immediately after cannulation, the kidney was isolated from the animal and perfused at a rate of approximately 18 ml/min for 20 min to obtain stable conditions. The renal arterial pressure

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was monitored through the experiment and maintained at 70–80 mmHg by adjusting the flow rate. The nonfiltering kidney experiment was accomplished by tying off the ureters with silk thread, raising the perfusate albumin concentration to 10%, and lowering the renal arterial pressure to 50–60 mmHg.

Indicator Dilution Experiment

Saline (0.14 ml) containing dissolved BSA (5%) and ^{111}In-labeled rhIL-11 ([^{111}In]rhIL-11) was introduced into the arterial catheter by pulse injection using a six-position rotary valve injector (Type 50 Teflon Rotary Valves, Rheodyne, Cotati, CA) (4). The venous outflow samples were collected into previously weighed tubes at 0.3- to 3-sec intervals for 20 sec. The collected sample volumes were calculated from the gain in weight of the tube assuming the density of the outflowing perfusate to be 1.0. The mean sampling time point was estimated from each sample volume assuming a constant flow rate. Urine samples were collected at 1- or 2-min intervals for 14 min. The urine volumes were calculated in the same way as the venous outflow samples. After perfusion, the wet weight of the excised kidney was measured and the renal cortex and medulla were separated. All the samples and the excised suborgans of the kidney were subjected to radioactivity counting. ^{111}In radioactivity was counted in a well-type NaI scintillation counter (ARC-500 Aloka, Tokyo, Japan).

Data Analysis

Statistical moment parameters for outflow pattern were calculated as follows:

$$AUC = \int_0^\infty C\,dt$$

$$MTT_{kid} = \int_0^\infty tC\,dt/AUC$$

where t is time and C represents the concentration of compounds normalized by the injection dose. AUC and MTT_{kid} denote the area under the concentration-time curve and mean transit time of the drug in the kidney, respectively. These parameters were calculated by numeric integration using a linear trapezoidal formula and extrapolation to infinite time based on a single-exponential equation (7,8). The steady state distribution volume was calculated from the moment parameters as follows:

$$F_0 = AUC \cdot Q$$

$$V_d = Q \cdot MTT_{kid}/F_0/\text{kidney weight}$$

where F_0 corresponds to the venous outflow recovery, Q represents the perfusion flow rate and V_d is the steady-state distribution volume normalized by kidney weight. MTT_{kid} and V_d were corrected by subtraction of the catheter transit time and catheter volume, respectively.

RESULTS

Isolated Rat Kidney Perfusion

In this perfusion system, values for perfusate flow rate (PFR; 16.1 ± 2.0 ml/min, mean ± SD; n = 12), glomerular filtration rate (GFR; 0.41 ± 0.10 ml/min; n = 4), perfusion pressure (77.5 ± 6.9 mmHg; n = 12), urine flow rate (50–100 μl/min), and glucose reabsorption ratio (>90%) remained largely constant, and were comparable to values reported in previous studies (9,10). Although erythrocytes were not added to the perfusate and PFR was three to four times greater than normal, kidney function was maintained (11).

Venous Recovery of rhIL-11

Fig. 1A shows a typical outflow concentration-time curve of [^{111}In]rhIL-11 after bolus injection together with curves of ^{111}In-labeled BSA and ^{14}C-inulin. Fig. 1B illustrates that of [^{111}In]rhIL-11 at different doses. In all cases, more than 90%–95% of the injected dose was recovered in the venous outflow during the first 10 sec after injection. Peak concentration of rhIL-11 was less than that of BSA and greater than that of inulin. The moment parameters and V_d values for rhIL-11 at different doses were calculated from the outflow patterns and

