COMPARATIVE DISTRIBUTION KINETICS OF CEFAZOLIN AND TOBRAMYCIN IN CHILDREN

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The time courses of drug concentration in serum after i.v. drip infusion of 2 mg/kg of tobramycin and 25 mg/kg of cefazolin in children were analyzed by model-independent moment analysis. The volume of distribution at the steady state per body weight (V_{dss}/BW) of tobramycin was in the range of 212 to 335 ml/kg and that of cefazolin was 119 to 156 ml/kg. A plot of the differences of V_{dss}/BW obtained in the same child for tobramycin and cefazolin against the value of V_{dss}/BW of tobramycin gave a linear regression line ($r = 0.971$). The magnitude of V_{dss} (l) of tobramycin could be well interpreted as corresponding to the extracellular water volume. In the case of cefazolin, the extracellular water space accounts for about 60% of the total distribution volume. The remaining 40% of the total V_{dss} of cefazolin was considered to be accounted for by the disposing organs.

Keywords—cefazoline; tobramycin; distribution volume; pharmacokinetics; intravenous administration; extracellular water volume

INTRODUCTION

Cefazolin and tobramycin, which are widely used as antibacterial drugs, are distributed in extracellular fluid in infected tissues.¹⁾ The distribution kinetics of cefazolin²⁾ and tobramycin³⁾ show different characteristics in patients of different ages. This effect has been attributed to changes in extracellular water content and in renal and/or hepatic disposition of these antibiotics. We have already reported that the volume of distribution at the steady state per body weight of cefazolin in animals^{4,5)} can be explained mathematically in terms of distribution into the extracellular water space and into the disposing organs. The age-related changes in kinetics of cefazolin could be interpreted as changes in extracellular water volume and changes in the serum protein binding in rats.^{6,7)} Although the volume of distribution of cefazolin in disposing organs such as liver and kidney was determined in rats⁷⁾ and rabbits,⁵⁾ there have been no such reports for humans. The volumes of distribution of tobramycin also vary widely among children.^{8,9)} However, the differences in the nature of distribution of cefazolin and tobramycin in children have not yet been clarified. Since it has been recognized that extracellular

water content in children changes interindividually during growth,¹⁰⁻¹²⁾ it is of great interest to compare the tissue distributions of cefazolin and tobramycin in the same child.

The purpose of this paper is to elucidate the relationship between the volumes of distribution at the steady state of cefazoline and tobramycin in children. This paper also describes the use of the volume of distribution as a guideline to determine the loading dose of both antibiotics.

MATERIALS AND METHODS

Subjects — Our subjects were patients admitted to Kanazawa University Hospital with diagnose of various heart diseases for the purpose of further examination by cardiac catheterization. All patients were in good physical condition without any apparent symptoms of heart failure. These patients received tobramycin and cefazolin for prevention of infection after examination. None showed clinical or laboratory evidence of renal, hepatic, endocrine or metabolic disorders or had a history of allergy to cephalosporins. The characteristics of subjects who participated in this study are given in Table I. The six patients ranged in age from 3 to 12 years, and each weighed within 10% of the ideal body weight as

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TABLE I. *Characteristics of the Study Population*

Patient No.	Name	Age	Sex	Height (cm)	Weight (kg)	Body surface area (m ²)
1	M.S.	3y	F	91.5	13.4	0.61
2	S.S.	4y3m	M	101.4	16.9	0.72
3	N.T.	5y11m	F	103.5	13.8	0.65
4	I.H.	6y	F	110.0	16.0	0.73
5	N.I.	9y4m	F	136.1	27.8	1.01
6	K.M.	12y3m	M	139.3	29.9	1.12

CL _{cr} /BW (ml/min/kg)	CL _{cr} /BSA (ml/min/m ²)	Laboratory data				
		Hct	Scr	T. protein	GOT	GPT
1.87	45.6	36	0.6	6.6	23	10
1.73	45.0	46	0.7	6.4	32	14
1.42	32.7	35	0.7	7.2	32	8
1.84	44.6	34	0.6	7.6	30	22
1.88	53.4	37	0.7	7.0	26	9
—	—	39	0.8	8.0	40	12

