Korean J. of Pharmacology
Vol. 23, No. 1, pp. 1-7,1987

Pharmacokinetic Study of Pyrazinamide Related to the Mechanism of the Renal Excretion

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ABSTRACT

The renal handling and tissue distribution of pyrazinamide were studied after administration of single dose intravenous injection for 15 min or constant infusion in New Zealand White rabbits. Peak pyrazinamide serum concentration ranged from 57.3 to 105.0 μg/ml (mean ± SD;83.0 ± 17.8). The mean half-life of the α phase was 0.143 ± 0.047 hr while the β phase ranged from 1.66 to 3.25 hr(mean ± SD;2.38 ± 0.57). The mean steady-state volume of distribution in non-compartmental model was 0.935 ± 0.362 L/kg.

Excretion ratio of pyrazinamide was dramatically reduced from 1.02 to 0.30 when unbound serum pyrazinamide concentration was increased from 6.04 to 60.9 μg/ml. The urine flow dependency of renal clearance of pyrazinamide was demonstrated in steady-state serum concentration.

The tissue/serum concentration ratio of pyrazinamide was highest in kidney and lowest in skeletal muscle among the tissues examined.

The results suggested that a large fraction of pyrazinamide filtered by glomerulus and secreted by renal tubule was reabsorbed and this tubular reabsorption of pyrazinamide might be greatly influenced by urine flow.

Key Words: Pyrazinamide, Pharmacokinetics, Renal excretion, Rabbit.

INTRODUCTION

Pyrazinamide is a synthetic compound discovered in 1952 but originally rejected for general use because of excessive hepatotoxicity when given in a dosage of 40 mg/kg/day(McDermott *et al.*, 1954). However it has now become an important drug for treatment of pulmonary tuberculosis because of its excellent tissue sterilizing ability when used in combination with other bactericidal drugs (Grosset, 1978). It is well absorbed from G-I tract, widely distributed throughout the body water (Acocella *et al.*, 1985). Stottmeier (1967) reported that pyrazinamide could be detected in serum, liver, lung and kidney for almost six hours in experimental animal treated only once with single dose (40 mg/kg) of pyrazinamide and could be found in the urine over periods of several hours at high levels. From this experimental findings, he suggested that the toxicity of pyrazinamide might be due both to slow excretion and to accumulation of drug in the liver.

Recently Park *et al.* (1986) analyzed the pharmacokinetic parameters in healthy volunteers and patients with renal failure with single oral dose (1 gm) of pyrazinamide and reported that about 28% of the dose administered was excreted in the urine up to 24 hr. And also they suggested the renal tubular secretion and passive reabsorption could play an important role in renal handling mechanism of pyrazinamide. It is important to elucidate the mechanism of renal handling of this drug because pyrazinamide reported to be excreted considerable amount in the urine but few studies on this aspect appear to have been reported.

Piug *et al.* (1983) determined the role of post-secretory reabsorption in regulation of urate excretion with pyrazinamide and probenecid test. Weiner

et al. (1961) only briefly analayzed the renal handling mechanism of drugs using standard clearance technique. However, standard clearance technique has several disadvantages such as long experimental periods and limited plasma levels for renal excretion analysis.

In this study renal handling mechanism of pyrazinamide was investigated by means of analysis of pharmacokinetic properties of pyrazinamide in rabbit with modified clearance method. In addition, pyrazinamide levels in several organs at postdistribution phase were measured to examine tissue distribution of the drug.

METHODS

Experimental procedures:

The New Zealand White rabbits weighted 2.3-3 kg(average 2.63 ± 0.24kg) were anesthetized with 0.75 g/kg urethane. A tracheal tube was intubated by tracheotomy and an arterial catheter was catheterized through carotid artery and then connected to pressure tansducer for blood pressure monitoring throughout experiment. A ureteral catheter was inserted up to pelvis of the both side kidney by medial abdominal incision.

