Arch. Pharm. Res. Vol. 17, No. 2, pp. 124-130, 1994

Nonlinear Renal Excretion of Theophylline and Its Metabolites, 1-Methyluric Acid and 1,3-Dimethyluric Acid, in Rats

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(Received December 10, 1993)

Plasma pharmacokinetics and renal excretion of theophylline (TP) and its metabolites were investigated in rats. Plasma concentrations of TP declined in a monoexponential manner, while those of 1-methyluric (MU) and 1,3-dimethyluric (DMU) declined in a biexponential manner upon respective iv bolus injection of each compound at 6 mg/kg dose. The total body clearances (CL_t) of the metabolites were 4-6 fold larger than that of TP, while the distribution volumes of them at steady-state (Vd_{ss}) were 40-50% smaller than that of TP. The metabolites showed their plasma peaks in 30 min after iv injection of TP indicating very rapid metabolism of TP. Metabolism of TP to DMU was more than fourfold faster than that to MU. Renal excretion of TP and its metabolites was studied in urine flow rate (UFR)-controlled rats. The renal clearance (CL_r) of TP was inversely related to plasma TP concentrations, and much smaller than the glomerular filtration rate (GFR) suggesting tubular secretion and profound reabsorption in the renal tubule. The CL_r of each metabolite also showed that inverse relationship, but far exceeded GFR suggesting that tubular secretion plays a major role in their elimination. The CL_r of the metabolites were reduced to less than GFR by ip injection of probenecid (142.7 mg/kg). It supports that the metabolites are secreted in the renal tubule, and suggests that they share a common transport system in their secretion processes with probenecid. On the other hand, the CL_r of TP was not affected significantly by the probenecid treatment. Considering the inverse relationship of TP between the CL_r and its plasma concentrations, no effect of probenecid on CL_r of TP is most likely due to negligible contribution of the secretion to the overall CL_r of TP.

Key words: Theophylline, 1-Methyluric acid, 1,3-Dimethyluric acid, Renal excretion, Secretion, Reabsorption, Nonlinearity, Pharmacokinetics

INTRODUCTION

Theophylline (TP) is widely used for the treatment of acute and chronic asthma in humans. Recently it has also been applied for the management of apnea in premature neonates (Hendeles *et al.*, 1986). TP is eliminated from body primarily through the hepatic metabolism. When TP is administered orally to human adults, 15 to 35% of the dose is excreted as 1-methyluric acid (MU), 29 to 39% as 1,3-dimethyluric acid (DMU) and 13 to 36% as 3-methylxanthine (MX) in 24 hr while 7 to 13% of the dose is eliminated unchanged in the urine (Lesko, 1984). Among the metabolites of TP, two major metabolites in the rat (MX and DMU) also have bronchodilating activity and thus may contribute to the therapeutic effect of TP (Persson and Andersson, 1977; Williams *et al.*, 1978).

Renal elimination pathways of TP and the metabolites are complicated, mainly due to a diuretic effect exerted by TP. It was documented that the renal clearance of TP is urine flow rate dependent (Levy and Koysooko, 1976; Agbaba *et al.*, 1990) and, therefore, alteration in the urine flow is likely to change the renal elimination of TP. In consistent with this view, Tan-Liu *et al.* (1982) and others (Gundert-Remy *et al.*, 1983; Bonnacker *et al.*, 1989) have demonstrated that high plasma concentration of TP elevated its renal clearance.

In literature, however, the negative relationship between TP plasma concentration and its CL_r has also been reported by our laboratory (Kuh *et al.*, 1991) and others (Agbaba *et al.*, 1990), indicating that the diuretic effect does not always promote an increase in the renal clearance. Because of these conflicting observations, contribution of diuresis to the TP renal elimina-

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tion is currently unknown. As a result, the underlying mechanism(s) of the renal clearance of TP is not fully understood. Similarly, the renal clearances of TP metabolites are urine flow rate dependent and the mechanisms are not clear.

For the investigation of the renal elimination pathways of TP and the metabolites, the control of the urine flow rate, independent of the diuresis exerted by TP, is requried. In this study, therefore, we examined the renal elimination of TP and the metabolites when a constant diuresis was induced by mannitol infusion. A constant urine flow rate allowed us to test contributions of active secretion and reabsorption processes involved in the renal elimination of TP and the metabolites.

ATERIALS AND METHODS

Materials

Theophylline (TP), 1-methyluric acid (MU), 1,3-dimethyluric acid (DMU) and inulin were purchased from Sigma Chem. Co. All other reagents were of analytical or HPLC grade. Male Wistar rats (Experimental Animal Center, Seoul National University) weighing 250± 10 g were used in all experiments.

IV Injection of TP, MU and DMU

In order to compare the plasma pharmacokinetics of TP with those of MU and DMU, plasma concentration profiles were studied in rats following iv bolus administration of each compound. The rats were placed at supine position during the experiment. Under ether anesthesia, femoral arteries and femoral veins were cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling and drug administration, respectively. After complete recovery (1 hr) from the anesthesia, TP, MU and DMU were administered as an iv bolus dose (6 mg/kg, 2 ml/kg) in saline through the venous catheter on separate occasions. Blood samples (0.2 ml) were collected in heparinized tubes from the femoral artery at 0, 5, 10, 20, 30, 40, 60, 90, 120, 180 and 240 min post-administration of TP, MU and DMU. The heparinization was conducted by treating the tubes with 10 μl of heparinized saline (150 IU/ml) and successive drying up the saline. Plasma samples were obtained by centrifuging the blood samples at 4,000×g for 10 min and stored at −20℃ until analysis.

