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Metabolic Kinetics of *p*-Aminobenzoic Acid in Rabbits

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ABSTRACT: The metabolic kinetics of *p*-aminobenzoic acid (PABA) in rabbits was studied. PABA is predominantly metabolized by acetylation and glycine conjugation to form *p*-acetamidobenzoic acid (PAABA), *p*-aminohippuric acid (PAHA), and *p*-acetamidohippuric acid (PAAHA). After PABA IV administration (20 mg/kg) to rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits, PABA was eliminated rapidly. The half-lives of PABA were 7.01 ± 0.32 min in rapid acetylation rabbits and 7.08 ± 0.78 min in slow acetylation rabbits. Significant differences were obtained in formation of PAABA and PAHA formed from PABA in both acetylation phenotype rabbits. The formation fraction of PAABA, formed by acetylation of PABA, was 0.8029 ± 0.0267 in rapid acetylators and 0.2385 ± 0.0428 in slow acetylators ($p < 0.001$). PAHA formed from PABA was 0.0462 ± 0.0102 in rapid acetylators and 0.6652 ± 0.0562 in slow acetylators ($p < 0.001$). Only 0.0156 ± 0.0030 of PABA could be detected as PAAHA in rapid acetylation rabbits which was obtained by acetylation of PAHA. After individual IV injection of PAHA, PAAHA, and PAABA to both phenotypes of rabbits, PAABA and PAAHA were eliminated in their unchanged forms whereas PAHA was further acetylated to form PAAHA. The formation fraction of PAAHA formed from the acetylation of PAHA was 0.4408 ± 0.0570 in rapid acetylators and 0.0539 ± 0.0084 in slow acetylators ($p = 0.002$). From the results obtained, metabolic pathways of PABA show significant differences in both acetylation phenotypes of rabbits. Acetylation is the major metabolic route of PABA in rapid acetylation rabbits, while glycine conjugation is more predominant in slow acetylation rabbits.

Key words: *p*-aminobenzoic acid; metabolites; metabolic kinetics

Introduction

p-Aminobenzoic acid (PABA) is widely included as a member of the vitamin B complex in nutritional supplements and is also an essential metabolite for certain microorganisms in the synthesis of folic acid. At present, it is popularly used as a sun-screen agent in cosmetics and a diagnostic agent of pancreatic function [1].

PABA is mainly metabolized by acetylation and glycine conjugation in the liver, forming PAABA, PAHA and PAAHA in humans, rabbits, and rats [2–4]. Like other aromatic amine and hydrazide drugs, PABA undergoes acetylation *in vivo*, but shows a different acetylation phenomenon [5]. Acetylation of sulfonamide and isoniazid can be classified as rapid or slow acetylation phenotypes in humans and rabbits. This bimodal distribution of acetylation is genetically controlled. During acetylation, rapid acetylation in humans and rabbits is dominant and slow acetylation is recessive. It has been well documented that acetylators differences are controlled by a simple autosomal Mendelian gene with two major alleles in rabbits [6]. Rabbits are divided phenotypically into rapid (RR, Rr) or slow (rr) acetylators according to the *in vivo* acetylation rate of drugs such as sulfadiazine (SDZ) or isoniazid. As regards the acetylation of PABA, only the monomorphic phenotype is found in both humans and rabbits. The reason for this is that monomorphic and polymorphic substrates may exhibit dissimilar patterns of hepatic *N*-acetyltransferase activity *in vitro* [7]. On the contrary, in hamsters, acetylation of PABA shows genetic polymorphism, but only monomorphism is found for the acetylation of sulfonamide and isoniazid [8].

During the past few years, several methods have been developed for the quantitation of PABA and its metabolites in biological fluids [9–11]. However, the sensitivity and specificity of these methods have not been high enough for the discrimination of PABA and its metabolites in biological fluids. In addition, no detailed pharmacokinetic study of PABA and its metabolites has yet been reported. To elucidate the metabolic kinetics of PABA, a specific and sensitive HPLC method with fluorescence detection for the quantitation of PABA and its metabolites (PAHA, PAABA, and PAAHA) in rabbit plasma has been developed [12]. In this report equal molar quantities of PABA, PAHA, PAABA, and

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PAAHA were IV administered to both acetylation phenotypes of rabbits and the metabolic kinetics of PABA were studied.