After loading a dose of 80 mg/kg inulin through marginal vein of ear, inulin, dissolved in physiological saline, was infused continuously with dose of 0.8 mg/kg/min at the rate of 0.43-0.50 ml/kg/min through same route in order to maintain continuous urine flow as well as constant plasma concentration of inulin. At the time when urine flow was stabilized, control urine sample was collected for 10 min and 2 ml of arterial blood was collected from arterial catheter. Pyrazinamide (40 mg/kg), dissolved in physiological saline, was administered through ear vein for 15 min with infusion pump. Blood samples (1 ml) were collected at 0.125, 0.25, 0.5, 0.75, 1 hr and thereafter at the interval of 0.5 hr up to 6 hr after administration of pyrazinamide. Urine samples were collected at 15 min intervals for the first 1 hr and thereafter at 30 min intervals up to 6 hr.

To evaluate the effect of urine flow on pyrazinamide excretion, a loading dose of pyrazinamide (11.2 mg/kg) was injected and then maintenance dose (66 μg/kg/min) was continuously infused at the rate of 0.5 ml/min or less. And 5% mannitol dissolved in 0.3% saline was infused to induce changes in urine flow. Blood and urine samples were collected 2 hr after inulin administration at 10-15 min intervals.

All experimental animals were sacrificed at the end of experiment and various organs (heart, liver, kidney, lung and thigh muscle) were excised for the determination of tissue/serum concentration ratio.

The concentrations of pyrazinamide in serum and urine samples were determined by sodium nitroprusside method according to Stottmeier *et al.* (1967). Excised tissues (1 gm) were disintegrated in 3 ml physiological saline with polytron at rheostat 5 for 15 s three times and subjected for determination of pyrazinamide content by same method.

Protein binding of pyrazinamide was determined by ultrafiltration tenchnique (Shah *et al.*, 1974) using Centricon (Amicon) with cut off M.W. 10,000 at 37°C. For determination of protein binding fractions, pyrazinamide was added to make 5-100 μg/ml to a 3 ml normal rabbit serum. Reaction mixtures were incubated for 1 hr at 37°C with shaking and then centrifuged at 5,000 rpm. The pyrazinamide levels in both of serum and filtrates were determined.

Inulin concentrations in serum and urine were determined by modification of Heyrovsky's method (1956) to exclude the interferences of serum glucose and urine chromogen by means of predigestion of samples in 1 N NaOH.

Analysis of data

Both of two-compartmental open model and non-compartmental model were applied for pharmacokinetic analysis of pyrazinamide. Serum drug concentration(C) during infusion and after termination of infusion can be expressed by following equation in two compartmental open model (Benet, 1972) (Fig. 1).

$$C = \frac{ko}{Vc} \frac{(k_{21}-\alpha)\,(1-e^{\alpha\tau})}{\alpha\,(\alpha-\beta)} e^{-\alpha t} + \frac{ko}{Vc} \frac{(\beta-k_{21})\,(1-e^{\beta\tau})}{\beta(\alpha-\beta)} e^{-\beta t} \quad \text{.....(1)}$$

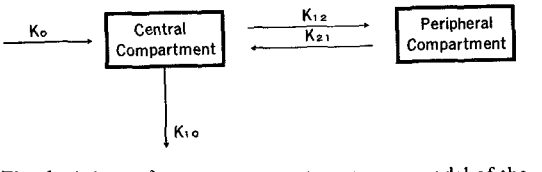

Fig. 1. Scheme for a two-compartment open model of the time course of concentration of pyrazinamide in the sera of rabbits given an intravenous infusion of constant rate (k_0); k_{12} and k_{21} are intercompartmental rate constants; k_{10} is elimination rate constant.

