EFFECT OF EXTRACELLULAR WATER VOLUME ON THE DISTRIBUTION KINETICS OF β-LACTAM ANTIBIOTICS AS A FUNCTION OF AGE

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The distribution kinetics of cefazolin in rats has been examined at four different ages (1, 7, 50 and 100 weeks). The steady state distribution volume of cefazolin, estimated from the plasma time course after i.v. injection of 20 mg/kg, varied between 136 ml/kg (50-week-old rats) and 297 ml/kg (1-week-old rats). The extracellular fluid volume, obtained from the steady state distribution volume of inulin, varied between 126 ml/kg (50-week-old rats) and 370 ml/kg (1-week-old rats). There was a good correlation between the steady state distribution volume of cefazolin and extracellular fluid volume (r = 0.977). The influence of changes on the value of the plasma unbound fraction and extracellular fluid volume on the tissue-to-plasma partition coefficient of β-lactam antibiotics was simulated by using a physiological pharmacokinetic model. The results of the simulation showed that extracellular fluid volume is an important factor affecting the distribution volume of β-lactam antibiotics and that plasma binding plays a minor role on it. The experimental and simulation results suggested that the change in the interstitial fluid volume is a determinant factor in the age-related changes in the distribution volume of β-lactam antibiotics.

Keywords — extracellular water; tissue distribution; distribution volume; aging; tissue-to-plasma partition coefficient (Kp); β-lactam antibiotics; cefazolin

INTRODUCTION

It has been shown in humans that administration of β-lactam antibiotics produces various characteristics in pharmacokinetic behavior among different ages, as reviewed by Ritschel. 1) This aging effect has been attributed to changes in extravascular water content, plasma albumin concentration, affinity to plasma albumin, and renal and/or hepatic drug disposing functions. Although creatinine clearance was found to be a good indicator for the prediction of the renal elimination rate constant for β-lactam antibiotics, 2) the determinant factor for changes in the volume of distribution with increasing age has not been elucidated yet.

In a previous paper, the mechanism of tissue distribution of β-lactam antibiotics was clarified by constructing a physiological pharmacokinetic model. 3) From the model analysis in rats, the interstitial fluid volume was found to be a factor affecting the distribution of β-lactam antibiotics in addition to albumin concentration and plasma binding. Since the volume of interstitial fluid is reported to fluctuate with increasing age in rats 4,5) and humans, 6) it is of great interest to investigate the effect of aging on the tissue distribution of β-lactam antibiotics.

The purpose of this paper was to study the relationship between the steady state distribution volume of cefazolin and the volume of extracellular water at four different ages in rats. This paper also describes the sensitivity of the previously established model to the tissue distribution kinetics of β-lactam antibiotics and dis-
cusses the determinant factor causing the age-related changes in the tissue distribution of the antibiotics.

MATERIALS AND METHODS

Materials — Cefazolin sodium (958 μg/mg) was generously supplied by Fujisawa Pharmaceutical Co., Ltd. Osaka, Japan, and used without further purification. 14C-Inulin was purchased from Amersham International, Ltd., Amersham, England. All other reagents and solvents were of reagent grade.

Pharmacokinetic Study — Wistar Rats (Sankyo Laboratory Animal Co., Toyama, Japan) of various ages were used. The animals were divided into four experimental groups: males and females of 1 ± 0.1 week, males of 7 ± 0.2 weeks, males of 50 ± 2 weeks and males of 100 ± 5 weeks. Under light ether anesthesia cefazolin or inulin was administered to 1-week-old rats via the femoral vein using a microliter syringe 705-LT (50 μl, 0.40 mm o.d., Hamilton Co., Reno, Nevada), and blood samples were collected from the jugular vein at appropriate times. The 1-week-old rats were kept at spine position for the drug administration and the blood collection. After the drug administration to 1-week-old rats, the animal was allowed to recover from ether anesthesia and put into cage. For 7-, 50- and 100-week-old rats the femoral artery and vein were cannulated with polyethylene tubing, SP45 (Natsurse Seisakusho Co., Tokyo, Japan), under light ether anesthesia for blood collection and administration of cefazolin or inulin, respectively. Cannulated rats were kept in restraining cages with water under normal housing conditions prior to the experiments. At least 3 h after the cannulation the drug was administered to 7-, 50- and 100-week-old rats under non-anesthetized condition. The body temperature of the rat was monitored in all cases by a digital thermometer, DIGIMULTI Model D611 (Takara Thermistor Instruments Co., Ltd., Yokohama, Japan). Animals, maintained by thermostatically controlled plates and/or heat lamps, were used only when their body temperature was greater than 36 °C. A single 20 mg/kg dose of cefazolin or inulin dissolved in saline was administered to the rat. Blood samples of 0.2 ml were put into a heparinized micro-centrifuge tube and centrifuged for 2 min by Centrifuge 5142, Eppendorf.