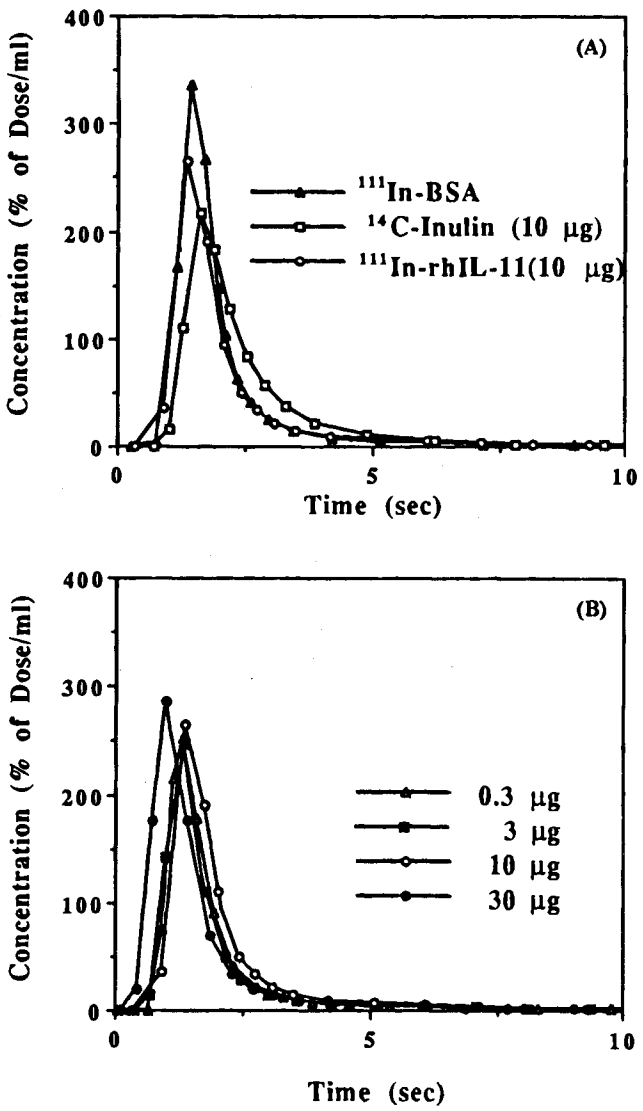

Fig. 1. Typical outflow curves for rhIL-11, BSA and inulin, in the perfused rat kidney after bolus injection. (A) [^{111}In]rhIL-11, [^{111}In]BSA, [^{14}C]Inulin. (B) rhIL-11; 0.3, 3, 10, 30 μg of dose/kidney.

Table I. Moments and Distribution Volumes of ^{111}In-rhIL-11, ^{111}In-BSA and ^{14}C-Inulin in the Kidney Perfusion Experiments[a]

	Dose (μg/kidney)	*AUC* (% of dose · sec/ml)	MTT_{kid} (sec)	V_d (ml/g kidney)
	0.3	361.8 ± 37.4	1.391 ± 0.322	0.402 ± 0.098
	3	359.1 ± 14.5	1.333 ± 0.393	0.353 ± 0.100
^{111}In-rhIL-11	10	320.9 ± 47.6	1.258 ± 0.329	0.395 ± 0.052
	30	334.3 ± 9.2	0.853 ± 0.086	0.228 ± 0.010
^{111}In-BSA	—[b]	325.6 ± 32.3	0.827 ± 0.077	0.254 ± 0.026
^{14}C-Inulin	10	337.8 ± 48.8	1.418 ± 0.095	0.450 ± 0.022

[a] Results are expressed as the mean ± SD of three experiments.
[b] Dose of BSA was not defined because the perfusate contained 5% BSA.

are summarized in Table I together with those of BSA and inulin. The V_d value of rhIL-11 at the doses of 0.3 to 10 μg/kidney was between 0.35 and 0.40 (ml/g kidney). However, V_d at the highest dose (30 μg/kidney) decreased to a value almost identical to that of BSA (0.25). Clearance (CL_{kidney}) value as calculated from the *AUC* was 30,000–40,000 μl/hr/g, which was in good agreement with the value obtained in the previous *in vivo* study, and accounted for almost 50% of total body clearance.

Tissue Accumulation and Urinary Excretion of rhIL-11

Figure 2 shows the tissue accumulation and urinary excretion of radioactivity after bolus injection of [^{111}In]rhIL-11 at different doses in the filtering and nonfiltering kidney perfusion experiments. In the filtering kidney, the amount recovered in the tissue was high (2.9%–3.8% of dose), while the total amount recovered in the urine was highly restricted (<0.3% of dose) at all doses. In the nonfiltering kidney, reduced but significant accumulation in the kidney (1.3%–1.8% of dose) was observed. These data indicate that the renal uptake of [^{111}In]rhIL-11 is due to both efficient tubular reabsorption after glomerular filtration and the uptake from the capillary side.

Suborgan Distribution of rhIL-11

Figure 3 shows the radioactivity concentration of rhIL-11 in the cortex, innermedulla and outermedulla after bolus injection of [^{111}In]rhIL-11 at different doses in the filtering and nonfiltering kidney perfusion experiments. In the filtering condition, the concentration of rhIL-11 in the cortex was significantly higher (3.8%–4.8% of dose/g tissue) than that in the outermedulla and innermedulla. In the nonfiltering condition, concentration in the cortex decreased (1.0%–2.7% of dose/g tissue). These data suggest that rhIL-11 is efficiently reabsorbed by renal proximal tubules in the cortex.