CL_{cr} = creatinine clearance, Hct = hematocrit (%), Scr = serum creatinine (mg/dl), T. Protein = serum total protein (g/dl), GOT = glutamic oxaloacetic transaminase (U), GPT = glutamic pyruvic transaminase (U).

estimated for individual height and sex. No patient had been given other antibiotics before this study. All had normal laboratory screening results, including white blood cell count, red blood cell count, hematocrit, platelet count, serum creatinine (S_{cr}), blood urea nitrogen, serum total protein, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and serum electrolytes (Na, K, Cl). Body surface area was estimated from the body weight and height of each patient by using the nomogram of Haycock *et al.*¹³⁾ Creatinine clearance (CL_{cr}) was estimated from the following formula: CL_{cr} (ml/min/kg) = $U \times V / S_{cr} \times BW$ (kg) $\times 60$, where V is urine volume (ml) in 60 min, U is creatinine concentration in urine (mg/ml) and S_{cr} is the creatinine concentration (mg/ml) of sampled serum during 60 min.

Drug Administration Protocol — The materials used in this work were commercial drugs: tobramycin sulfate (Tobracin; Shionogi Pharmaceutical Co., Ltd., Osaka, Japan) and cefazolin sodium (Cefamezin; Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan). Each patient received a single 25 mg/kg dose of cefazolin by intravenous infusion over 30 min. Subsequently, with an interval of 10 min, tobramycin 2.0 mg/kg was given over 30 min in an identical manner. Each

dose of cefazolin and tobramycin was diluted in 30 ml of sterile water and administered through an indwelling catheter (HEMAQUET; USCI Co., Billerica, MA) placed in a femoral vein during the cardiac catheterization. The infusion rate was controlled by a constant flow pump (STC-521; Terumo Co., Ltd., Tokyo, Japan). Any remaining drug in the infusion line was washed out with several ml of saline at the end of administration.

Sampling — An indwelling needle was placed in a vein of the dorsal manus and blood samples were withdrawn through the needle at the following periods: 30, 50, 70, 90, 110, 130, 160, 190, 220 and 280 min after the start of the cefazolin infusion (corresponding to 10, 30, 50, 70, 90, 120, 150, 180 and 240 min after the start of the tobramycin infusion). Serum samples were separated and assayed on the same day or frozen at -20°C until analyzed. No glass instruments were used in order to avoid adhesion of tobramycin.

Cefazolin Assay — Serum concentration of cefazolin was determined by high-performance liquid chromatography. Fifty microliters of serum sample, 50 μl of isotonic phosphate buffer (pH 7.4) and 100 μl of methanol were put into a polyethylene centrifuge tube (1 ml).

The tube was mixed for 30 s with a vortex mixer, allowed to stand in an ice bath for 30 min, then centrifuged at 12000 rpm for 30 min at 4 °C in a refrigerated centrifuge (MR-15A; Tomy Seiko Co., Ltd., Tokyo, Japan). The supernatant fluid was filtered through a membrane filter (TM-2; Toyo Roshi Co., Ltd., Tokyo, Japan). The injection volume of sample was 15 μ l and the flow rate was 1.5 ml/min. A solvent delivery system (TRI-ROTTER; Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with a reverse-phase column (μ -Bondapak C18 column; 30 cm $\times$ 4.0 mm i.d., Waters Associates, Milford, MA) was used with a variable-wavelength UV detector (UVIDEC-100-VI; Japan Spectroscopic Co., Ltd.) set at 270 nm (range 0.08 AFS). The peak area was recorded with a data collector (DS-L300; Japan Spectroscopic Co., Ltd.). The mobile phase for this assay was 10% acetonitrile in 0.01 M ammonium acetate. To prepare a standard curve, 50 μ l of pooled human serum were added to 50 μ l of various concentrations of cefazolin and tested in triplicate. The serum cefazolin concentrations were correlated in a linear fashion with the peak areas from 5 to 200 μ g/ml ($r = 0.999$).