From equation (1), α, β, Vc and k_{21} were determined by iterative least square non-linear regression using simple algorithm. The parameters, k_{10}, k_{12}, steady state volume of distribution (Vdss), total clearance (CLt), half-life of distribution phase($t_{1/2}\,\alpha$) and half-life of elimination phase($t_{1/2}\,\beta$), were calculated by following equations(Gibaldi *et al.*, 1982). Initial estimation of pharmacokinetic parameters were deduced by ESTRIP program (Brown *et al.*, 1976) based on stripping method.

$$k_{10}=\alpha\cdot\beta/k_{21}, \quad k_{12}=\alpha+\beta-k_{21}-k_{10}$$
$$CLt=k_{10}\cdot Vc, \quad Vdss=Vc(1+k_{12}/k_{21}) \quad \text{.....(2)}$$
$$t_{1/2}\alpha=0.693/\alpha, \quad t_{1/2}\beta=0.693/\beta$$

Data also were analyzed using non-compartmental method. Area under the serum concentration-time curve(AUC) was estimated by equation (3),

$$AUC(\mu g/ml\cdot hr) = \int_0^t Cp\,dt + Cp(t)/\beta \quad \text{.... (3)}$$

where Cp(t) is a serum concentration at 6 hr after drug administration. The area up to 1.5 hr was estimated using linear trapezoidal rule, thereafter estimated using log-linear trapezoidal rule.
AUMC(area under the first moment curve) was calculated by

$$AUMC=\int_0^\infty t\cdot Cp\cdot dt \quad \text{..... (4)}$$

and mean residence time of intravenous injection (MRTiv) and that of intravenous infusion (MRTinf) were calculated by

$$MRTinf=\frac{AUMC}{AUC}$$

$$MRTiv=MRTinf-\frac{\tau}{2} \quad \text{.....(5)}$$

where τ is the infusion time.
Total(CLt) and renal(CLr) clearance of pyrazinamide were calculated by equation (6),

$$CLt=\frac{dose}{AUC}, \qquad CLr=\frac{\Delta Ae}{AUC_0^6} \quad \text{..... (6)}$$

where ΔAe is the amount of excreted pyrazinamide in the urine during 0 to 6 hr and AUC_0^6 is the AUC during 0 to 6 hr. The volume of distribution at steady state (Vdss) was calculated by equation (7),

$$Vdss=\frac{dose\cdot AUMC}{(AUC)^2}-\frac{dose\cdot\tau}{2AUC} \quad \text{..... (7)}$$

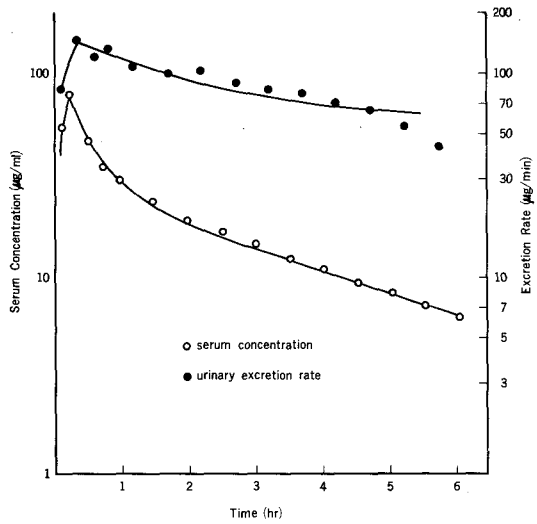

Fig. 2. Semilogarithmic plots of mean pyrazinamide serum concentration vs. time and excretion rate vs. time over 6 hr period.

1. Pyrazinamide pharmacokinetics in rabbits.

With 15 min intravenous infusion of pyrazinamide (40 mg/kg), peak serum concentration was achieved as 57.30 to 105.00 μg/ml (mean ± SD; 83.00 ± 17.82).
Serum concentration curve revealed definite distribution phase up to 1.5 hr and thereafter declined by first order kinetic pattern. However, there was some discrepancy between slope of serum pyrazinamide change and that of urinary excretion rate. The rate of decrement of serum pyrazinamide concentration was faster than that of urinary excretion rate (Fig. 2).