Analytical Procedure — Concentrations of cefazolin in plasma were determined by a high-performance liquid chromatographic (HPLC) assay. Plasma samples of cefazolin were treated before HPLC assay as follows. Aliquots (0.1 ml) of plasma, 0.3 ml of methanol and 0.2 ml of phosphate buffer (pH 7.4) were put into a polyethylene tube (1.5 ml) and mixed vigorously. The mixture was cooled at 4 °C for 15 min, then centrifuged for 2 min by Centrifuge 5142, Eppendorf. The supernatant filtered through membrane filter TM-2 (Toyo Roshi Co., Ltd., Tokyo, Japan) was applied to the HPLC. Assays were carried out using a constant-flow high-performance liquid chromatograph, consisting of a solvent delivery system, BIP-1 or TRI ROTOR-II (Japan Spectroscopic Co., Tokyo, Japan), a guard column, C18/CORASIL, a reversed phase column, μ Bondapak C18 (30 cm × 3.9 mm i.d.; Waters Associates, Inc. Milford, Mass), packed in this laboratory, and a variable ultraviolet (UV) detector, UVIDEC 100-V or UVIDEC 100-III (Japan Spectroscopic Co., Tokyo, Japan) set at 270 nm. Cefazolin in plasma was eluted in a mobile phase of 10% acetonitrile in 0.01 M ammonium acetate. The column and solvent were kept at ambient temperature. The flow rate was controlled at 1.5 ml/min and the injection volume was 10–80 μl. Peak area, recorded by CHROMATOPAC C-R1B (Shimadzu Co., Kyoto, Japan), was used for quantification.

Concentration of inulin in plasma was determined by radiochemical assay according to the method described previously.3) Pharmacokinetic Analysis — The cefazolin and inulin data were analyzed according to the model independent moment analysis.7) The last determined plasma concentration was extrapolated to time infinity using the terminal slope
of the log-time disposing curve. The area under the plasma concentration versus time curve was estimated by trapezoidal rule. The steady state distribution volume per body weight ($V_d_{ss}/BW$) and the total body clearance per body weight ($Cl_{tot}/BW$) were estimated as described by Yamaoka, et al.

**RESULTS**

**Comparison of Pharmacokinetics of Cefazolin among 1-, 7-, 50- and 100-Week-Old Rats**

Figure 1 represents the mean plasma concentration versus time profile of cefazolin after an intravenous bolus administration of 20 mg/kg to 1-, 7-, 50- and 100-week-old rats. The age of the rats had a marked influence on the distribution and elimination of cefazolin. The half-lives of the terminal phase in 1-, 7-, 50- and 100-week-old rats were 117, 34.8, 45.9 and 141 min, respectively. The plasma level was analyzed by the model independent moment analysis. Table I lists the area under the plasma concentration versus time curve ($AUC$), mean residence time ($MRT$), volume of distribution at steady state per body weight ($V_d_{ss}/BW$) and total body clearance per body weight ($Cl_{tot}/BW$) of cefazolin in rats as a function of age. There were remarkable differences in these pharmacokinetic parameters depending on the age of the rat (Table I). The value of distribution volume at steady state per body weight varied about 2-fold, from 136 ml/kg (50 weeks) to 297 ml/kg (1 week); and the total body clearance per body weight varied about 5-fold, from 0.94 ml/min/kg (100 weeks) to 4.48 ml/min/kg (7 weeks) (Table I). It is noteworthy that the volume of distribution at steady state per body weight in 100-

![Graph showing plasma concentration versus time](image)

**TABLE I. Pharmacokinetic Parameters of Cefazolin in 1-, 7-, 50- and 100-Week-Old Rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1 Week</th>
<th>7 Weeks</th>
<th>50 Weeks</th>
<th>100 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>4–9</td>
<td></td>
<td>3</td>
<td>3–7</td>
</tr>
<tr>
<td>$AUC^{c)}$</td>
<td>µg min/ml</td>
<td>10500±497</td>
<td>4460±92</td>
<td>8500±461</td>
</tr>
<tr>
<td>$MRT^{c)}$</td>
<td>min</td>
<td>156±14</td>
<td>46.3±2.0</td>
<td>57.7±6.4</td>
</tr>
<tr>
<td>$V_d_{ss}/BW^{c)}$</td>
<td>ml/kg</td>
<td>297±37</td>
<td>208±12</td>
<td>136±20</td>
</tr>
<tr>
<td>$Cl_{tot}/BW^{c)}$</td>
<td>ml/min/kg</td>
<td>1.90±0.09</td>
<td>4.48±0.09</td>
<td>2.35±0.13</td>
</tr>
</tbody>
</table>