Tobramycin Assay — Tobramycin concentrations in the serum were assayed by a fluorescence polarization immunoassay (TDX; Dinabot Co., Ltd., Tokyo, Japan). All samples were assayed in duplicate or triplicate and the mean concentrations were used for pharmacokinetic analysis.

Determination of Cefazolin Binding to Serum Protein — To investigate the binding of cefazolin to serum protein, the ultrafiltration technique was adopted. Disposable Centrifree MPS devices (Amicon Co., Denver, MA) were used. A portion (0.5–1.0 ml) of each sample withdrawn at appropriate intervals after the administration of cefazolin was added to the sample reservoir. The ultrafiltrate was obtained by centrifugation at 37 °C for 7 min (2400 rpm), using a KR-702 Centrifuge equipped with a 45 ° angle rotor, Type RA-360 (Kubota Co., Ltd., Tokyo, Japan). The percentage of cefazolin bound to serum protein was calculated with reference to the initial sample concentration. For tobramycin, no appreciable binding with serum protein was observed.

Data Analysis — The serum concentration data in children were analyzed by model-independent moment analysis. The area under

FIG. 1. Serum Concentration-Time Profiles after a 30 min Intravenous Drip Infusion of Tobramycin 2 mg/kg in Six Children

○, 1; ●, 2; ▲, 3; △, 4; ■, 5; □, 6. The numbers at the right of symbols identify the patients. The pharmacokinetic parameters are listed in Table II.

the serum concentration to infinite time *versus* time curve was estimated by means of the trapezoidal rule by using the terminal slope of the log serum concentration-time curve. The mean residence time (MRT), the distribution volume at the steady state per body weight (V_{dss}/BW) and the total body clearance per body weight (CL_{tot}/BW) were estimated as described by Yamaoka *et al.*¹⁴ Since drugs were given by a constant rate intravenous infusion for 30 min, MRT was corrected by subtracting the mean infusion time (15 min) from the MRT after the infusion administration.¹⁵

RESULTS

Figure 1 shows the serum concentration *versus* time profile of tobramycin after intravenous drip infusion into six children. The half-life of the terminal phase ranged from 79.6 to 153 min. Table II lists the model-independent kinetic parameters, MRT, V_{dss}/BW , and CL_{tot}/BW of tobramycin. The values of V_{dss}/BW and CL_{tot}/BW were in the range from 212 to 335 ml/kg and 1.30 to 2.18 ml/min/kg, respectively, being coincident with those previously reported in children.⁹

The time-courses of the serum levels of cefazolin after intravenous drip infusion are illustrated in Fig. 2. The half-life of the terminal phase ranged from 67.9 to 137 min. Table III

TABLE II. Pharmacokinetic Parameters of Tobramycin after 2.0 mg/kg Intravenous Administration

Patient No.	$t_{1/2}$ (min)	MRT (min)	V_{dss}/BW (ml/kg)	Total V_{dss} (l)	CL_{tot}/BW (ml/min/kg)	CL_{tot}/BSA (ml/min/m ²)
1	79.6	115	251	3.81	2.18	53.1
2	84.6	120	212	4.04	1.77	46.0
3	153	201	261	3.86	1.30	29.9
4	140	169	335	5.86	1.98	48.0
5	111	142	261	8.03	1.84	52.2
6	108	140	259	8.55	1.85	52.5
Mean (S.D.)	113(29)	148(32)	263(40)	5.69(2.16)	1.82(0.29)	47.0(8.8)

TABLE III. Pharmacokinetic Parameters of Cefazolin after 25 mg/kg Intravenous Administration