DISCUSSION

rhIL-11 is potentially useful in the treatment of neutropenia and thrombocytopenia associated with cancer chemotherapy and/or radiotherapy. A previous study showed that the rapid disappearance of rhIL-11 from the circulation was mainly due to clearance by the kidney (3). It was also suggested that glomerular filtration, a non-saturable process, was a major route of elimination and that filtered rhIL-11 underwent highly efficient reabsorption in the tubule. The objective of the present study was to clarify the renal disposition characteristics of rhIL-11 at the organ level using perfused rat kidney.

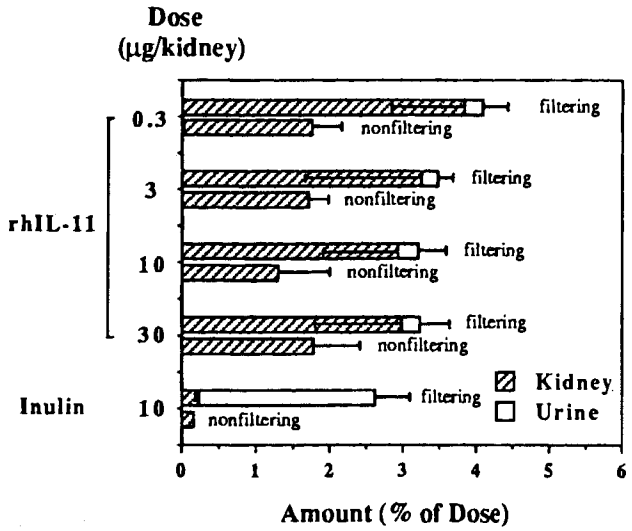

Fig. 2. Amount recoveries in the kidney and urine for [^{111}In]rhIL-11 in filtering and nonfiltering kidney perfusion experiments. Results are expressed as the mean ± SD of three rats.

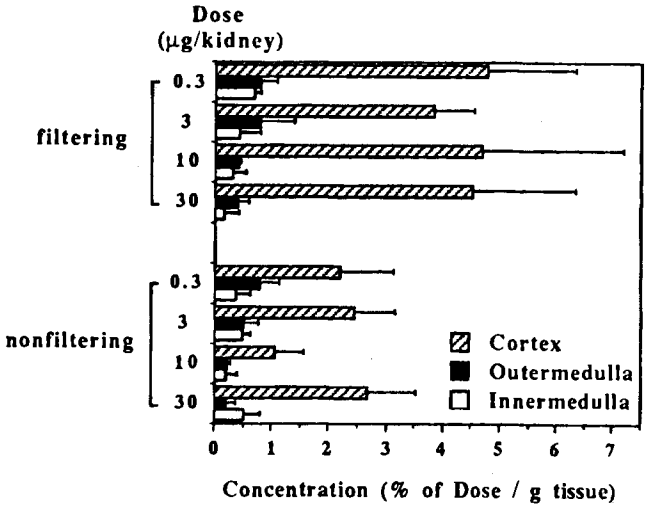

Fig. 3. Concentration of [^{111}In]rhIL-11 in the kidney cortex, outermedulla, and innermedulla. Results are expressed as the mean ± SD of three rats.

Since radioactivity in the organ was retained, rhIL-11 was labeled with ^{111}In using DTPA anhydride to allow evaluation of the initial phase of tissue uptake without any significant effect of metabolic degradation. It has been reported that ^{111}In-labeled proteins are delivered to the lysosome where the proteins can be degraded to yield ^{111}In-DTPA-amino acid(s) and that these metabolites remain within the lysosome with only slow release from the cell (12).

The kidney perfusion experiments allowed us to elucidate the total renal disposition processes of macromolecules and proteins. By employing filtering and nonfiltering kidneys, tubular reabsorption could be calculated by subtracting the tissue accumulation in the nonfiltering kidney from that in the filtering kidney. The amount of drug undergoing glomerular filtration was calculated as the sum of the amount recovered in the urine and the tubular reabsorption amount. The venous recovery was calculated by subtracting the amount for glomerular filtration and that for uptake from capillary side from the injected dose. Thus, we could quantitatively determine the renal disposition process of rhIL-11. Fig. 4 schematically summarizes the renal disposition process of rhIL-11 together with that of inulin and of BSA previously reported (4), i.e., glomerular filtration, tubular reabsorption, and capillary side uptake.

rhIL-11 (19kDa) showed efficient glomerular filtration (1.5% to 2.4% of injected dose) during single passage through the kidney, with the filtered amount close to that of inulin (2.5%), a marker of the glomerular filtration rate (Fig. 4). It is well known that the permselectivity of the glomerular capillary wall to macromolecules is primarily based upon molecular size (1). In general, proteins with a molecular mass of less than 30 kDa are susceptible to glomerular filtration (13). In our previous studies, relatively small proteins as neocarzinostatin (NCS; 12 kDa) and soybean trypsin inhibitor (STI; 20 kDa) showed similar values (2.4% and 2.2%, respectively). In addition, no significant change in the total filtered amount of rhIL-11 was observed within the tested doses (Fig. 2), indicating the linearity of glomerular filtration.

