—Regular Articles—

Estimating Pediatric Doses of Drugs Metabolized by Cytochrome P450 (CYP) Isozymes, Based on Physiological Liver Development and Serum Protein Levels

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(Received October 26, 2009; Accepted December 10, 2009; Published online December 14, 2009)

We established a method for estimating pediatric doses of drugs metabolized by cytochrome P450 (CYP) isozymes, using the free fraction of drug in plasma (fu), serum protein level (P), liver volume (LV), and CYP activity ($V\mathrm{max}/Km$) as indices of physiological and biochemical development in children up to 15 years old. This method allows the child/adult dose ratio (D_C/D_A) = child/adult oral clearance ratio ($CL_{(PO)_C}/CL_{(PO)_A}$) of drugs mainly metabolized in the liver to be estimated by the following equation:

$$\frac{D_C}{D_A} \cong \left(\frac{1}{fu_A + (1 - fu_A)\dfrac{P_C}{P_A}} \right) \cdot \left(\frac{BSA_C}{BSA_A} \right)^{1.176} \cdot \left(\frac{V\mathrm{max}_C/Km_C}{V\mathrm{max}_A/Km_A} \right)$$

Major metabolism of drugs was ascribed to CYP1A2 for theophylline and caffeine, and CYP1A2 and CYP2D6 for propranolol and mexiletine. For theophylline and caffeine, $CL_{(PO)_C}/CL_{(PO)_A}$ calculated from the child/adult body surface area ratio (BSA ratio) and the value calculated by our method were compared, using $\widehat{CL}_{(PO)_C}/\widehat{CL}_{(PO)_A}$ calculated from the clearance ratio based on population pharmacokinetics (PPK ratio) as a reference. For all drugs, pediatric doses calculated from the Crawford equation and our equation were compared, with predetermined doses as the reference. For theophylline and caffeine, the relative accuracy of our method was significantly higher than that of BSA-based estimation when the PPK ratio was used for reference. For theophylline, caffeine, and propranolol, the relative accuracy of our method was significantly higher than that of BSA-based estimation when predetermined doses were used for reference. These findings indicate the validity of our method which considers the physiological and biochemical development (*i.e.*, fu, P, LV, and CYP activity) for pediatric dose estimation.

Key words——pediatric dose estimation; free fraction of drug in plasma; liver volume; CYP1A2; human serum albumin; α_1-acid glycoprotein

INTRODUCTION

Since pharmacokinetics and pharmacodynamics of drugs have not been fully elucidated in children, pediatric doses have not been established for many drugs. When using such drugs in pediatric patients, it is difficult for medical professionals to determine the appropriate doses. Therefore, when a physician prescribes a drug without a recommended dose for children, the adult dose is modified based on the age, weight, and body surface area (BSA) of the child in question using the Augsberger equation, Young equation, Clark equation, Crawford equation, or conversion table of von Harnack. Among them, the Crawford equation is based on the assumption that the pediatric dose is proportional to the child/adult BSA ratio. Augsberger equation and conversion table of von Harnack are the simplified expression of the Crawford equation, and thus used routinely in clinical situations. However, these methods of estimation do not take the physiological and biochemical development of children into account. We previously reported that pediatric dose estimation incorporating measures of physiological/biochemical development was more accurate than conventional estimation for drugs mainly excreted *via* the kidneys. Our method included the free fraction of drug in plasma (fu), serum protein level (P), glomerular filtration rate (GFR), and tubular secretion clearance (Sc), which directly affect the clearance (CL) of drugs mainly ex-

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creted *via* the kidneys.[1,2] With regard to metabolism in the liver, the development of various metabolizing enzymes cytochrome P450 (CYP isozymes) occurs at different rates.[3] For example, it takes about 3 years after birth before CYP1A2 reaches 80% of its activity in adults. Therefore, variations of CYP activity should be taken into consideration for pediatric dose estimation.