Experiments

Chemicals and Reagents

All chemicals were reagent grade and all solvents were HPLC grade. Sulfamethazine (SMZ), sulfadiazine (SDZ), PABA, PAABA, PAHA, salicylic acid (SA), and heparin were obtained from Sigma (St. Louis, MO, USA). PAAHA was prepared according to the method of Yung-Jato [13].

Instruments

Chromatographic analysis was performed using an LC-10AD high-pressure pump, an RF-551 fluorescence detector, an SPD-10A ultraviolet detector, a CR-7A integrator, and an SIL-9A autoinjector, all manufactured by Shimadzu, Kyoto, Japan.

Analytical Conditions

Analytical Conditions for SDZ. The method for SDZ analysis was modified from that of Ho [14]. The analytical column used was a Nucleosil C_{18} column (250×4.6 mm, ID, 5 μm). The mobile phase was H_2O–CH_3CN–CH_3COOH (100:10:1, v/v). Samples were monitored at a flow rate of 1 mL/min and a UV detection wavelength of 269 nm.

Analytical Conditions for PABA and its Metabolites. A Cosmosil MS-C_{18} column (250×4.6 mm, ID, 5 μm) was used for analysis, and the column temperature was set at 40°C. The mobile phase was H_2O–CH_3CN–CH_3COOH (100:3:1) adjusted to pH 4.0 with a 10 N NaOH solution. PABA and its metabolites were separated under a flow rate of 1.5 mL/min. The excitation and emission wavelengths of fluorescence were set at 270 and 350 nm, respectively.

Sample Preparation

Sample Preparation of SDZ. An aliquot of 200 μL of rabbit plasma was spiked with 500 μL of CH_3CN, containing an internal standard (SMZ), and vortexed for 30 s. After centrifugation at $10000 \times g$ for 5 min, 200 μL of the supernatant was added to a clean vial containing 1 mL of H_2O. After vortexing for 10 s, a constant volume (20 μL) of the supernatant was injected onto the column.

Sample Preparation of PABA and its Metabolites. An aliquot of 200 μL rabbit plasma was spiked with 500 μL of CH_3OH, containing an internal standard (SA) and vortexed for 30 s. After centrifugation at $10000 \times g$ for 5 min, 700 μL of the supernatant was added to a clean vial containing 500 μL of H_2O. After vortexing for 10 s, a constant volume (30 μL) of the supernatant was injected onto the column.

Animal Experiments

Twenty-four male white New Zealand rabbits, weighing $1.6 \sim 2.5$ kg, were used in these experiments. There was a washout period of at least 7 days and an overnight fast between each experiment. Before each experiment, a blank blood sample was drawn for calibration curve usage. All syringes and microtubes were rinsed with heparin (500 units/mL in 0.9% NaCl) before use to prevent clotting.

Determination of the Acetylation Phenotype in Rabbits. SDZ (20 mg/kg) was intravenously injected into the marginal ear vein of the rabbits. Blood samples (0.5 mL) were then obtained from the other marginal ear vein at 5, 10, 20, 30, 40, 60, 80, 100, and 120 min after injection. For analysis, an aliquot of 0.2 mL of plasma was used after centrifugation. Following the plasma SDZ concentration determination and PCNONLIN computer program treatment [15], rabbits were classified as rapid or slow acetylators depending on the half-life of the elimination of SDZ in rabbit plasma. Rabbits with an SDZ elimination half-life greater than 80 min were classified as slow acetylators, and those with an SDZ elimination half-life of less than 60 min were classified as rapid acetylators [4].

Pharmacokinetic Study of PABA and its Metabolites. PABA (20 mg/kg) was IV administered into the marginal ear vein of rabbits. Blood samples were obtained from the other marginal ear vein at 1, 3, 5, 7, 9, 12, 15, 20, 30, 45, 60, 80, 100, 120, 140, 160, and 180 min after IV injection of PABA.

In order to elucidate the metabolic kinetics of PABA, an equivalent molarity (0.1458 mmol/kg) of PABA of its three metabolites, PAABA (26.1 mg/kg), PAHA (28.3 mg/kg), and PAAHA (34.5 mg/kg), were also IV administered individually according to the above experimental procedures.

