Kinetic Parameters of $\alpha_2$-macroglobulin in Rats Induced Nephropathy by Administration of Gentamicin

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ABSTRACT
The elimination phase of $\alpha_2$-macroglobulin ($\alpha_2$M), a typical acute phase protein, in nephropathic rats was investigated.

Methods
Renal failure was induced by injection of gentamicin. Acute inflammation was induced by injection of turpentine oil in rats. Serum concentrations of $\alpha_2$M were measured by enzyme-linked immunosorbent assay. Half-life was calculated as 0.693/elimination rate constant (K). The serum concentrations of $\alpha_2$M were significantly higher during renal failure than in the control. Moreover, the maximum concentrations and total area under the blood concentration vs. time curve were significantly elevated during renal failure. However, the rate of elimination in nephropathic rats was equivalent to that in control rats. Therefore, no significant difference in the $\alpha_2$M half-life was observed between nephropathic and control rats.

INTRODUCTION
$\alpha_2$-macroglobulin ($\alpha_2$M) is a typical acute phase protein in rats. Serum $\alpha_2$M concentrations increased more sensitively than AAG levels in rats following the induction of acute inflammation. While certain characteristics of $\alpha_2$M production in rats have been reported, there is a limited amount of information regarding the kinetic of $\alpha_2$M in disease models. However, alterations in the serum $\alpha_2$M concentrations in rats with hepatic impairment have been reported. Nephropathy is known to be a serious adverse event related to aminoglycoside antibiotics. Thus, we used a high dose of an aminoglycoside antibiotic to induce the impairment of renal function in rats in this study. The aim of this study was to clarify the influence of nephropathy on $\alpha_2$M elimination rates in rats.

MATERIALS AND METHODS
Animals
Ten Sprague-Dawley rats (body weight; 120g~150g) were purchased from CLEA Japan, Inc. (Tokyo, Japan). Rats were housed individually at a temperature of 23 ± 2°C, with a relative humidity of 55 ± 10% on a 12/12 dark (20:00-8:00)/light (8:00-20:00) cycle. The air was exchanged 12 or more times per hour. Rats were fed MF diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and allowed free access to water. The present animal experiments were approved by the Institutional Animal Care and Use Commit-

**Table 1 Biochemical parameters and kinetic parameters of α₂-macroglobulin in rats treated with gentamicin (30mg/kg) once a day for five days**

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Cmax (µg/ml)</th>
<th>K</th>
<th>AUC (mg·h/ml)</th>
<th>t¹/₂ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephropathy</td>
<td>30* ± 7</td>
<td>0.33* ± 0.05</td>
<td>2418.1* ± 1051.7</td>
<td>0.0065 ± 0.0025</td>
<td>216.7* ± 69.2</td>
<td>121.2 ± 48.7</td>
</tr>
<tr>
<td>Control</td>
<td>20 ± 3</td>
<td>0.24 ± 0.04</td>
<td>1351.3 ± 607.3</td>
<td>0.0065 ± 0.0010</td>
<td>92.9 ± 26.4</td>
<td>108.7 ± 18.3</td>
</tr>
</tbody>
</table>

Each value were represented mean ±standard deviation., Cmax: maximum concentrations, K: elimination rate constant, AUC: area under the blood concentration vs. time curve, t₁/₂: half-life time, *: Values differs significantly from control rats (p<0.05).

Nephropathy was induced by daily intravenous administration of gentamicin sulfate (Schering-Plough Corporation, NJ, USA) at a dose of 30 mg/kg for 5 days. Sterile saline was similarly administered in the control group. Acute inflammation was induced by injection of 3 ml/kg body weight turpentine oil (Wako Pure Chemical Industries, Co., Ltd.). Ventricular blood was collected before turpentine oil administration and at 24, 48, 72, 96, 144, 192, 240, 312, and 384 hours post-administration. Blood was collected under slight anesthesia by intravenous injection of 6 mg/kg pentobarbital (Kyoritsu Seiyaku Corporation, Tokyo, Japan). Serum was stored at -80°C until analysis.

**α2M, BUN and Cr. Measurement**

Serum concentrations of α2M were measured according to the procedure of Honjo et al. Blood urea nitrogen (BUN) was measured using the urease • GLDH method. Creatinine (Cr.) was measured using an enzymatic method.

**Pharmacokinetic Parameters**

Maximum concentrations (Cmax) were determined in individual rats. The area under the blood concentration vs. time curve (AUC) from 0 to 384 hours was calculated using the trapezoidal rule. The linear slope of serum α2M concentration vs. time was then plotted on log-linear regression for individual animals. The elimination rate constant (K) was calculated using a minimum of 3 measured serum concentrations. Half-life (t₁/₂) was calculated from the formula:

\[
K(h^{-1}) = (-2.303) \times \text{slope}
\]

\[
t_{1/2} (h) = \frac{0.693}{K}
\]

where, K is the elimination rate constant, CA is serum concentration at Time(A), CB is serum concentration at Time(B)

**Statistical Analysis**

Unpaired Student’s t-tests were performed to evaluate differences in AUC, K, t₁/₂, BUN and Cr. between the nephropathy and control groups; p values of <0.05 were considered to be significant.

**RESULTS**

Serum levels of BUN and Cr. in control and gentamicin-treated rats are shown in Table 1. Both BUN and Cr. were significantly higher in rats treated with gentamicin than in control rats. Serum concentrations and kinetic parameters of α2M are shown in Table 1 and Figure 1, respectively. Except for pre-turpentine oil treatment levels, serum concentrations of α2M were significantly higher in rats treated with gentamicin than in control rats. Cmax and AUC in rats treated with gentamicin were significantly higher than in control rats. However, no significant differences were observed in both K and t₁/₂ between gentamicin-treated and control rats.

**DISCUSSION**

Renal failure is a well-known, serious adverse event associated with the use of aminoglycoside antibiotics. Serum levels of BUN and Cr. in rats treated with gentami-
cin were significantly higher than in control rats, which is indicative of the induction of nephropathy. Serum concentrations of α2M have been shown to increase in humans with renal failure.\textsuperscript{9, 10} Serum concentrations of α2M in nephropathic rats were significant higher than in control rats. Moreover, Cmax and AUC were significant higher than in control rats. These results are congruent with the elevation of α2M observed in nephropathic humans. However, the rate α2M elimination in nephropathic rats was not different from control rats, with both K and t\textsubscript{1/2} not significantly different between groups. Thus, renal failure resulted in increased α2M levels, but did not influence its elimination rate. The mechanism of aminoglycoside-induced renal failure is thought to be due to tubular cell toxicity.\textsuperscript{11} Elimination rates are not influenced by impairment of the renal tubule. Alterations in elimination rates associated with renal failure are presumed to result from failure of non-tubule sites in the kidney. Thus, further studies aimed at investigating glomerular injury are required.

**REFERENCES**


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**Figure 1** Serum concentration of α\textsubscript{2}-macroglobulin (α2M) in nephropathy rats treated with gentamicin (30mg/kg). *: Values differs significantly from control rats (p<0.05).