In this study, we established a new method of pediatric dose estimation for drugs metabolized by CYP, in children aged 15 years or younger that incorporated factors to allow for the influence of physiological/biochemical development. We employed *fu*, *P*, *LV*, and CYP activity as indices of physiological and biochemical development in children up to 15 years old, and designated this method as the ''method for estimating pediatric doses based on physiological and biochemical development'' (ePPBD). For drugs with the ePPBD and estimation based on the *BSA* ratio were compared, using the mean oral clearance ratio $\hat{CL}_{(PO)_C}/\hat{CL}_{(PO)_A}$ (PPK ratio) calculated based on population pharmacokinetic analysis ($\hat{CL}_{(PO)}$) for reference. The validity of the ePPBD was also examined by comparison with the Crawford equation, using predetermined dose such as those listed in package inserts or reported in the literature as the reference.

METHODS

Establishing a Method of Pediatric Dose Estimation that Incorporates *fu*, *P*, *LV*, and Hepatic CYP Activity (Namely ePPBD) The maintenance dose (D) is expressed by Eq. (1), which incorporates bioavailability (F), systemic clearance (clearance: CL) (l/h/body), mean steady state blood concentration in ($\overline{Css}$), and administration interval (τ).

$$D=\frac{CL\cdot\overline{Css}\cdot\tau}{F} \qquad \text{Eq. (1)}$$

Equation (2) is derived from Eq. (1), because F is the production of the fraction of drug absorbed (Fa), and the fractions of drug which avoids first-pass extraction through the gut and liver (Fg and Fh, respectively).

$$D=\frac{CL\cdot\overline{Css}\cdot\tau}{Fa\cdot Fg\cdot Fh} \qquad \text{Eq. (2)}$$

The clearance of drugs mainly metabolized in the liver can be approximated by the hepatic clearance (CLh) $\cong CL$, so $Fg\fallingdotseq 1$. When it is assumed that $Fa_C=Fa_A$, the dose ratio that yields the equivalent $\overline{Css}$ after oral administration (with the τ being common in child and adult) can be estimated by Eq. (3), where $CL_{(PO)}$ is the systemic clearance after oral administration, and subscripts C and A represent a child and an adult, respectively.

$$\frac{D_C}{D_A}=\frac{CL_{(PO)_C}}{CL_{(PO)_A}}\cong\left(\frac{CLh_C}{Fh_C}\right)\Big/\left(\frac{CLh_A}{Fh_A}\right) \qquad \text{Eq. (3)}$$

Equation (3) can then be expressed as Eq. (4), because $CLh/Fh=fu\cdot CL\text{int}$ (CLint: intrinsic hepatic clearance).

$$\frac{D_C}{D_A}=\frac{CL_{(PO)_C}}{CL_{(PO)_A}}\cong\frac{fu_C\cdot CL\text{int}_C}{fu_A\cdot CL\text{int}_A} \qquad \text{Eq. (4)}$$

It has been reported that fu_C can be expressed in terms of fu_A and the P, as shown in Eq. (5).[3]

$$fu_C=\frac{1}{1+\dfrac{(1-fu_A)\,P_C}{P_A\cdot fu_A}} \qquad \text{Eq. (5)}$$

Since fu_C depends on the type of protein to which a drug is bound, *i.e.*, it depends on whether a drug mainly binds to human serum albumin (HSA) or to α_1-acid glycoprotein (AGP), fu_C is estimated as shown below.

For drugs mainly bound to HSA, P is substituted by the HSA level $[HSA]$. Then, Eq. (5) can be expressed as Eq. (6).

$$fu_C\cong\frac{1}{1+\dfrac{(1-fu_A)\,[HSA_C]}{[HSA_A]\,fu_A}} \qquad \text{Eq. (6)}$$

It has been reported that the relationship between $[HSA_C]$ and age can be expressed as Eq. (7).[3]

$$[HSA_C]\,(\text{g/dL})=1.1287\cdot\ln(Age)+33.746 \qquad \text{Eq. (7)}$$

On the other hand, for drugs mainly binding to AGP, P is substituted by the AGP level $[AGP]$. Then, Eq. (5) can be expressed as Eq. (8).