Patient No.	$t_{1/2}$ (min)	MRT (min)	V_{dss}/BW (ml/kg)	Total V_{dss} (l)	CL_{tot}/BW (ml/min/kg)	CL_{tot}/BSA (ml/min/m ²)	Protein binding (%)
1	67.9	106	119	1.82	1.12	27.3	82.3
2	77.9	118	120	2.30	1.02	26.5	75.0
3	137	192	142	2.11	0.74	17.1	79.7
4	103	138	156	2.77	1.13	27.4	79.7
5	90.5	124	134	4.17	1.08	30.6	—
6	76.6	120	124	4.14	1.03	29.2	77.7
Mean(S.D.)	92.2(25.2)	133(31)	133(15)	2.89(1.03)	1.02(0.14)	26.3(4.8)	78.1(2.5)

lists the model-independent kinetic parameters, MRT, V_{dss}/BW and CL_{tot}/BW of cefazolin. The

FIG. 2. Serum Concentration-Time Profiles after a 30 min Intravenous Drip Infusion of Cefazolin 25 mg/kg in Six Children

○, 1; ●, 2; ▲, 3; △, 4; ■, 5; □, 6. The numbers at the right of symbols identify the patients. The pharmacokinetic parameters are listed in Table III.

values of V_{dss}/BW and CL_{tot}/BW ranged from 119 to 156 ml/kg and 0.74 to 1.13 ml/min/kg, respectively. As also listed in Table III, the serum protein binding of cefazolin in children ranged from 75 to 82.3%.

The values of CL_{tot}/BW of tobramycin were almost equal to the creatinine clearance in the same child, and those of cefazolin were about 60% of the creatinine clearance (Tables I and II). Relationship between of tobramycin and the body weight led to the equation of (V_{dss})_{TOB} (l) = $0.261 \times BW$ (kg) ($r = 0.944$). The values of V_{dss}/BW of cefazolin and tobramycin were not related with the value of hematocrit or protein binding of cefazolin. However, there was an apparent correlation between the values of V_{dss}/BW of cefazolin and tobramycin in the same child.

DISCUSSION

It has recently been indicated that β -lactam antibiotics after administration are localized in the extracellular water space and bound to albumin of both intravascular and interstitial fluids in non-disposing organs, although, in disposing

organs such as the liver and kidney, these antibiotics are distributed in intracellular fluid.⁴⁻⁶⁾ On this basis, V_{dss}/BW can be expressed by the following equation⁶⁾:

$$V_{dss}/BW = (V_p + \sum V_{is,non} (AR + (1 - AR)f_p) + \sum K_{p,dis} V_{t,dis})/BW \quad (1)$$

where V_p , $V_{is,non}$ and $V_{t,dis}$ are the serum volume, the interstitial fluid volume of one of the non-disposing organs, and the volume of one of the disposing organs, respectively. f_p is the fraction of unbound drug in serum, AR is the interstitial-to-serum albumin concentration ratio and $K_{p,dis}$ is the tissue-to-serum concentration ratio in the corresponding disposing organ.

Although the accumulation of aminoglycosides in the peripheral tissues was suggested after repeated dosing,¹⁶⁾ the values of V_{dss}/BW , which are better estimates of the extracellular space, can be obtained from the 2-compartment analysis of serum data after a single dose.^{17,18)} For V_{dss}/BW of tobramycin, therefore, Eq. 1 may also be adopted. From the fact that (1) no serum protein binding of tobramycin was observed (*i.e.* $f_p = 1$) and that (2) the distribution of tobramycin in liver and kidney in rats was less than 15% of the volume of distribution at steady state,¹⁸⁾ the above equation for V_{dss}/BW of tobramycin can be simplified to the following form:

$$(V_{dss}/BW)_{TOB} = (V_p + \sum V_{is,non})/BW = V_{ecw}/BW \quad (2)$$

where $(V_{dss}/BW)_{TOB}$ and V_{ecw} represent the V_{dss}/BW of tobramycin and extracellular water volume. In our results shown in Table II, the value of $(V_{dss}/BW)_{TOB}$ was coincident with the value of V_{ecw}/BW in children.¹⁰⁾

