Regular Article

**Effect of Co-administration of Cationic Macromolecules on the In Vivo Disposition and In situ Renal Disposition Characteristics of rhIL-11**

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**Summary:** The effects of co-administration of cationic proteins on the in vivo disposition characteristics of recombinant human interleukin-11 (rhIL-11) in mice and on the renal disposition in the perfused rat kidney were investigated. Following a bolus intravenous injection of 10 μg/kg ¹¹¹In-labeled rhIL-11, along with cationic proteins at high dose (50 mg/kg), the plasma clearance of ¹¹¹In-labeled rhIL-11 was significantly decreased mainly due to a reduction in the hepatic clearance of ¹¹¹In-labeled rhIL-11. The effect on the renal clearance was relatively small, suggesting that the kidney has a high clearance capacity. The urinary excretion ratio increased by a factor of 2 or 4 with co-administration, suggesting that the cationic character of rhIL-11 is involved in tubular re-absorption. An in situ renal disposition study supports these postulations. Thus, the renal and hepatic disposition of rhIL-11 is based on nonspecific cationic interaction. These data suggest that an efficient delivery system for this cytokine would require the reduction of electrostatic interaction of this molecule with these tissues in order to reduce the plasma clearance rate. These findings provide useful information for the construction of an rhIL-11 delivery system.

Key words: recombinant human interleukin-11 (rhIL-11); hepatic disposition; tubular reabsorption; urinary excretion; cationic protein

**Introduction**

Various peptides and proteins are increasingly becoming a very important class of therapeutic agents given the recent progress in the clarification of their pathophysiological roles. However, in many cases, the clinical application of proteins is often limited by their rapid clearance in vivo. Recombinant human IL-11 (rhIL-11), manufactured using genetic recombination techniques, has a molecular mass of 19 kDa. rhIL-11 has potential utility in the treatment of neutropenia and thrombocytopenia associated with cancer chemotherapy and/or radiotherapy.¹ A previous study showed that rhIL-11 rapidly disappeared from the circulation, and that the kidney and liver were major contributors to this rapid elimination.² It was also demonstrated that the renal disposition of rhIL-11 was due to efficient reabsorption after glomerular filtration and uptake from the capillary side.³ It has been suggested that these disposition characteristics of rhIL-11 could be based on electrostatic interaction with the tissues, and that the highly cationic character of rhIL-11 may be involved. The objective of this study was to clarify these postulations in order to facilitate the construction of a rational strategy for the development of a delivery system.

**Methods**

**Animals:** ddY male mice (5 week) and Wistar strain male rats (weight 215–230 g) were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Mice and rats were maintained under conventional housing conditions. Water and laboratory diet were provided ad libitum. All animal experiments were done in compliance with the “Principles of Laboratory Animal Care”, established by NIH.

**Chemicals:** rhIL-11 was kindly supplied by Genetics Institute, Inc., Massachusetts, USA. [¹¹¹In]Cl₃ (74 MBq/ml) was a gift from Nihon Mediphysics Co., Takarazuka, Japan. All other chemicals were of reagent
Radio-labeling of rhIL-11: rhIL-11 was radio-labeled 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. It has been confirmed that the plasma release of rhIL-11 was not greatly changed by radio-labeling and the pharmacokinetic behavior of $^{111}$In-labeled rhIL-11 was the same as that of the non-labeled molecule.

Synthesis of Cationic Protein: Cationized bovine serum albumin (cBSA) and cationic superoxide dismutase (cSOD) were synthesized by a method reported previously. Molecular weights of cationized proteins and rhIL-11 were determined by size-exclusion chromatography using a TSKgel G2000SWXL column (TOSOH, Tokyo, Japan). Isoelectric points of proteins were determined by isoelectric focusing gel electrophoresis. However, all the proteins migrated rapidly toward the cathode but were too basic to be retained on the gel. The physicochemical properties of these proteins are summarized in Table I together with other proteins used in this study. It was confirmed by SDS-PAGE that there was no interaction between the cationic proteins and rhIL-11 in the mixture (data not shown).