$$fu_C\cong\frac{1}{1+\dfrac{(1-fu_A)\,[AGP_C]}{[AGP_A]\,fu_A}} \qquad \text{Eq. (8)}$$

It has been reported that the relationship between $[AGP_C]$ and age can be expressed as Eq. (9).[3]

$$[AGP_C]\,(\text{g/dL})=\frac{0.887\times Age^{0.38}}{8.89^{0.38}+Age^{0.38}} \qquad \text{Eq. (9)}$$

When the ratio of CYP activity (maximum rate of metabolism per gram of liver tissue: Vmax) to the Michaelis-Menten constant (Vmax/Km) and liver volume (LV) (g) are introduced into Eq. (4), D_C/D_A and $CL_{(PO)_C}/CL_{(PO)_A}$ can be expressed as Eq. (10), assuming that the drug concentration is lower

than Km and thus the metabolism is considered to be linear.

$$\frac{D_C}{D_A}=\frac{CL_{(PO)_C}}{CL_{(PO)_A}}=\frac{fu_C \cdot CLint_C}{fu_A \cdot CLint_A}\cong\left(\frac{fu_C}{fu_A}\right)\times\left(\frac{LV_C}{LV_A}\right)\cdot\left(\frac{V\max_C/Km_C}{V\max_A/Km_A}\right) \quad \text{Eq. (10)}$$

When there are more than one important metabolic pathways, D_C/D_A is calculated from the weighted average obtained as enzyme activity multiplied by its contribution ratio of X_i, as expressed in Eq. (11).

$$\frac{D_C}{D_A}=\frac{CL_{(PO)_C}}{CL_{(PO)_A}}\cong\left(\frac{fu_C}{fu_A}\right)\cdot\left(\frac{LV_C}{LV_A}\right)\times\sum_i\left(\frac{V\max,\ i_C/Km,\ i_C}{V\max,\ i_A/Km,\ i_A}\right)X_i \quad \text{Eq. (11)}$$

Table 1 shows the child/adult ratio $((V\max_C/Km_C)/(V\max_A/Km_A))$ of CYP activity per gram of liver tissue for each age bracket according to the literature.[3] It has been reported that LV can be expressed as Eq. (12) in terms of BSA (m^2).[3]

$$LV=0.722\times BSA^{1.176} \quad \text{Eq. (12)}$$

BSA (m^2) is calculated by the Dubois equation (Eq. (13)), based on the standard height (HT) and standard weight (Wt) of Japanese children published in 2000.[4,5]

$$BSA\,(m^2)=71.84\times HT\,(cm)^{0.725}\times Wt\,(kg)^{0.425}\times 0.0001 \quad \text{Eq. (13)}$$

The final equations for calculating the oral clearance ratio and pediatric dose are Eqs. (14) and (15), respectively.

$$\frac{CL_{(PO)_C}}{CL_{(PO)_A}}\cong\left(\frac{1}{fu_A+(1-fu_A)\dfrac{P_C}{P_A}}\right)\cdot\left(\frac{BSA_C}{BSA_A}\right)^{1.176}\times\left(\frac{V\max_C/Km_C}{V\max_A/Km_A}\right) \quad \text{Eq. (14)}$$

$$D_C\cong D_A\cdot\left(\frac{1}{fu_A+(1-fu_A)\dfrac{P_C}{P_A}}\right)\cdot\left(\frac{BSA_C}{BSA_A}\right)^{1.176}\times\left(\frac{V\max_C/Km_C}{V\max_A/Km_A}\right) \quad \text{Eq. (15)}$$

Evaluation of the New Dose Estimation Method (ePPBD) Incorporating Physiological Development

Selection of Drugs We selected drugs to evaluate our method according to the following criteria.