*a) Determined after intravenous bolus injection of a 20 mg/kg dose.  b) Data are presented as the mean ± S.D.  c) Calculated from a set of mean plasma concentration and its S.D. at each time after drug administration.*
Comparison of Pharmacokinetics of Inulin among 1-, 7-, 50- and 100-Week-Old Rats

Figure 2 illustrates the mean plasma concentration versus time profile of inulin after an intravenous bolus administration of 20 mg/kg to 1-, 7-, 50- and 100-week-old rats. The age of the rat also had a marked influence on the distribution and elimination of inulin, showing a tendency similar to that of cefazolin with increasing age. Table II lists the pharmacokinetic parameters resulting from the moment analysis of inulin levels of plasma among rats of various ages. There was also a marked difference in these parameters for inulin depending on the age of the rat (Table II). The value of distribution volume at steady state per body weight, i.e. extracellular water volume per body weight, $V_{ECW}/BW$ varied about 3-fold, from 126 ml/kg (50 weeks) to 370 ml/kg (1 week); and glomerular filtration rate per body weight varied about 3.5-fold, from 1.87 ml/min/kg (100 weeks) to 6.47 ml/min/kg (7 weeks) (Table II).

**Correlation between Distribution Volume of Cefazolin at Steady State and Extracellular Water Volume in Rats of Various Ages**

Figure 3 shows the correlation between distribution volume of cefazolin at steady state per body weight and extracellular water volume per body weight in rats of different ages. There was a linear relationship with the correlation coefficient of 0.977. The slope of the line was 0.608, and the Y-ordinate intersection was 74.9 ml/kg.

**TABLE II. Pharmacokinetic Parameters of Inulin in 1-, 7-, 50- and 100-Week-Old Rats a)**

<table>
<thead>
<tr>
<th>Parameters b)</th>
<th>1 Week</th>
<th>7 Weeks</th>
<th>50 Weeks</th>
<th>100 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>g</td>
<td>16.1±1.6</td>
<td>241±12</td>
<td>757±83</td>
</tr>
<tr>
<td>$n$</td>
<td>3–4</td>
<td>3–8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$AUC$ c)</td>
<td>μg min/ml</td>
<td>6180±347</td>
<td>3090±116</td>
<td>6170±344</td>
</tr>
<tr>
<td>$MRT$ c)</td>
<td>min</td>
<td>114±15</td>
<td>33.9±29</td>
<td>38.9±5.0</td>
</tr>
<tr>
<td>$V_{ECW}/BW$ c)</td>
<td>ml/kg</td>
<td>370±61</td>
<td>219±24</td>
<td>126±20</td>
</tr>
<tr>
<td>$GFR/BW$ c)</td>
<td>ml/min/kg</td>
<td>3.24±0.18</td>
<td>6.47±0.24</td>
<td>3.24±0.18</td>
</tr>
</tbody>
</table>

a) Determined after intravenous bolus injection of a 20 mg/kg dose. b) Data are presented as the mean ± S.D. c) Calculated from a set of mean plasma concentration and its S.D. at each time after drug administration.
DISCUSSION

By means of a physiologically based pharmacokinetic approach to the distribution and elimination of β-lactam antibiotics, it has recently been indicated that β-lactam antibiotics are localized in extracellular water space (ECW) and bound to albumin of both intravascular and interstitial fluid in the non-disposing organ after administration. These tissue distribution characteristics can be expressed by the following equation:

\[ K_p = \frac{K_d + (C_{fp})_{ss} + n AR P}{K_d + (C_{fp})_{ss} + n P} \] (1a)

FIG. 3. Correlation between Steady State Distribution Volume of Cefazolin and Extracellular Water Volume in Several Aged Rats
Key: ○, 1 week; ●, 7 weeks; △, 50 weeks; ▲, 100 weeks.