In Vivo Disposition Study: $^{111}$In-labeled rhIL-11 at a dose of 10 µg/kg was co-administered with various cationic peptides to male ddY mice through a tail vein. The mice were then housed in metabolic-testing cages for urine collection. At indicated time points (2, 5, 10, 30, 60 min) after dosing, blood was collected from the vena cava under ether anesthesia, and then the mice were sacrificed. The kidneys and liver were excised, rinsed with saline, weighed, and subjected to radioactivity counting. The $^{111}$In radioactivity levels were counted in a well-type NaI scintillation counter (ARC-500 Aloka, Tokyo, Japan). The plasma volume of each organ was determined from the distribution data on $^{111}$In-labeled bovine serum albumin at 3 min after intravenous injection in order to correct for the contamination of plasma in tissue samples.

Calculation of Organ Uptake Clearance: The tissue distribution data were evaluated by using a tissue uptake rate index calculated in terms of clearance. In the early phase post-dosing, the efflux of $^{111}$In radioactivity from the organ is considered to be negligible since the degradation products of $^{111}$In-labeled proteins remain in the lysosome with only slow release from the cell. With the assumption described above, CL$_{org}$ can be calculated as follows:

$$CL_{org} = \frac{T(t_1)/AUC_{0-t_1}}{W}$$

where $t_1$ (h) is the time of sampling after injection. $T(t_1)$ (% of dose/g) represents the amount of radioactivity in 1 g of the tissue. W is the total organ weight. Urinary clearance (CL$_{urine}$) was also calculated using the accumulated amount excreted in urine. In this study, these parameters were calculated from the data obtained up to 60 min after dosing.

Isolated Rat Kidney Perfusion: In kidney perfusion experiments, the perfusate consisted of Krebs-Henseleit bicarbonate buffer, pH 7.45, containing glucose (5 mM) and BSA (5%). The perfusate was normally oxygenated with 95% O$_2$/5% CO$_2$. The kidneys of Wistar strain male rats weighing 215–255 g were isolated according to the method of Nishiitsu-Uwo et al. and perfused in situ under filtering kidney conditions, as described previously. Saline (0.14 m/) containing dissolved BSA (5%) and $^{111}$In-labeled rhIL-11 (0.3 µg/kidney) was introduced into the arterial catheter by pulse injection using a six-position rotary valve injector (Type 50 Teflon Rotary Valves, Rheodyne, Cotati, CA) together with the cationic proteins (1.5 mg/kidney). Urine samples were collected for 14 min. 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.

Results

In Vivo Tissue Distribution of $^{111}$In-labeled rhIL-11 in Co-administration with Cationic Proteins: Fig. 1A shows the plasma concentration-time profile of $^{111}$In-labeled rhIL-11 after bolus intravenous administration to mice along with cationic proteins. The plasma clearance of rhIL-11 was decreased by co-administration of a high dose (50 mg/kg) of cationic proteins, however, it was unchanged by co-injection of low dose (1 mg/kg) of cationic proteins other than rhIL-11. Fig. 1B, C show the liver and kidney distribution-time profile of [$^{111}$In]rhIL-11, respectively. The hepatic accumulation of rhIL-11 was significantly decreased by the co-administration of cationic proteins (high dose). The renal accumulation of rhIL-11 was slightly changed, but the changes varied among the cationic proteins used in this study. The effect of co-administration of cationic proteins was not significant in the kidney distribution-time profile of rhIL-11.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular Weight</th>
<th>Isoelectric Point (pI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhIL-11</td>
<td>19000</td>
<td>11.7*</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>14300</td>
<td>11</td>
</tr>
<tr>
<td>cat-SOD</td>
<td>34000</td>
<td>&gt;10</td>
</tr>
<tr>
<td>cat-BSA</td>
<td>70000</td>
<td>&gt;10</td>
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</table>

*; calculated value

Table I. Physicochemical Properties of Proteins Used in These Experiments

grade and commercially produced.
proteins on the renal accumulation of rhIL-11 was unclear compared with the hepatic accumulation. This phenomenon was explained by the difference in the capacity of hepatic uptake versus that of renal uptake, as the former would be low and the latter high.