(1) Urinary excretion of the unchanged drug (Ae) is less than 10%.[6] (2) The drug mainly binds to HSA or AGP.[7–10] (3) The drug is metabolized by hepatic enzymes including CYP1A2, the development of which is relatively slow compared with CYP2C8, 2C9, 2C19, 2D6, and 3A. For example, about half of the adult CYP1A2 activity was reached at 1-year after birth, while about 80% the adult CYP3A activity was reached at 1-year after birth. (4) Population mean clearance data for children and adults have been published.[3,11,12] (5) Doses have been published in package inserts or literature from Japan or overseas.[3,13–17] Theophylline and caffeine were selected according to criteria (1)–(4), while propranolol and mexiletine were selected according to criteria (1), (2), (3), and (5) (Table 2).

Table 1. Equations Used to Generate Hyperbolic Function Describing the Development of Individual Cytochrome P450 Activity

Enzyme	Hyperbolic function (fraction of adult CYP abundance) $\left(\frac{V\max_C/Km_C}{V\max_A/Km_A}\right)$
CYP1A2	$\left(\frac{1\times Age^{1.41}}{1.13+Age^{1.41}}\right)$
CYP2D6	$\left(\frac{1.01\times Age}{0.101+Age}\right)+0.036$

It was assumed that theophylline and caffeine are for the most part metabolized by CYP1A2 (Eq. (10)). In addition, it was assumed that propranolol and mexiletine are metabolized by CYP1A2 and CYP2D6, so the dose was estimated by using the weighted average contribution of each enzyme (Eq. (11)). Their contributions were estimated to be CYP1A2 : CYP2D6=28% : 72% for propranolol and 18% : 82% for mexiletine.[18,19]

For the above-mentioned drugs, estimation by the ePPBD and estimation by the conventional BSA-based method were compared, using the population mean oral CL ($\hat{CL}_{(PO)}$) and standard D_C (predetermined dose) for references.

Evaluation of Pediatric Dose Estimation Compared with the PPK Ratio As shown in Table 2, $\hat{CL}_{(PO)}$ for adults and for children in each age bracket were calculated using the population mean oral clearance ($\hat{CL}_{(PO)}$) of theophylline and caffeine, after which the $\hat{CL}_{(PO)_C}/\hat{CL}_{(PO)_A}$ ratio (PPK ratio) was calculated. Standard values of HT (cm) and Wt (kg) were used for children in each age bracket, while the age of adults was assumed to be 20. Mean HSA_A was set at 45.5 (g/L)[20] and mean AGP_A was at 0.60 (g/L).[21]

Based on the allometric principle, $CL_{(PO)_C}/CL_{(PO)_A}$

Table 2. Major Enzyme, Urinary Excretion Ratio of the Unchanged Drug (Ae), Unbound Fraction of Drug in Plasma (fu), Major Binding Protein, Predetermined Doses for Pedeiatric and Adult, Population Mean Oral Clearance for Pediatric and Adult

Drug	Major Enzyme	Ae (%)[6] fu[6]	Major Binding Protein	Predetermined Doses[a]		Population $CL_{(PO)}$ (l/h/kg)	
				Pediatric	Adult	Pediatric	Adult
Theophylline	CYP1A2	8 0.44	HSA[7]	0.5 to <1 age (y) ***6*** mg/kg/day[13] 1–15 age (y) ***8***～10 mg/kg/day[13]	***400*** mg/day[13]	0 to <1 age (y): 0.0322±0.0047[11] 1 to <2 age (y): 0.0461±0.0062[11] 2 to <3 age (y): 0.0537±0.0149[11] 3 to <4 age (y): 0.0682±0.0135[11] 4 to <5 age (y): 0.0621±0.0117[11] 6 to <10 age (y): 0.0745±0.0158[11] 10 to <15 age (y): 0.0755±0.0267[11]	13 to <53 age (y): 0.0557±0.0394[12]
Caffeine	CYP1A2	1.1 0.64	HSA[8]	0.083–0.33 age (y), 0.5–1.6 age (y) ***2***～10 mg/kg/day[3]	***200***～900 mg/day[14]	0.083–0.33 age (y): 0.034±0.018[3] 0.5–1.6 age (y): 0.102±0.04[3]	22–68 age (y): 0.092±0.029[3]
Propranolol	CYP1A2 CYP2D6	0.5 0.13	AGP[9]	(maintenance dose of Arrhythmias) ***2***～4 mg/kg/day[15]	(maintenance dose of Arrhythmias) ***40***～320 mg/day[15]		
Mexiletine	CYP1A2 CYP2D6	9.5 0.37	AGP[10]	***6***～15 mg/kg/day[16]	***300*** mg/day[17]		