Data Analysis

The calibration curve was calculated using the least-square linear regression with a weighting factor of $1/C$ (C: spiked concentration). Concentrations of plasma samples were calculated from this linear equation.

Plasma concentrations of PABA and its three metabolites obtained from the experiments were fitted into the compartment model by the PCNONLIN computer program [15]. By using the sum of squared residuals, the sum of weighted squared residuals, the correlation between observed and calculated function values, and AIC criteria as the

best-fitting conditions, pharmacokinetic parameters of elimination half-life, volume of distribution (V_d), and systemic clearance were obtained. The area under the plasma concentration–time curve (AUC) was obtained by using the trapezoid rule plus the last measured concentration divided by the elimination rate constant.

The area analysis method was used for the metabolic kinetic model of PABA in this study [16]. The formation of the metabolite after parent compound IV administration is described as follows:

$$F_{p\to m} = \frac{AUC_{p\to m}}{AUC_m},$$

where $F_{p\to m}$ is the formation fraction of the metabolite after IV administration of the parent compound, $AUC_{p\to m}$ is the AUC of the metabolite after IV administration of the parent compound, and AUC_m is the AUC of IV administration of the metabolite alone. The pharmacokinetic parameters and definitions are shown in Table 1. All of the parameters for solving the metabolic kinetics of PABA are shown below:

$$F_{PABA\to PAABA} = \frac{AUC_{PABA\to PAABA}}{AUC_{PAABA}},$$

$$F_{PABA\to PAHA} = \frac{AUC_{PABA\to PAHA}}{AUC_{PAHA}},$$

$$F_{PAHA\to PAAHA} = \frac{AUC_{PAHA\to PAAHA}}{AUC_{PAAHA}},$$

$$F_{PABA\to PAAHA} = F_{PABA\to PAHA} \times F_{PAHA\to PAAHA},$$

$$F_{PABA} = 1 - F_{PABA\to PAHA} - F_{PABA\to PAABA},$$

$$F_{PAHA} = 1 - F_{PAHA\to PAAHA},$$

$$k_{PABA} = \frac{0.693}{\text{elimination half-life of PABA}} = k_{10} + k_{12} + k_{13},$$

$$k_{PAABA} = \frac{0.693}{\text{elimination half-life of PAABA}} = k_{30},$$

$$k_{PAHA} = \frac{0.693}{\text{elimination half-life of PAHA}} = k_{20} + k_{24},$$

$$k_{PAAHA} = \frac{0.693}{\text{elimination half-life of PAAHA}} = k_{40},$$

$$k_{13} = F_{PABA\to PAABA} \times k_{PABA},$$

$$k_{12} = F_{PABA\to PAHA} \times k_{PABA},$$

$$k_{10} = k_{PABA} - k_{12} - k_{13},$$

$$k_{30} = k_{PAABA},$$

$$k_{24} = F_{PAHA\to PAAHA} \times k_{PAHA},$$

$$k_{20} = k_{PAHA} - k_{24},$$

$$k_{40} = k_{PAAHA}.$$

All data were expressed as means $\pm$ S.E.M.

Results

Acetylation Phenotyping of Rabbits

After IV administration of SDZ (20 mg/kg) to 24 male white New Zealand rabbits, as shown in Table 2, 16 rabbits were classified as rapid acetylators,

Table 1. Definition of the notations and parameters

k_{PABA}	Elimination rate constant of PABA
k_{PAABA}	Elimination rate constant of PAABA
k_{PAHA}	Elimination rate constant of PAHA
k_{PAAHA}	Elimination rate constant of PAAHA
k_{10}	PABA remaining elimination rate constant
k_{20}	PAHA remaining elimination rate constant
k_{30}	PAABA elimination rate constant
k_{40}	PAAHA elimination rate constant
k_{12}	Formation rate constant of PAHA from PABA
k_{13}	Formation rate constant of PAABA from PABA
k_{24}	Formation rate constant of PAAHA from PAHA
$F_{PABA\to PAABA}$	Formation fraction of PAABA formed from PABA IV administration
$F_{PABA\to PAHA}$	Formation fraction of PAHA formed from PABA IV administration
$F_{PABA\to PAAHA}$	Formation fraction of PAAHA formed from PABA IV administration
F_{PABA}	Remaining fraction of PABA after PABA IV administration
$F_{PAHA\to PAAHA}$	Formation fraction of PAAHA formed from PAHA IV administration
F_{PAHA}	Remaining fraction of PAHA after PAHA IV administration
$AUC_{PABA\to PAABA}$	AUC of PAABA after PABA IV administration
$AUC_{PABA\to PAHA}$	AUC of PAHA after PABA IV administration
$AUC_{PAHA\to PAAHA}$	AUC of PAAHA after PAHA IV administration
AUC_{PAHA}	AUC of PAHA after PAHA IV administration
AUC_{PAABA}	AUC of PAABA after PAABA IV administration
AUC_{PAAHA}	AUC of PAAHA after PAAHA IV administration