Fig. 2 shows the urinary excretion of radioactivity after co-administration with cationic proteins. In all cases, the amount recovered in the urine increased according to the co-administered amount of the proteins. The most significant enhancement was obtained by co-administration of cSOD (6.6% of dose).

Calculation of Organ Clearance: Table II summarizes the AUC, total body, hepatic, renal and urinary clearances after iv co-injection of $^{111}$In-rhIL-11 with cationic proteins. The total body clearance (CL$_{tot}$) was 20200–22100 µl/hr in the case of co-administration of cationic protein at low dose (1 mg/kg), and was 12000–14500 µl/hr at high dose (50 mg/kg). As shown in Fig. 3, the renal and hepatic clearances contributed mainly to the CL$_{tot}$; the former slightly changed along with the dose of cationic protein, while the latter significantly decreased. The urinary clearance also was increased by the co-administration of cationic protein (high dose).

Effect of Co-administration of Cationic Proteins on Renal Disposition of rhIL-11 in situ Isolated Rat Kidney: Fig. 4 shows the tissue accumulation and urinary excretion of radioactivity after bolus injection of rhIL-11 with cationic proteins in the filtering kidney experiments. The amount recovered in the tissue decreased by 4% to 2% of the dose, which is almost identical to the value obtained in the non-filtering kidney experiments.
Table II. AUC and Clearance for $^{111}$In-rhIL-11 in Mice after Intravenous Co-administration of Various Proteins

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Dose (mg/kg)</th>
<th>AUC$_{0-60}$ (% of dose·min/ml)</th>
<th>CL$_{tot}$ (μl/hr)</th>
<th>CL$_{kidney}$ (μl/hr)</th>
<th>CL$_{liver}$ (μl/hr)</th>
<th>CL$_{urine}$ (μl/hr)</th>
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<tr>
<td>control</td>
<td>—</td>
<td>259</td>
<td>22100</td>
<td>11100</td>
<td>6660</td>
<td>163</td>
</tr>
<tr>
<td>rhIL-11 (pI = 11.7)</td>
<td>1</td>
<td>436</td>
<td>12500</td>
<td>9260</td>
<td>2240</td>
<td>622</td>
</tr>
<tr>
<td>Lysozyme (pI = 11)</td>
<td>1</td>
<td>266</td>
<td>21300</td>
<td>11600</td>
<td>6380</td>
<td>183</td>
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<td></td>
<td>50</td>
<td>395</td>
<td>14500</td>
<td>7700</td>
<td>2120</td>
<td>679</td>
</tr>
<tr>
<td>cBSA (pI &gt; 10)</td>
<td>1</td>
<td>266</td>
<td>20800</td>
<td>11000</td>
<td>6380</td>
<td>367</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>474</td>
<td>12000</td>
<td>7880</td>
<td>1010</td>
<td>404</td>
</tr>
<tr>
<td>cSOD (pI &gt; 10)</td>
<td>1</td>
<td>295</td>
<td>20200</td>
<td>10400</td>
<td>5330</td>
<td>294</td>
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<td></td>
<td>50</td>
<td>417</td>
<td>13700</td>
<td>8650</td>
<td>1870</td>
<td>946</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of Co-administration of Various Cationic Proteins on the Organ Clearance of $^{111}$In-rhIL-11 after Intravenous Administration in Mice.