On the other hand, since it was reported that the changes in V_{dss}/BW of cefazolin are related directly to the changes in extracellular water space,⁷⁾ the V_{dss}/BW of cefazolin should be given by the following modified equation:

$$(V_{dss}/BW)_{CEZ} = R V_{ecw}/BW + \sum K_{p,dis} \cdot V_{t,dis}/BW \quad (3)$$

where $(V_{dss}/BW)_{CEZ}$ represents the V_{dss}/BW of cefazolin. R is defined as follows:

$$R = (V_p + \sum V_{is,non} (AR + (1 - AR)f_p))/V_{ecw} \quad (4)$$

FIG. 3. Correlation between $(V_{dss}/BW)_{TOB} - (V_{dss}/BW)_{CEZ}$ and $(V_{dss}/BW)_{TOB}$ in Children
 $Y = -52.2 + 0.694X$, $r = 0.97$, $p < 0.01$

According to these two equations, the difference of V_{dss}/BW between tobramycin and cefazolin is expressed as follows:

$$(V_{dss}/BW)_{TOB} - (V_{dss}/BW)_{CEZ} = (1 - R)(V_{dss}/BW)_{TOB} - \sum K_{p,dis} \cdot V_{t,dis}/BW \quad (5)$$

Equation 5 was verified by Fig. 3, which is consistent with a linear relationship between the value of $(V_{dss}/BW)_{TOB} - (V_{dss}/BW)_{CEZ}$ and the value of $(V_{dss}/BW)_{TOB}$ in children. The results supported the idea that changes in extracellular space could be a major determinant for the interindividual changes in V_{dss}/BW of tobramycin and cefazolin in children. The Y -ordinate intersection in Fig. 3 may represent the value of $-K_{p,dis} V_{t,dis}/BW$; the value thus obtained ($= 52.2$ ml/kg) agrees quite well with that obtained in rats (74.9 ml/kg).⁷⁾ In the excretory tissues (liver and kidney), β -lactam antibiotics can be taken up by carrier-mediated transport systems¹⁹⁻²³⁾ and then bind strongly to water-soluble intracellular protein, ligandin.^{24,25)} High tissue-to-serum concentration ratios in liver and kidney, exceeding the value of the extracellular space, were obtained in rats⁴⁾ and rabbits.⁵⁾ On the other hand, in our preliminary experiments, [³H]tobramycin was not detected in isolated hepatocytes after a 30 min incubation (data not shown). These results suggest that the volume expressed by the Y -intersection is attributable to the distribution of cefazolin in the disposing organs (liver and kidney).

In human adults,¹⁸⁾ the R value was estimated to be 0.6 and the value determined experimentally was also 0.6 in rats.⁷⁾ However, by taking the f_p value of 0.219 ± 0.025 determined in the children who participated in this study, the R value was evaluated to be 0.3 from the magnitude of the slope in Fig. 3. This discrepancy in the R value between adults and children may be due to differences in the f_p , AR and/or the ratio of serum volume to extracellular volume.

In conclusion, the value of V_{dss} of tobramycin could be interpreted by assuming it is equal to V_{ecw} , and V_{dss} of cefazolin expressed as follows:

$$(V_{dss})_{CEZ} (l) = 0.3 \times V_{ecw} + 0.052 \times BW (kg) \quad (6)$$

where the first and second terms on the right side correspond to the distribution volumes of the extracellular water space and the disposing organs in children, respectively. The extracellular water space accounts for about 60% of the total distribution volume of cefazolin, though this value may be expected to change markedly with fluctuation of both the extracellular water space itself and the unbound fraction in serum. The other 40% of the total distribution of cefazolin is accounted for by the disposing organs and is not influenced by changes of the extracellular water space and the serum protein binding.

The difference in the tissue distribution characteristics between cefazolin and tobramycin is interesting, since it implies that the dosage regimen of tobramycin should be changed simply according to the amount of extracellular water space expected, for example, in the treatment of obese children. However, in the case of cefazolin, if the volume of the disposing organs increases proportionally with the increase in body weight, the dosage regimen of cefazolin should take into account changes in the distribution volumes of both the disposing organs and extracellular water space. In a subsequent paper, we will demonstrate the usefulness of the above equations in the prediction of the distribution volume of cefazolin and tobramycin in obese children.

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