Table 2. Pharmacokinetic parameters after IV administration of SDZ (20 mg/kg) to rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits

Pharmacokinetic parameters	Rapid acetylation rabbits (mean ± S.E.M.)	Slow acetylation rabbits (mean ± S.E.M.)
Half-life (min)	49.46 ± 2.55	91.15 ± 4.08 ($p < 0.001$)
AUC (μg · min/mL)	4250.14 ± 256.29	9065.40 ± 1255.33 ($p < 0.001$)
Clearance (mL/min/kg)	5.00 ± 0.32	1.81 ± 0.43 ($p < 0.001$)
V_d^a (mL/kg)	342.35 ± 12.70	231.96 ± 48.12 ($p = 0.008$)

[a] Volume of distribution.

with a mean elimination half-life of 49.46 ± 2.55 min, and eight rabbits were classified as slow acety lators with a mean elimination half-life of 91.15 ± 4.08 min ($p < 0.001$).

Pharmacokinetic Study of PABA and its Metabolites

After IV administration of PABA (20 mg/kg) to both phenotypes of rabbits, PABA was eliminated following a one-compartment open model. The metabolites of PABA were detected as PAABA, PAHA, and PAAHA in plasma. The concentration–time profiles of PABA are presented in Figure 1. After IV administration of PAHA and PAAHA to rabbits, these two metabolites were also eliminated following a one-compartment open model, as shown in Figures 2 and 3. As Figure 4 shows, owing

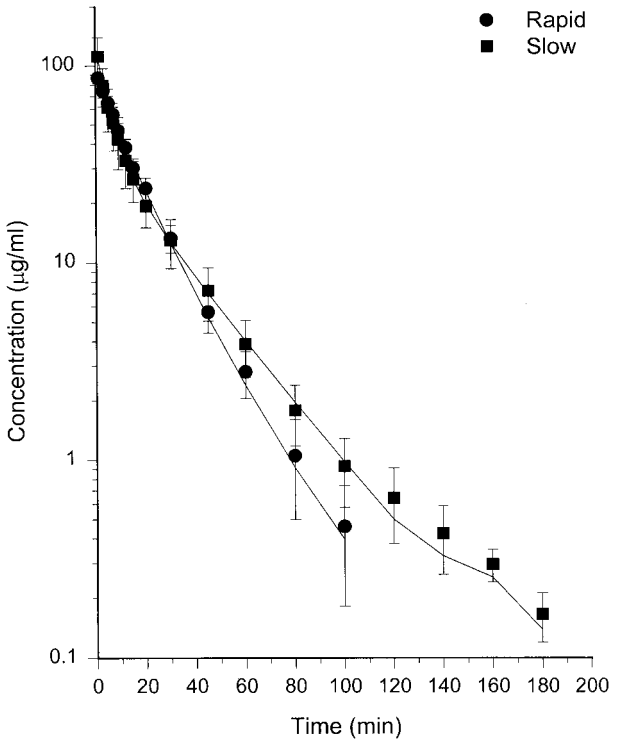

Figure 2. Plasma concentration–time profile of PAHA (28.6 mg/kg), after PAHA IV administration on rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits

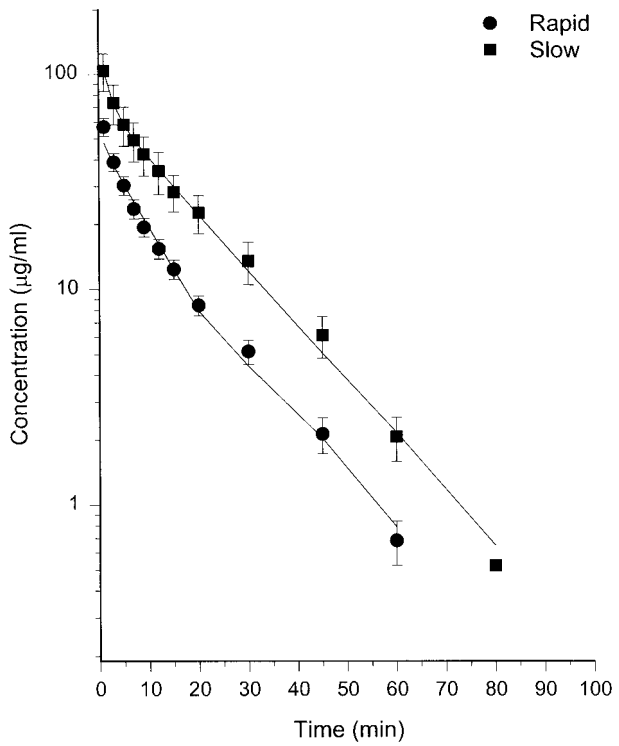

Figure 1. Plasma concentration–time profile of PABA (20 mg/kg), after PABA IV administration on rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits

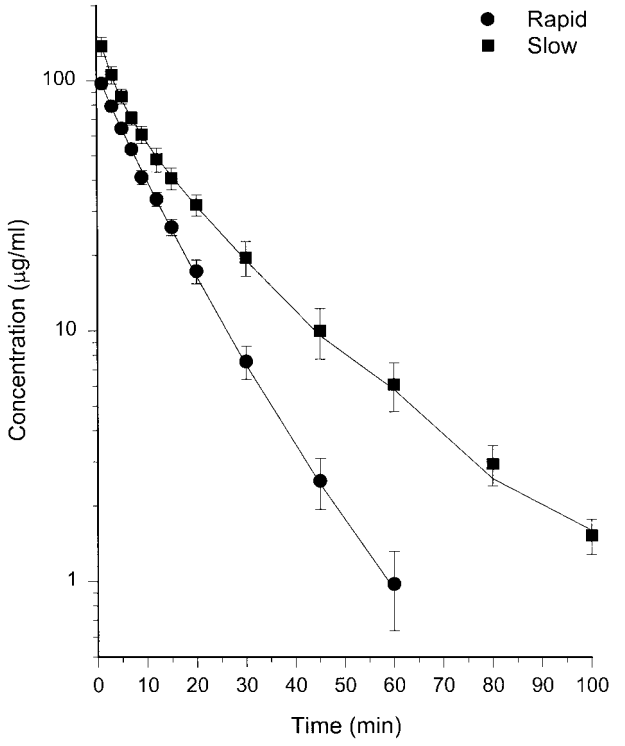

Figure 3. Plasma concentration–time profile of PAAHA (34.5 mg/kg), after PAAHA IV administration on rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits

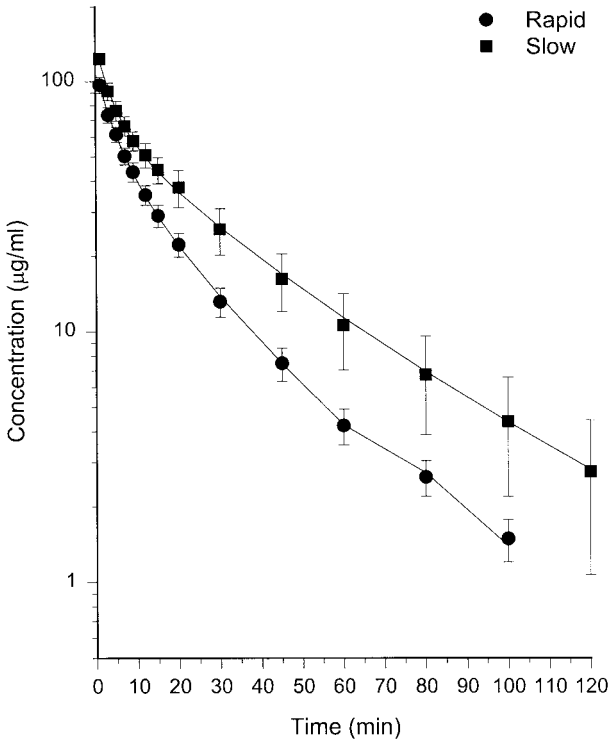

Figure 4. Plasma concentration–time profile of PAABA (26.1 mg/kg), after PAABA IV administration on rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits

to the hydrophobic property of PAABA, it was eliminated as a two-compartment model after IV injection of PAABA. PAABA and PAAHA were eliminated in unchanged forms only, whereas PAHA was further acetylated to form PAAHA after IV administration. The metabolic model of PABA is described in Figure 5. The pharmacokinetic parameters of PABA and its metabolites are listed in Table 3. The elimination half-lives of PABA, PAABA, PAHA, and PAAHA were 7.01 ± 0.32, 21.37 ± 2.85, 10.05 ± 0.96, and 7.39 ± 0.40 min in rapid acetylators, and 7.08 ± 0.78, 23.83 ± 4.75, 18.40 ± 1.91, and 15.34 ± 1.46 min in slow acetylators, respectively. Like previous reports [5], no difference was obtained in PABA elimination in both phenotypes of rabbits. But significant differences were detected in PAHA and PAAHA elimination between the two acetylation phenotype rabbits ($p \leq 0.002$). Using the area analysis method [16], the metabolic kinetics of PABA and its metabolites, PAABA, PAHA, and PAAHA, are calculated as shown in Table 4. The formation fractions of PAHA and PAABA after PABA IV administration were 0.0462 ± 0.0102 and 0.8029 ± 0.0267 in rapid acetylators, and 0.6652 ± 0.0562 and 0.2385 ± 0.0428 in slow acetylators, respectively. The formation of PAHA and PAABA formed from PABA showed significant differences ($p < 0.001$) between rapid and slow acetylation rabbits. Formation of PAAHA formed from PABA was detected in rapid acetylators only. The formation fraction was 0.0156 ± 0.0030. The formation of PAAHA after IV adminisration of PAHA was significantly different in both phenotypes also. The formation fractions were 0.4408 ± 0.0570 in rapid acetylators and 0.0539 ± 0.0084 ($p = 0.002$) in slow acetylators. The remaining fractions (F_{PABA}) of PABA (the elimination of unchanged PABA) after IV administration of PABA were 0.1509 ± 0.0260 in rapid acetylators and 0.0962 ± 0.0276 in slow acetylators. According to the results obtained above, acetylation was concluded to be the major pathway for PABA metabolism in rapid acetylation rabbits, while glycine conjugation of PABA was predominant in slow acetylation rabbits.

Discussion

In the early 1970s, PABA was found to be a monomorphic character in acetylation in rabbits and humans [7], but was shown to be polymorphic in hamsters [8]. *N*-Acetyltransferase, enzymes of slow

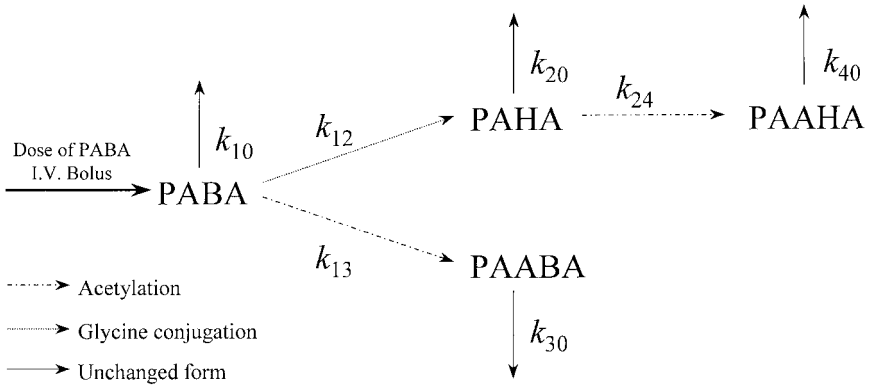

Figure 5. Metabolic pathway of PABA

Table 3. Pharmacokinetic parameters after IV administration of PABA (20 mg/kg), PAABA (26.1 mg/kg), PAHA (28.3 mg/kg) and PAAHA (34.5 mg/kg) to rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits

Pharmacokinetic parameters	Rapid acetylation rabbits Slow acetylation rabbits (mean ± S.E.M.)			
	PABA IV[a]	PAABA IV[b]	PAHA IV[a]	PAAHA IV[a]
α-half-life (min)		4.28 ± 0.70 2.83 ± 0.50		
β-half-life (min)		21.37 ± 2.85 23.83 ± 4.75		
Half-life (min)	7.01 ± 0.32 7.08 ± 0.78		10.05 ± 0.96 18.40 ± 1.91 ($p = 0.002$)	7.39 ± 0.40 15.34 ± 1.46 ($p < 0.001$)
MRT (min)	8.72 ± 0.54 1.99 ± 0.02 ($p < 0.001$)	17.59 ± 1.20 29.83 ± 5.79 ($p = 0.008$)	15.63 ± 1.11 1.97 ± 0.01 ($p < 0.001$)	11.22 ± 0.72 17.13 ± 2.45 ($p = 0.009$)
AUC (μg · min/mL)	528.83 ± 54.27 1371.50 ± 273.82 ($p < 0.001$)	1459.22 ± 139.50 2620.04 ± 552.43 ($p = 0.001$)	1546.56 ± 143.91 1505.25 ± 348.78	1179.76 ± 79.43 2035.25 ± 165.67 ($p < 0.001$)
Clearance (mL/min/kg)	55.06 ± 14.65 17.34 ± 3.47	20.60 ± 2.18 11.51 ± 1.91	23.82 ± 3.01 22.88 ± 4.67	30.90 ± 1.80 17.45 ± 1.53 ($p = 0.002$)
V_d^c (mL/kg)	551.84 ± 145.04 452.62 ± 64.46	672.82 ± 166.49 345.15 ± 7.48	304.19 ± 16.33 598.00 ± 120.62 ($p = 0.001$)	321.07 ± 17.96 379.14 ± 31.68
AUC of PAHA (μg · min/mL)	69.74 ± 15.95 1299.64 ± 331.77 ($p < 0.001$)			
AUC of PAAHA (μg · min/mL)	148.08 ± 19.20 *		494.66 ± 69.24 128.27 ± 23.67 ($p = 0.037$)	
AUC of PAABA (μg · min/mL)	1260.67 ± 120.70 696.53 ± 61.52 ($p = 0.020$)			

[a] One-compartment open model.
[b] Two-compartment open model.
[c] Volume of distribution.
* PAAHA could not be found in these rabbits.

Table 4. Metabolic kinetic parameters after IV administration of PABA (20 mg/kg) in rapid ($n = 16$) and slow ($n = 8$) acetylation rabbits

Metabolic kinetic parameters	Rapid acetylation rabbits ($n = 16$) (mean ± S.E.M.)	Slow acetylation rabbits ($n = 8$) (mean ± S.E.M.)	p
$F_{PABA \rightarrow PAABA}$	0.8029 ± 0.0267	0.2385 ± 0.0428	< 0.001
$F_{PABA \rightarrow PAHA}$	0.0462 ± 0.0102	0.6652 ± 0.0562	< 0.001
$F_{PABA \rightarrow PAAHA}$	0.0156 ± 0.0030		
F_{PABA}	0.1509 ± 0.0260	0.0962 ± 0.0276	0.326
$F_{PAHA \rightarrow PAAHA}$	0.4408 ± 0.0570	0.0539 ± 0.0084	0.002
F_{PAHA}	0.5592 ± 0.0570	0.9461 ± 0.0084	0.002
k_{PABA} (1/min)	0.1019 ± 0.0048	0.0921 ± 0.0117	0.985
k_{PAABA} (1/min)	0.0389 ± 0.0039	0.0317 ± 0.0048	0.511
k_{PAHA} (1/min)	0.0769 ± 0.0064	0.0594 ± 0.0105	0.010
k_{PAAHA} (1/min)	0.0984 ± 0.0062	0.0687 ± 0.0108	0.001
k_{10} (1/min)	0.0156 ± 0.0027	0.0088 ± 0.0020	0.240
k_{20} (1/min)	0.0463 ± 0.0081	0.0372 ± 0.0037	0.625
k_{30} (1/min)	0.0389 ± 0.0039	0.0333 ± 0.0055	0.511
k_{40} (1/min)	0.0984 ± 0.0062	0.0469 ± 0.0045	0.001
k_{12} (1/min)	0.0051 ± 0.0013	0.0686 ± 0.0101	< 0.001
k_{13} (1/min)	0.0812 ± 0.0038	0.0247 ± 0.0052	< 0.001
k_{24} (1/min)	0.0306 ± 0.0040	0.0020 ± 0.0002	0.002