Average urinary excretion up to 6 hr after pyrazinamide administration was 12.74 mg/kg body weight which was about 31.85% of administered dose.
The time-concentration curve of pyrazinamide could be interpreted as well as two-compartment open model. Pharmacokinetic parameters calculated according to non-compartment model were quite comparable to those of 2-compartment open model anlaysis (Table 1).

2. Protein binding of pyrazinamide

Pyrazinamide showed relatively low and non-linear protein binding, it was 1.80-12.50% at the pyrazinamide concentration ranged 5-100 μg/ml and it was expected that the protein binding of

Table 1. Pharmacokinetic constants of the two-compartment model and non-compartment model in 8 rabbits following intravenous administration of 40 mg/kg pyrazinamide

A. Two-compartmental pharmacokinetic parameters.

	α hr^{-1}	$t_{1/2}\alpha$ hr	β hr^{-1}	$t_{1/2}\beta$ hr	k_{21} hr^{-1}	k_{12} hr^{-1}	k_{10} hr^{-1}	Vc L/kg	Vdss[a] L/kg	CLr [b] ml/kg/hr
mean	5.42	0.143	0.306	2.28	1.89	2.96	0.88	0.344	0.907	302.27
±SD	2.21	0.047	0.073	0.57	0.82	1.49	0.09	0.071	0.256	66.62

a; calculated by $(1+k_{12}/k_{21})Vc$

b; calculated by $k_{10}\cdot Vc$

B. Non-compartmental pharmacokinetic parameters.

	AUC $\mu g/ml\cdot hr$	AUMC $\mu g/ml\cdot hr^2$	MRT hr	ΔA^{∞}_{o} mg	Vdss L/kg	CLr [a] ml/kg/hr	CLr [b] ml/kg/hr
mean	143.94	486.26	3.43	33.57	0.953	284.24	112.26
±SD	22.85	105.72	0.83	8.41	0.362	46.36	33.20

a; calculated by dose/AUC

b; calculated by ΔA^{∞}_{o}/AUC

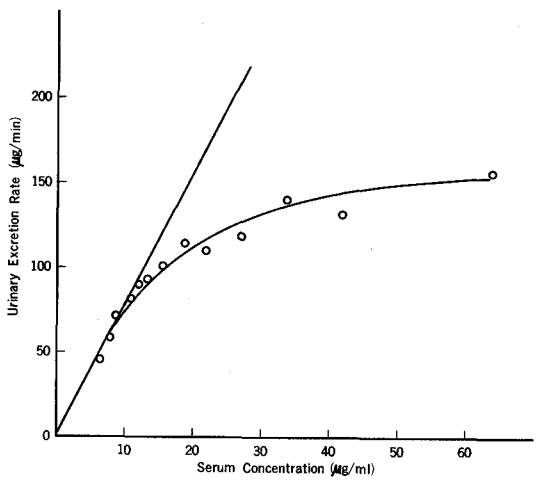

Fig. 3. Non-linear relationship between urinary excretion rate of pyrazinamide and serum concentration of the drug determined at the midpoints of each urine collection interval after cessation of intravenous infusion of 40mg/kg dose to 8 rabbits.

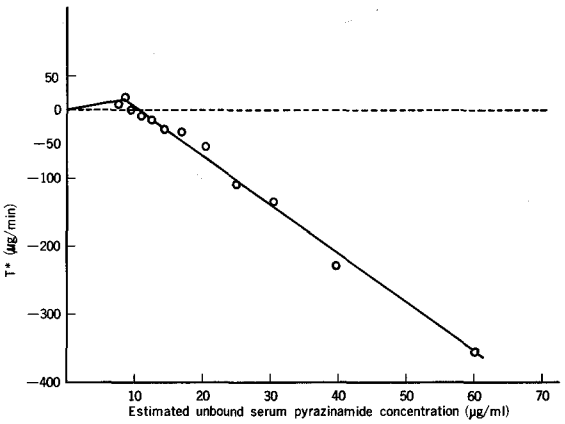

Fig. 4. Apparant tubular transport of pyrazinamide. Each point is the mean value of 8 rabbits.

pyrazinamide at therapeutic serum concentration of 20 μg/ml would be 7.16%.