[a] The bold and Italic numbers are the values employed for the prediction of doses.

can be expressed as Eq. (16), *i.e.*, as the ratio of the mean BSA_A in adults to the standard BSA_C for children of each age bracket.

$$\frac{CL_{(PO)_C}}{CL_{(PO)_A}} \cong \frac{BSA_C}{BSA_A} \qquad \text{Eq. (16)}$$

The $\widehat{CL}_{(PO)}$ ratio (PPK ratio) was compared with $CL_{(PO)_C}/CL_{(PO)_A}$ calculated from the ePPBD (Eq. (14)) and with BSA_C/BSA_A (BSA ratio) (Eq. (16)) to evaluate the relative accuracy of the ePPBD.

Evaluation of Pediatric Dose Estimation Compared with Predetermined Doses The pediatric dose (D_C) calculated from the ePPBD (Eq. (15)) and that calculated from the Crawford equation (estimation based on BSA) (Eq. (17)) were also compared to evaluate the relative accuracy of the ePPBD, using predetermined doses for reference. The predetermined dose was defined as the minimum dose when both maximum and minimum doses are stipulated in the package inserts, because the maximum dose is often determined from the maximum-tolerated dose.

$$D_C = D_A \times \frac{BSA_C}{BSA_A} \qquad \text{Eq. (17)}$$

Statistical Validation of the ePPBD In order to evaluate the relative accuracy of the ePPBD, the relative root mean squared prediction error ($RMSE$)[22] was calculated according to Eq. (18).

$$\text{Relative } RMSE\ (\%) = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{z_i - \bar{z}_i}{\bar{z}_i}\right)^2} \times 100 \qquad \text{Eq. (18)}$$

The PPK ratio or predetermined dose was used for z, and estimation from ePPBD or based on BSA was used for $\bar{z}$. "i" in the above equation represents the age points defined in this study.

To calculate relative $RMSE$ (%), 181 age points were used, which were obtained by equally dividing the population aged 0 to 15. However, some age brackets lacked a predetermined PPK ratio or predetermined dose. Thus, in the case of theophylline, 169 age points were used for the PPK ratio (no data for age 5), and 175 age points were used for comparison with the predetermined dose (no data for age 6 months or younger). In the case of caffeine, 18 age points were used for comparison with the PPK ratio and with the predetermined dose (data were available only for ages 0.083 to 0.33 and 0.5 to 1.6). In the

case of mexiletine, 84 age points were used for comparison with the predetermined dose (data were available only for ages 7 to 13).

For comparison of estimates obtained from the ePPBD with *BSA*-based estimates, the paired *t*-test was conducted to assess relative *RMSE* (%) values (SPSS® 11.0 J for Windows, SPSS Inc.). Before testing, the relative *RMSE* values were divided into 15 to 18 age points, in consideration of the age range.

RESULTS

Evaluation of Pediatric Dose Estimation Compared with the PPK Ratio Figure 1 shows the relative RMSE of $CL_{(PO)_C}/CL_{(PO)_A}$ calculated from the *BSA* ratio based on the allometric principle and that calculated with the ePPBD, in comparison with the PPK ratio. For theophylline, the relative *RMSE* of $CL_{(PO)_C}/CL_{(PO)_A}$ estimated by the ePPBD was lower than that obtained with the *BSA*-based method for boys and girls in every age bracket, except the age bracket of 6 to 10 years. The relative *RMSE* of $CL_{(PO)_C}/CL_{(PO)_A}$ calculated by the *BSA*-based method and that calculated by the ePPBD was respectively 67.3% and 17.8% for boys, and 59.5% and 17.4% for girls of age 0 to 15. For caffeine, the relative *RMSE* of $CL_{(PO)_C}/CL_{(PO)_A}$ calculated with the *BSA*-based method and that calculated with the ePPBD was respectively 198% and 48.8% for boys, and 181% and 50.7% for girls of ages combining 0.083–0.33 and 0.5–1.6. For both theophylline and caffeine, the predictive relative accuracy of the ePPBD was significantly ($p<0.05$) higher than that of the *BSA*-based method for both boys and girls. These results indicated that the ePPBD was superior to the *BSA*-based method when the PPK ratio was used for reference.