FIG. 4. Influence of Changes in the Plasma Unbound Fraction (fp) and Interstitial Fluid Space (ISi) on the Tissue-to-plasma Partition Coefficient (Kp)
Panel a, ARi is 0.5; panel b, ARi is 0.6; panel c, ARi is 0.9; panel d, ARi is 1.0. The number next to each line is the plasma unbound fraction, which varied from 0 to 1.0.
where $K_p$ is the tissue-to-plasma partition coefficient of $\beta$-lactam antibiotics for the non-disposing organ, $IS$ is the interstitial fluid space (i.e. ratio of interstitial fluid volume to organ volume, which was calculated as the ratio of each tissue to plasma concentration of inulin at steady state), $AR$ is the interstitial-to-plasma albumin concentration ratio, $K_d$ is a dissociation constant for drug binding to albumin, $(C_{f_p})_{ss}$ is the unbound concentration in plasma at steady state, and $n$ and $P$ are the number of binding sites and albumin concentration in plasma, respectively. In the case of linear binding of $\beta$-lactam antibiotics to plasma albumin, a more simpler equation can be obtained:

$$K_p = IS(AR + (1 - AR)f_p) \quad \text{(1b)}$$

where $f_p$ is the fraction of unbound drug in plasma. Previous work\(^3\) used $AR$ values in rats of 0.5 for lung, heart, fat and others, 0.6 for muscle, 0.9 for gut and 1.0 for skin and bone marrow. Figures 4a, b, c and d illustrate the influence of changes in the values of $f_p$ and $IS$ on the $K_p$ value when the $AR$ values are 0.5 (in Fig. 4a), 0.6 (in Fig. 4b), 0.9 (in Fig. 4c) and 1.0 (in Fig. 4d), respectively. Figure 4a suggests that the $IS$ value changes the $K_p$ value proportionally at any value of $f_p$, even if a 1000-fold or greater increase in the $f_p$ value produces only a twofold increase in the $K_p$ value in rats. Figure 4d suggests that the $K_p$ value is equal to the $IS$ value and that plasma binding produces no influence on the changes in the $K_p$ value for non-disposing organs such as the skin, as the $AR$ value is 1.0. In addition, it was demonstrated that the value of $IS$ varies proportionally with the $K_p$ value at any value of $AR$, while the $f_p$ value plays a minor role in the changes in the $K_p$ value when the $AR$ value approaches 1.0 (Fig. 4a–d).

The volume of distribution at steady state per body weight, $Vd_{ss}/BW$, is given\(^8\) by:

$$Vd_{ss}/BW = (V_p + \Sigma K_p V_i)/BW \quad \text{(2a)}$$

where $V_p$ and $V_i$ are the plasma volume and the volume of other tissues in the body, respectively. The $K_p$ values for skin, bone marrow and gut were suggested to be slightly dependent on or independent of the $f_p$ value (Fig. 4c, d). Since the sum of $V_p$ and $K_p V_i$ values per body weight for these three tissues was reported to be almost 78% of $Vd_{ss}/BW$ for cefazolin in adult rats,\(^9\) the increase of $Vd_{ss}/BW$ resulting from the changes in $f_p$ values would be less than 22%. There was a marked change in $Vd_{ss}/BW$ for cefazolin among the different ages below 50-week-old in the rat (Table I); e.g., the value of $Vd_{ss}/BW$ in 1-week-old rats decreased to about half in comparison with that in 50-week-old rats. Our preliminary study has obtained the values of serum unbound fraction to be 0.637 ±0.025 ($n=5$, the mean ±S.E.M.) for 1-week-old rats and 0.339 ±0.020 ($n=6$) for 50-week-old rats in the concentration range of the terminal phase of plasma cefazolin time course after i.v. injection. (The result for the age-related changes of the serum protein binding will be published elsewhere.) Since it has not been elucidated yet whether the $AR$ values change with increasing age or not, we assumed that the $AR$ value is constant in all aged rats. Under this assumption the decrease of $Vd_{ss}/BW$ resulting from the changes in serum protein binding would be less than 18%, since the decrease of $K_p/IS$ value from 1-week-old rats to 50-week-old rats was estimated to be 18% from Eq. 1b by using the $ARi$/value of 0.5 and the unbound fractions described above for the respective rats. However, the observed difference in $Vd_{ss}/BW$ was greater than that predicted from the changes in serum protein binding with increasing age. Thus, it was suggested that the $f_p$ value moderately influences the changes in steady state distribution volume of $\beta$-lactam antibiotics per body weight with increasing age.

According to Eqs. 1b and 2a, the distribution volume of $\beta$-lactam antibiotics at steady state per body weight can be expressed as follows:

$$Vd_{ss}/BW = (V_p + \Sigma V_{i,non} (AR + (1 - AR)f_p) + \Sigma K_{p,dis} V_{i,dis})/BW \quad \text{(2b)}$$
where $V_{\text{dis,non}}$ is the interstitial fluid volume of the non-disposing organ, $K_{p,\text{dis}}$ and $V_{t,\text{dis}}$ are tissue-to-plasma partition coefficients of $\beta$-lactam antibiotics for the disposing organ and the volume of the disposing organ (e.g., liver and kidney), respectively. This equation also suggests that the extracellular water volume per body weight directly affects the steady state distribution volume of $\beta$-lactam antibiotics per body weight, whereas plasma binding has a minor influence on changes in the steady state distribution volume.