and rapid acetylation, show structurally distinct isoenzymes with different catalytic properties for the acetylation of aromatic amines and hydrazines [17]. Acetyl coenzyme A is necessary and is the acetyl donor for acetylation [18]. For given physiological conditions, the activity of *N*-acetyltransferases may be saturated for different substrates. Consequently, a monomorphism, or nearly monomorphic pattern of acetylation is observed in rapid and slow acetylator rabbits after administration of these drugs. On the contrary, if an excess concentration of acetyl coenzyme A is given during a normal physiological condition *in vitro*, a polymophism is observed in the acetylation of PABA [19,20]. The elimination half-life for PABA and the acetylation status in rabbits were first reported by Weber [5]. PABA shows acetylation monomorphism, and the elimination half-life is between 15 and 51 min as determined by the Bratton–Marshall method. The Bratton–Marshall method is not a specific and sensitive analysis method for the quantitation of PABA in biological fluids. In this study, the elimination half-life of PABA also shows monomorphic phenomena as reported by Weber [5], but it occurred more rapidly. The reason for these differences may be due to the different analytical methods used. The HPLC-fluorescence method is more specific than the Bratton–Marshall method in the PABA study.

Earlier studies [2–4] have shown that PABA undergoes glycine conjugation and acetylation in humans, rabbits, and rats. However, there are no detailed studies about the metabolic kinetics of PABA. In this study, these reactions were also detected in rabbits, and the metabolic kinetics obtained from PABA in rabbits was studied by using a simple and specific analytical method. The metabolism of PABA after IV administration in both acetylation phenotypes of rabbits is clearly elucidated by the study. In rapid acetylation rabbits, the major metabolic pathway of PABA is acetylation, and the major metabolite formed is PAABA (0.8029 ± 0.0267). Only a small fraction of PABA (0.0462 ± 0.0102) was metabolized by glycine conjugation to form PAHA. On the other hand, glycine conjugation is the major metabolic pathway in slow acetylation rabbits. The 0.6652 ± 0.0562 fraction of PABA is conjugated to form PAHA and the 0.2385 ± 0.0428 fraction of PABA is acetylated to form PAABA in slow acetylation rabbits. The third metabolite of PABA, PAAHA, was obtained by further acetylation of PAHA in rapid acetylation rabbits only. After IV administration of PABA, PAABA was eliminated in its unchanged form only. The formation fraction of PAAHA ($F_{\mathrm{PABA}\rightarrow\mathrm{PAAHA}}$) was obtained from the product of the formation fraction of PABA to PAHA ($F_{\mathrm{PABA}\rightarrow\mathrm{PAHA}}$) and the formation fraction of PAHA to PAAHA ($F_{\mathrm{PAHA}\rightarrow\mathrm{PAAHA}}$). This formation fraction ($F_{\mathrm{PABA}\rightarrow\mathrm{PAAHA}}$) is only about 0.0156 ± 0.0030 in the metabolism of PABA. The acetylation fraction ($F_{\mathrm{PABA}\rightarrow\mathrm{PAABA}}$, 0.8029 ± 0.0267) obtained in this study was consistent with the acetylation of sulfadiazine in rapid acetylation rabbits [21,22]. This means that PABA, a monomorphic acetylation drug, like most other polymorphic acetylation drugs is predominantly acetylated in rapid acetylation rabbits. Glycine conjugation requires ATP and coenzyme A, as well as the availability of intracellular glycine. The enzyme involving glycine *N*-acyltransferase (catalyses glycine conjugation) is located in the hepatocytes. After PABA administration, it is quickly eliminated from plasma by glycine conjugation [20]. In this study, PAHA also rapidly disappeared from the plasma. The major metabolic pathway of PABA in slow acetylation rabbits was glycine conjugation.

In conclusion, although the elimination half-life of PABA shows no differences in rapid and slow acetylation rabbits, different metabolism pathways of PABA in both acetylation phenotypes exist. Acetylation is the major metabolic pathway of PABA in rapid acetylation rabbits, while glycine conjugation is predominant in slow acetylation rabbits.

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