Evaluation of Pediatric Dose Estimation Compared with Predetermined Doses Figure 2 shows the relative *RMSE* of the pediatric dose (D_C) calculated from the Crawford equation ($D_A \cdot BSA_C/BSA_A$) and that calculated by the ePPBD, in comparison with the predetermined dose. For theophylline, the relative *RMSE* of D_C calculated by the Crawford equation and that obtained with the ePPBD was respectively 19.0% and 13.6% for boys, and 34.9% and 17.5% for girls of age 0 to 15. In the case of caffeine, the relative *RMSE* of D_C calculated from the Crawford equation and that from the ePPBD was respectively 16.3% and 4.42% for boys, and 21.8% and 5.12% for girls of age 0 to 15. For propranolol, the relative *RMSE* of D_C calculated from the Crawford equation and that obtained with the ePPBD was respectively 41.8% and 32.3% for boys, and 33.0% and 21.3% for girls of age 0 to 15. For theophylline, caffeine, and propranolol, the predictive relative accuracy of the ePPBD was again significantly ($p<$

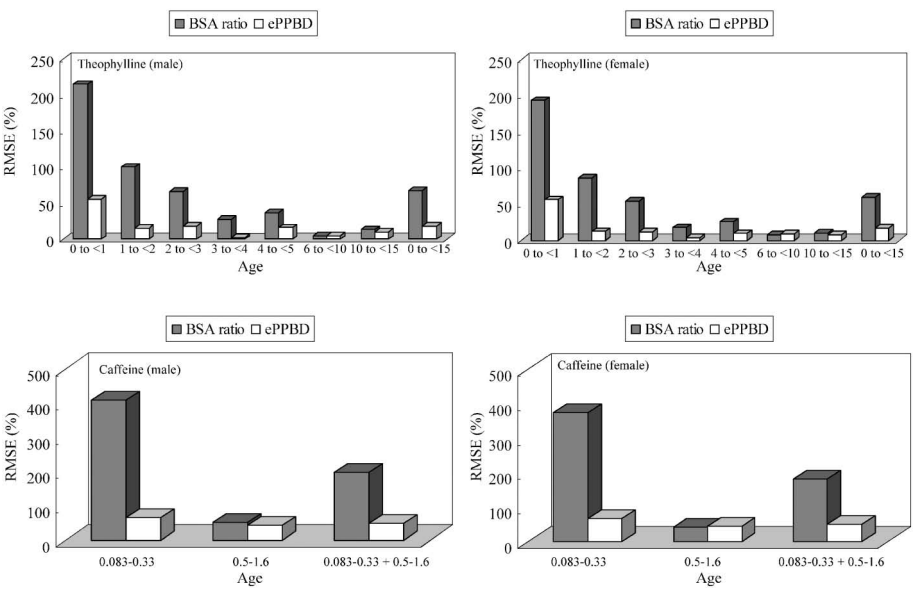

Fig. 1. Relative *RMSE* (%) of Clearance Ratios ($CL_{(PO)_C}/CL_{(PO)_A}$) of Theophylline, Caffeine Estimated from ePPBD and Allometric Approach (BSA_C/BSA_A) in Comparison with the $\hat{CL}_{(PO)_C}/\hat{CL}_{(PO)_A}$ Ratio of Theophylline Calculated from Population Mean Clearance (*po*), for Children