Renal tubular epithelial cells, particularly those in the proximal tubule, have the ability to reabsorb proteins from the tubular lumen. A number of studies have demonstrated that important determinants for their reabsorption may be the physicochemical properties of proteins such as the net charge of molecule and/or number of free amino groups (1). It is well known that cationic proteins are more susceptible to reabsorption than anionic proteins (14). The present study has demonstrated that rhIL-11 shows a high reabsorption ratio (80%–91%) (Fig. 2) at all doses. It has not been reported that a receptor for rhIL-11 exists in the kidney. Accordingly, this result may be explained by the highly basic character of the rhIL-11 molecule, the sequence of which is rich in basic amino acids such as arginine. Within the dose range tested in this study, the reabsorption of IL-11 did not seem to be saturable. The concentration of rhIL-11 accumulated in the cortex was much higher than that in the medulla at all doses (Fig. 3). These findings suggest that the renal uptake of rhIL-11 occurred mainly at the proximal tubule as reported for other proteins (14). The differences in suborgan distributions in the filtering and nonfiltering conditions support this speculation. It is generally accepted that proteins and large peptides are taken up by the tubular cells as intact molecules. In contrast, small linear peptides such as angiotensin II and bradykinin (composed of 8 and 9 amino acids, respectively) are known to be degraded at the luminal surface of the brush border of the proximal tubules, a region which contains a variety of hydrolytic enzymes, by the process of membrane digestion with reabsorption of the breakdown products (15). We assessed this possibility through size exclusion chromatographic analysis of the urine and tissue samples, but evaluation could not be done precisely due to the very low radioactivity derived from [^{111}In]rhIL-11. However, involvement of this phenomenon in the luminal sequestration of ^{111}In-labeled rhIL-11 is unlikely, since rhIL-11 is a polypeptide composed of 177 amino acids. Accordingly, it was speculated that rhIL-11 could be adsorbed on the membrane surface as a result of electrostatic interaction followed by the adsorptive endocytic internalization. In addition to efficient reabsorption, the marked accumulation of rhIL-11 from the capillary side was observed in this study (Fig. 4). This finding also suggested this uptake process has high capacity.

The steady-state distribution volume (V_d) of rhIL-11 (0.35–0.40 ml/g) was similar to those of NCS (0.38–0.41 ml/g) and STI (0.41–0.43 ml/g) within the dose range of 0.3 to 10 μg/kidney. However, the V_d value decreased at the highest dose of 30 μg/kidney and reached a value closely similar to that of BSA (Table I). These results suggest the existence of a reversible and saturable interaction of rhIL-11 with the renal capillary wall based on the highly basic character of the protein molecule. Our preliminary inhibition experiments using cationic peptides, such as lysozyme, support these postulations. In spite of the change in the distribution volume determined from the outflow patterns in the early phase after injection, no significant effect of the injected dose was observed on tissue accumulation and urinary excretion at 14 min.

In conclusion, the present study has demonstrated that rhIL-11 accumulates in the kidney to a great extent due to both tubular reabsorption after efficient glomerular filtration and uptake from the capillary side. We previously reported that cationic derivatives of superoxide dismutase (pI ≥ 9) showed similar behavior in the kidney based on non-specific electrostatic interaction with the tissue. The highly cationic character of rhIL-11 (pI ≥ 11) probably plays an important role. Thus, the results obtained in the present study agree well with previous observations in an *in vivo* disposition study in mice (3). These findings will provide useful information in facilitating the clini-

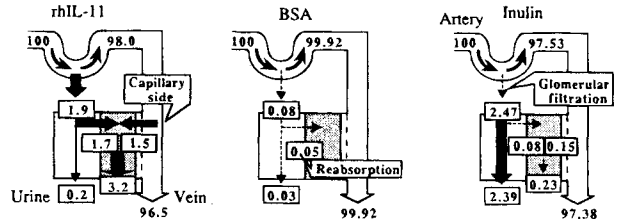

Fig. 4. Renal disposition profile of rhIL-11, BSA and inulin in kidney perfusion experiments. Values at each step are expressed as a percentage of dose. Values for rhIL-11 are the average of four doses tested. The amount for tubular reabsorption was calculated by subtracting the tissue accumulation in the nonfiltering kidney from that in the filtering kidney. The amount of drug undergoing glomerular filtration was calculated as the sum of the amount recovered in the urine and the tubular reabsorption amount. The venous recovery was calculated by subtracting the amount for the glomerular filtration and that for the uptake from capillary side from the injected dose.

cal application of rhIL-11 and also in constructing a rational strategy for its delivery systems.

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