3. Mode of urinary excretion

The glomerular filtration rate(GFR) and pH of urine were relatively constant when the urinary volume was constantly maintained. Under this condition, the relationship between serum pyrazinamide concentration and urinary excretion rate was not linear but hyperbolic pattern (Fig. 3).

Unbound serum pyrazinamide concentration (Cpf mid) was estimated from mid-point serum pyrazinamide concentration of each urine collection intervals and serum protein binding fraction of pyrazinamide. The clearance of unbound pyrazinamide (CLf) was calculated from Cpf and urinary excretion rate of pyrazinamide. The renal apparent transport(T*) was obtained by substract a filtered amount per minute

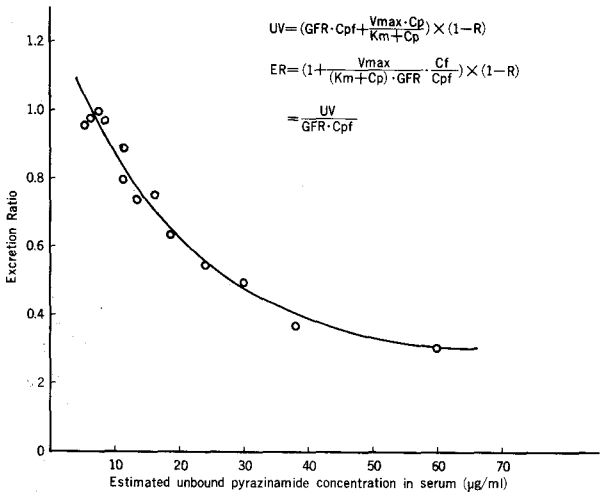

Fig. 5. Experimental renal clearance data for pyrazinamide in the rabbits.
Each point represents the mean of 8 rabbits.

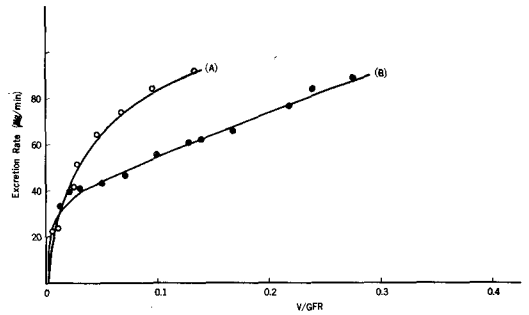

Fig. 6. Urine flow dependence of renal clearance for pyrazinamide
(A); serum pyrazinamide concentration and GFR were in the range of 15.17 to 16.67 μg/ml and 6.00 to 6.99 ml/min during experiment respectively.
(B); serum pyrazinamide concentration and GFR were in the range of 0.13 to 10.24 μg/ml and 7.23 to 9.89 ml/min respectively.

through glomerulus from amount of urinary excretion amount per minute. The relationship between renal apparent transport per minute and unbound serum pyrazinamide concentration revealed slight positive balance at low concentration but at high concentration than 10 μg/ml, renal apparent transport was markedly declined (Fig. 4). Excretion ratio was reduced markedly according to the increase of estimated unbound pyrazinamide concentration, and reached to 0.3 at unbound serum pyrazinamide concentration of 60.90 μg/ml. These suggested that more than 70% of filtered pyrazinamide could be reabsorbed by passive reabsorption in renal tubule (Fig. 5).

Effect of urine flow on pyrazinamide excretion was analyzed at the steady-state serum pyrazinamide concentration. When serum pyrazinamide concentration maintained in relatively stable state, renal clearance of pyrazinamide was hyperbolically depended on the increase of urine flow (Fig. 6).