In the present experiment in rats, there was a linear relationship between the steady state distribution volume of cefazolin per body weight and extracellular water volume per body weight (Fig. 3). The result supports the idea that changes in extracellular water space could be a major determinant for the changes in $Vd_{ss}/BW$ of $\beta$-lactam antibiotics with increasing age, as suggested by Eq. 2b. The Y-ordinate intersection of Fig. 3 may represent the sum of $K_{p,\text{dis}} \cdot V_{t,\text{dis}}/BW$ and may be attributed, in part, to the fluctuations of plasma protein binding or interstitial-to-plasma albumin concentration ratio among different ages in the rat.

There have been many experimental reports$^{10,11}$ concerning the aging effect on the distribution volume of $\beta$-lactam antibiotics in humans, and Ritschel$^{13}$ Nightingale$^{12}$ and Brogard and Strasbourg$^{13}$ have presented reviews. In comparing of $Vd_{ss}/BW$ between adults, newborns and infants, the younger the age, the greater the value of $Vd_{ss}/BW$ that can be obtained for cefazolin, cephalothin and cefmetazole.$^{10-13}$ Furthermore, extracellular water volume per body weight ($V_{ECW}/BW$) in the human has also been reported to change markedly with increasing age.$^6$ A rapid decrease of $V_{ECW}/BW$ in the very early stage of life (44.5% of body weight for 0 to 1-day-old newborns, 25.6% of body weight for 1 to 2-year-old babies) followed by a smaller decrease in $V_{ECW}/BW$ later in childhood (18.7% of body weight for 10 to 15-year-old children).$^6$ Our findings concerning the aging effect on steady state distribution volume in rats from newborn (1 week) to adult (7 weeks) suggested that the decrease of $Vd_{ss}/BW$ in humans is predominantly attributable to the remarkable decrease in $V_{ECW}/BW$.

According to our results for $Vd_{ss}/BW$ in 7-, 50-, and 100-week-old rats, it would be expected that in humans $Vd_{ss}/BW$ of $\beta$-lactam antibiotics would decrease until middle age and then increase with increasing age. However, there was no such general rule found in the comparison of the distribution volume per body weight, $Vd/BW$, between adult and elderly men, as reviewed by Ritschel.$^{13}$ These results indicate i) no change in $Vd/BW$, ii) decrease in $Vd/BW$, iii) increase in $Vd/BW$ and iv) both a decrease and increase in $Vd/BW$, with increasing age. One possible explanation for the complicated results in the changes of $Vd_{ss}/BW$ of $\beta$-lactam antibiotics between adult and elderly men is that the extent of inter-individual difference of the $V_{ECW}/BW$ would be greater than that of experimental animals as rats used in this study. It has been reported that there is no significant difference in $V_{ECW}/BW$ between young (16–39 years old) and old (59–89 years old) humans, while the value of $V_{ECW}/fat-free$ body mass of the elder group is significantly greater than that of the younger group.$^{14}$ This behavior in humans is very similar to the change of $V_{ECW}/BW$ in rats evaluated in this study from insulin distribution volume. Thus, age dependence of the volume of adipose tissue in humans was suggested to be one of the most important factor which influences the inter-individual difference of $V_{ECW}/BW$. Accordingly, the value of $Vd_{ss}/fat-free$ body mass should be compared for the study of age-related changes on the distribution of $\beta$-lactam antibiotics in humans.

Total body clearance is also important factor influencing the drug distribution. There was a marked difference of total body clearance per body weight of cefazolin among different aged rats (Table I). Renal excretion is a major elimination route of cefazolin in rats and human.$^{12}$ As seen from the data in Tables I and II, there was a good correlation between cefazolin total body
clearance per body weight and glomerular filtration rate per body weight in the different age \( r = 0.991 \). The renal function of 1-week-old rats increased 2-fold in 7-week-old rats and then decreased with increasing age. This physiological change as a function of age would be one of the dominant factors for the changes of total body clearance of cefazolin per body weight.

In conclusion, it was demonstrated that the changes in distribution volume per body weight at steady state of \( \beta \)-lactam antibiotics are predominantly ascribed to the changes in extracellular water volume per body weight with increasing age, whereas the changes in plasma protein binding could have a modest influence on the changes in distribution volume of the antibiotic per body weight at steady state.

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