Fig. 4. Effect of Cationic Proteins on the Amount Recoveries of $^{111}$In-rhIL-11 in the Kidney and Urine in the Perfused Rat Kidney. Results are expressed as the mean ± SD of the results from three experiments.
Fig. 5. Effect of Cationic Proteins on the Concentration of $^{111}$In-rhIL-11 in the Kidney Cortex, Outermedulla, and Innermedulla in the Perfused Rat Kidney. Results are expressed as the mean ± SD of the results from three experiments.

by co-administration. The total amount recovered in the urine increased in the case of co-injection with lysozyme. Fig. 5 shows the radioactivity concentration of rhIL-11 in the cortex, inner-medulla and outer-medulla after bolus injection of rhIL-11 along with cationic proteins. These data suggest that tubular re-absorption of rhIL-11 after glomerular filtration and uptake from the capillary side may be based on non-specific electrostatic interaction with the tissues.

**Discussion**

rhIL-11 has potential utility in the treatment of neutropenia and thrombocytopenia associated with cancer chemotherapy and/or with radiotherapy. Previous studies have shown that the rapid clearance of rhIL-11 could be attributed to electrostatic interaction with tissues such as liver and kidney, and the highly cationic character of rhIL-11 may also play an important role. The objective of this study was to clarify these postulations.

In this study, we used three kinds of cationic proteins: lysozyme, cBSA and cSOD. Lysozyme has a physicochemical character similar to rhIL-11 and has been reported to be filtered by glomeruli, and this is followed by tubular reabsorption. We previously reported that cBSA showed large tissue uptake rate indices in the liver and kidney, and that the highly cationic character of this molecule was involved. We also reported that the uptake of cSOD, both from the renal proximal tubules and the capillary side, was remarkable.

In the in vivo disposition study, the total clearance of rhIL-11 was reduced by co-administration of high doses of cationic macromolecules. Fig. 3 summarizes the contribution of each organ to the total body clearance ($CL_{tot}$). The reduction in $CL_{tot}$ could be mainly due to the decrease of hepatic accumulation, which is a saturable process based on adsorptive endocytosis. The effect on renal clearance was small due to the high capacity of this organ. Urinary excretion was significantly enhanced by co-administration, but the precise mechanism of this enhancement may be different among the proteins. Hepatic accumulation was reduced by all cationic proteins. These effects due to the cationic proteins were not observed when rhIL-11 was co-administered with BSA which has a pI of about 5 (data not shown). Although accurate pI values could not be measured for all the proteins, rhIL-11 has the highest pI among the cationic proteins used in this study. This could be one of the possible reasons why rhIL-11 itself had the most effect on the pharmacokinetic behavior of rhIL-11 at the low dose (1 mg/kg). It was reported that mRNA of the IL-11 receptor (IL-11R) was expressed also in the liver and kidney of rodents. Although protein level expression of IL-11R has not yet been reported, and a non-linear pharmacokinetic profile of rhIL-11 was not observed at the clinically relevant dose range (10–100 mg/kg), the contribution of this receptor to the clearance should be investigated further. Tubular reabsorption was reduced by cSOD and by lysozyme, and uptake from the capillary side was reduced by cBSA and by cSOD. The renal disposition study using perfused rat kidney partly supported these speculations. However, the effects of cSOD on the recoveries in the kidney and urine were relatively smaller than expected. Further investigations are needed to elucidate these speculations. For instance, the effect of pretreatment with these cationic proteins on the pharmacokinetic behavior of rhIL-11 should be examined. In this case a proper dosing schedule should be considered with respect to the pharmacokinetic profile of each cationic protein.

Thus, the present study has demonstrated that the highly cationic character of rhIL-11 (pI>11), which is
rich in basic amino acids, such as arginine, in its sequence, plays an important role. These findings, therefore, suggest that it is necessary to control the renal and hepatic clearance of this cytokine in order to ensure its effective delivery. One possible approach to efficient delivery of rhIL-11 would be its chemical modification with macromolecular carriers such as dextran and polyethylene glycol.\textsuperscript{6,11,12,13} This method may be also useful for reducing the hepatic uptake of rhIL-11.

References