4. Tissue/serum pyrazinamide concentration ratio

Tissue drug concentrations at 6 hr after administration of pyrazinamide disclosed highest value in the kidney and lowest in the muscle. Tissue/serum concentration ratio ranged 1.16 to 1.71.

When the serum pyrazinamide concentration was maintained at stable state, tissue/serum concentration ratio ranged 1.10-1.68. There were no significant differences between 15 minute infusion data and

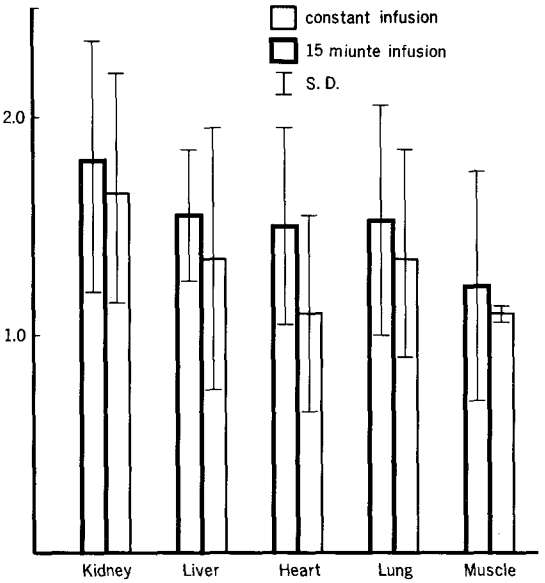

Fig. 7. Pyrazinamide tissue/serum ratio at 6 hour after 15 minute infusion of a dose of 40 mg/kg (n = 5) and at steady state by constant infusion of 66 μg/kg/min after 11.2 mg/kg loading dose (n = 3). (The serum concentration is represented as 1)

constant infusion data in tissue/serum concentration ratio (Fig. 7).

DISCUSSION

In order to reevaluate the possibility of renal

tubular secretion of pyrazinamide which suggested by Park *et al.*(1985), we analyzed the renal excretory mechanism of pyrazinamide by modified clearance method (Hori *et al.*, 1976) in rabbit.

For the analysis of renal excretory mechanism, constant appreciable volume of urine flow is needed since sometimes drug amount of urinary excretion is critically changed by urine flow in vivo study. In this study, continuous infusion of physiological saline (0.45 ml/kg/min) and single intravenous injection of pyrazinamide was adopted as suggested by Hori *et al.*

The serum concentration of pyrazinamide showed distinctive two phase, slow elimination phase after rapid distribution phase. In this result, the serum pyrazinamide concentration during elimination phase was little bit higher than that reported by Sttotmeier (1967) with intravenous bolus injection of 40 mg/kg. Pharmacokinetic parameters calculated according to two compartmental open model were almost same as those calculated according to non-compartmental model.

The rapid distribution phase showed in serum pyrazinamide concentration-time curve was a result of rapid equillibrium between serum and tissue because pyrazinamide showed very low protein binding and it might be expected that almost all pyrazinamide would be present as undissociated form in the serum because of low pKa of pyrazinamide(0.5). Ellard(1969) suggested that the pyrazinamide penetrates readily tissue membrane. Chen *et al.* (1979) also suggested rapid tissue distribution of pyrazinamide from the result of physiological pharmacokinetic model.

Ellard(1969) reported that pyrazinamide serum and urine concentration fell exponentially after 3 hours and half-life of about 10 hours was obtained. And he suggested that about 98% of the pyrazinamide filtered by kidney was reabsorbed.

In the present study, steady state volume of distribution was 0.953 L/kg and renal clearance was 112 ml/kg/hr which was 39.5% of total clearance. This results suggested that about 60% of administered pyrazinamide would be eliminated by non-renal route. And substantial amount of reabsorption was expected from average glomerular filtration rate of 9 ml/min in this experiment.