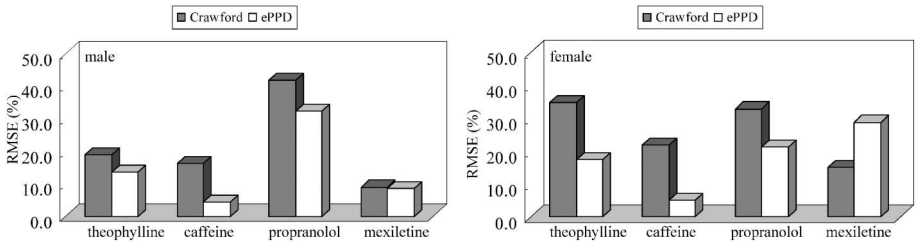

Fig. 2. Relative *RMSE* (%) of Dosages of Theophylline, Caffeine, Propranolol, Mexiletine for Children Calculated from Crawford and ePPBD Equations in Comparison with the Dosages Described in the Predetermined Doses

0.05) higher than that of the Crawford equation for both boys and girls of age 0 to 15 when the predetermined doses were used for reference. With mexiletine, however, the relative *RMSE* of D_C calculated from the Crawford equation and that from the ePPBD was respectively 8.97% and 8.41% for boys, and 15.0% and 28.8% for girls of age 7 to 13. The predictive relative accuracy of the ePPBD, in the case mexiletine, was significantly ($p<0.05$) lower than that of the Crawford equation for girls of age 7 to 13.

DISCUSSION

In general, *BSA* is well correlated with physiological function and thus drug doses can be appropriately estimated based on *BSA* values. However, the rate of development varies among hepatic metabolizing enzymes, and a some period is necessary for children to acquire adult levels of enzyme activity. Therefore, dose estimation based only on *BSA* is not adequate, especially for infants and neonates. This study was focused on CYP1A2, which takes 3 years after birth to reach 80% of adult activity, and we considered that the pediatric dose could be more appropriately estimated by incorporating *fu*, *LV*, and CYP activity in the calculations. In this study, we estimated the pediatric dose at each stage of development from the adult dose.

Figure 3 shows the changes with age of *fu* (*HSA*, *AGP*), *V*max/*Km* (CYP1A2, CYP2D6), *LV* and *BSA*, expressed as child/adult ratios.[3–5] *HSA*, *AGP, and V*max/*Km* only vary with age, so that the values for boys and girls are the same. However, *LV* and *BSA* are calculated from the standard height and weight of boys and girls, so that the values for boys and girls are different. The *fu* ratio depends on the adult *fu* value, so the median value, $fu_A=0.5$ was used for this calculation as an example. The value of *fu* is higher in children than in adults due to lower protein levels in children, and there is considerable

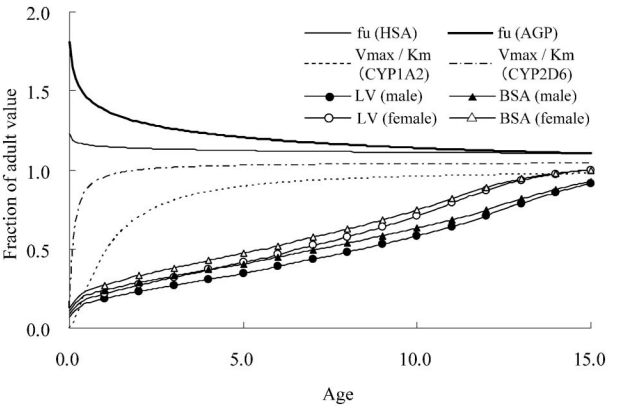

Fig. 3. Age-related Changes in Human Serum Albumin (*HSA*), α_1-Acid Glycoprotein (*AGP*), Liver Volume (*LV*), Body Surface Area (*BSA*), and Cytochrome P450 Expression/Activity as a Fraction of Adult Values

variation when dose estimation is attempted for neonates and infants. *LV* and CYP1A2 are lower in children than in adults because of their less developed hepatic function, and *LV* increases to the adult level faster in girls than in boys because of the relatively rapid growth in girls compared with boys. Therefore, our ePPBD that incorporates these factors may be a useful method for dose estimation because it allows for significant variation of development in neonates and infants, and for differences in development between boys and girls.