The result of hyperbolic relationship between serum pyrazinamide concentration and urinary excretion rate suggested that there should be also substantially active renal tubular secretion of pyrazinamide with saturation kinetics. Therefore we try to estimate the secretory and reabsorption magnitude with urinary excretion data and estimated unbound concentration according to the equations on fig. 6. The Km value of renal tubular secretion was expected to be lesser than 15 μg/ml for every experimental rabbit and reabsorption fractions were more than 90%. However, higher toxic serum concentration might be needed for accurate estimation of parameters of secretory and reabsorptive process, because secretory Vmax and Km values were greatly influenced by minor error in curve fitting.

When serum pyrazinamide concentration maintained at stable level renal clearance of pyrazinamide was hyperbolically increased according to the increase of urine flow. This result suggested that renal tubular reabsorption is quite dependent on urine flow and is not a rapid equilibrium process.

In conclusion, it is suggested from present experiment that a great fraction of pyrazinamide filtered by glomerulus and secreted by renal tubule is reabsorbed and this renal tubular reabsorption of pyrazinamide might be greatly influenced by urine flow.

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=국문초록=

Pyrazinamide의 신배설기전에 관한 약동학적 연구

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최응상 · 신상구 · 이선희 · 최철희 · 김용식 · 임정규 · 박찬웅

가토에서 체중 kg당 40mg 용량을 15분간 단기 주입 또는 일정 혈중농도 유지를 위한 지속 주입 방법으로 pyrazinamide를 투여 후 혈청농도 및 뇨중 배설량을 측정하여 이의 신장 배설 기전을 약동학적으로 분석하고 분포기 이후의 조직농도 측정으로 다음과 같은 결과를 얻었다.

1. 가토에서 pyrazinamide의 약동학적 분석을 compartmental model에 적용시 분포기의 반감기는 2.38±0.57시간이었으며 V_c(central compartment의 용적)는 체중 kg당 349.96±70.99mℓ이었고 총 분포 용적(V_{dss})은 907.00±255.61mℓ이었다.
2. Non-compartmental model parameter는 총 분포 용적(V_{dss})은 체중 kg당 953.34±362.02 mℓ이었으며 평균 체내 잔류 시간(MRT_{iv})은 3.30±0.84시간이었고 신 청소율은 112.26mℓ/kg/hr 로 총 청소율의 39.5%에 해당하였다.
3. pyrazinamide의 혈청 단백 결합율은 5~100μg/mℓ의 범위에서 12.50-1.80%의 결합율을 보였다.
4. pyrazinamide의 분포기 이후 조직/혈청 농도비는 약물 투여 방법에 따라 지속 정맥주입에 따른 steady-state 유지시 15분 정주 방법시 보다 낮은 값을 보였으며 steady-state 상태의 조직 분포율은 신장에서 가장 높았고(1.68±0.53), 근육에서 가장 낮은 값(1.10±0.46)을 보였다.
5. 가토에서의 pyrazinamide의 신장 청소율은 혈청 농도 변화에 비직선적인(non-linear) 관계를 보였다.
6. pyrazinamide의 신장 현성 운반량(apparent transport)은 혈청 pyrazinamide농도 10μg/mℓ까지는 양성 균형을 보였으나 이후 혈청 농도의 증가에 따라 현저한 음성균형을 보였다.
7. pyrazinamide의 신장을 통한 분비율(excretion ratio; ER)은 혈청농도 증가에 따라 현저히 감소하는 양상을 보이며 60μg/mℓ의 농도에서 약 0.3의 ER치를 보여 70% 이상의 재흡수율을 나타냈다.
8. 혈청 pyrazinamide농도를 일정히 유지하고 뇨량 변화시 pyrazinamide의 신장 청소율은 뇨량 증가에 의존적인 상승을 보였다.

이상의 실험 결과로 pyrazinamide의 신장 배설은 사구체 여과와 더불어 세뇨관 분비 기전이 존재하고 비교적 빠른 확산속도를 보이는 세뇨관 재흡수 기전에 의해 신장 배설이 조절됨을 추정할 수 있었다.