Theophylline and caffeine are the only substrates of CYP1A2 for which PPK values have been reported for adults and children, to our knowledge at best. Therefore, the reliability and relative accuracy of the ePPBD were evaluated by comparing ePPBD and *BSA*-based estimates with the *CL* value of PPK as the reference. As a result, for both theophylline and caffeine, the ePPBD produced estimates closer to the PPK ratio than the *BSA*-based method. Especially for theophylline, the value estimated by the ePPBD was significantly closer to the PPK ratio than that estimat-

ed by the *BSA*-based method in children aged 5 or younger. This finding suggests that because *BSA*-based estimation does not take the development of CYP activity into account, *BSA*-based estimation is inadequate for infants. The ePPBD method gave better estimates of the PPK ratio for theophylline as compared with the *BSA*-ratio, suggesting that the age-dependent changes of theophylline clearance is relatively close to the CYP1A2 growth curve. On the other hand, the age-dependent changes of caffeine clearance lies approximately in the center between the ePPBD estimates and *BSA*-based estimates and thus the ePPBD method gave similar estimates of the PPK ratio with the *BSA*-ratio for caffeine in terms of the *RMSE* comparison. The ePPBD incorporates *fu*, *P*, *LV*, and CYP activity to estimate the doses of drugs metabolized by CYP1A2 and thus is more accurate than the *BSA*-based method, especially in infants for whom dose estimation is often difficult. These findings suggest that the ePPBD is superior to the *BSA*-based method.

In addition to evaluation by comparison with the PPK ratio, the relative accuracy of the ePPBD was evaluated by comparison with the predetermined dose. As a result, for theophylline, caffeine and propranolol, the predictive relative accuracy of the ePPBD was higher in both boys and girls. The contribution of CYP2D6 is as high as 82% for mexiletine. Since the growth of CYP2D6 is rapid (*i.e.*, 90% of the adult level at 1-year), our ePPBD method does not show superiority against the Crawford equation, in the case of mexiletine. These findings indicate that the ePPBD could also estimate doses more accurately when the predetermined dose was used for a reference instead of the PPK ratio.

In the present study, our method (the ePPBD) was compared with the population mean *CL* value or with the predetermined doses. Accordingly, this study has a limitation that the relative accuracy and precision of *CL* estimates in the literatures will significantly influence the comparison. Also, we used predetermined doses as a reference for estimating pediatric doses, but rationale for setting the predetermined doses was not always clear. Therefore, the relative accuracy and precision of the predetermined doses may be another problem with our study. In this study, dose estimation was based solely on pharmacokinetic considerations; however, pharmacodynamics may also differ between adults and children. Thus, it appears, regarding mexiletine, that the predictive relative accuracy of the ePPBD may be lower in both boys and girls compared with that of the *BSA*-based estimation. Moreover, our study has a limitation that the number of drugs surveyed is only four.

The results of this study suggested that our method for pediatric dose estimation employing *fu*, *P*, *LV*, and CYP activity is valid because it approximates the pharmacokinetics of drugs metabolized by CYP1A2 together with other hepatic enzymes. Unlike *BSA*-based estimation, which was considered to be an adequate method based on the development of children, our method incorporating *fu*, *P*, *LV*, and CYP activity to estimate pharmacokinetics is a novel approach that has not been tried in practice. However, our method seems especially useful for neonates and infants, who show marked differences of physiology. When setting the dose of a new drug that has never been used in children, our method could be superior to the conventional method that lacks allowance for pharmacokinetic property of the drug. Future study should be performed in order to take pharmacodynamics (*i,e.*, effects and toxicity) of drugs into consideration. Moreover, we await to demonstrate the significance and usefulness of our new calculation method in clinical practice.

Acknowledgement This study was supported by the High-Technology Research Project of Showa University.

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