IV Injection of TP under Mannitol Infusion

Effect of plasma concentration of each compound on their CL_r was studied in the rats receiving iv infusion of 3% (w/v) mannitol-saline after iv administration of TP. The mannitol, a potent diuretic, was infused in order to make the UFR constant. Under constant UFR, interpretation of the CL_r change would become simpler. Under ether anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubings as described above. The ureters were cannulated at 2 cm from the both kidneys with PE-10 polyethylene tubings for urine collection. The rats were kept warm at supine position during the experiment. Mannitol infusion was started more than 1 hr after the operation allowing for recovery from the anesthesia.

A loading dose of 2 ml/kg of 5% (w/v) inulin solution in 3% (w/v) mannitol-saline was followed by a sustaining dose of 2% (w/v) inulin solution in 3% (w/v) mannitol-saline at the constant rate of 1.0 ml/hr using microautomatic infusion pump (model KN, Natsume, Japan). After the steady-state of plasma inulin was attained (60 min), infusion was stopped temporarily and TP was injected into the femoral vein at a dose of 6 mg/kg. After the injection, the infusion of inulin-mannitol-saline was continued again.

Blood samples (0.2 ml) were withdrawn at 0, 0.5, 1.5, 3, 5, 7 and 9 hr after the TP injection from the femoral artery. Urine samples in 0-1, 1-2, 2-4, 4-6, 6-8 and 8-10 hr were collected. Plasma and urine samples were stored at −20℃ until analysis for inulin, TP, MU and DMU.

IV Infusion of TP and ip Injection of Probenecid under Mannitol Infusion

The effect of probenecid on the plasma concentration and CL_r level of each compound was studied as follows. The rats were anesthetized with ether and the femoral vein and artery were cannulated using polyethylene tubing as described above. The procedures used for ureteral cannulation and infusion of mannitol and inulin were identical to those described above. After the steady-state of plasma inulin concentration was attained (60 min), the infusion was stopped temporarily and a loading dose of TP (8.6 mg/kg) followed by a continuous infusion of TP (720 μg/hr) was administered. The infusion of the mannitol-inulin solution was then resumed. Blood samples (0.2 ml) were withdrawn at 90, 140, 190 min after the infusion-start of the inulin solution. Urine samples in 65-115, 115-165, and 165-215 min were collected. Probenecid (142.7 mg/kg) was then administered by intraperitoneal injection at 220 min. Then blood samples (0.2 ml) were withdrawn at 250, 300 and 350 min, and urine samples during 225-275 and 275-325 min after the probenecid administration were collected. Blood and urine samples were treated in the same way described above. They were stored at −20℃ until analysis for inulin, TP, MU and DMU.

Analytical Method

The concentrations of TP, MU and DMU in the pla-

sma and urine samples were determined by modified HPLC method (Kuh *et al.*, 1991). To 70 μl plasma and urine samples, 250 μl of acetonitrile was added and vortexed for the deproteinization. After the centrifugation at 5000 rpm for 10 min, 200 μl aliquots of the supernatants were transferred and evaporated to dryness under nitrogen stream. A 100 μl of mobile phase solution was added to dissolve the residue for the plasma samples. Twenty μl aliquots were injected onto HPLC precolumn (μ Bondapak C18).

For the urine samples, the residue was dissolved with 1 ml of the mobile solution and 20 μl aliquots were injected onto the precolumn.

The mobile phase solution was prepared by dissolving 100 mmole (13.97 g) of sodium acetate, 4 mmole (2.56 ml as 40% w/v aqueous solution) of tetrabutylammonium hydroxide, and 30 ml of methanol in 1 liter of deionized water. The pH of the mobile phase was adjusted to 4.5 with acetic acid. The mobile phase was filtered using 0.45 μm millipore filter and degassed ultrasonically before use. TP, MU and DMU were separated on a reversed phase column (RP-18 Speri 5, 5 μm, 250×3.9 mm id) kept warm at 45± 1℃ using electrical heating jacket. The flow rate of the mobile phase was 1.0 ml/min. The analytes were monitored by UV absorbance at 280 nm.

The peaks of MU, DMU, and TP were separated and their retention times were 6.7, 8.5, and 18.0 min, respectively. Linearity of the standard curves for each compound was confirmed over the concentration range of 0.2~32.0 μg/ml for TP, MU and DMU in plasma samples, and 5.0~400 μg/ml for those in the urine samples. The respective recoveries of TP, MU, and DMU were 69, 50, and 75% from the plasma samples, and 100, 97, and 99% from the urine samples. The detection limits of each compound were 0.2, 0.1, and 0.1 μg/ml, respectively in the plasma samples, and 5 μg/ml for all the compounds in the urine samples. Intraday and interday variation of the assay were below 4.0% for all the compounds in the plasma and urine samples.

Inulin in the plasma and urine samples was analyzed by the photometric method (Waugh, 1977).

Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated in a time-averaged manner. Total body plasma clearance (CL_t), distribution volume at steady-state (Vd_{ss}) and plasma half life ($t_{1/2}$) of TP, MU and DMU were calculated from the respective plasma concentration data after *iv* injection (6 mg/kg) of each compound as follows:

$$CL_t = Dose/AUC \quad (1)$$

$$Vd_{ss} = Dose \cdot AUMC/AUC^2 \quad (2)$$

$$t_{1/2} = 0.693/K \quad (3)$$

$$t_{1/2\alpha} = 0.693/\alpha \quad (4)$$

$$t_{1/2\beta} = 0.693/\beta \quad (5)$$

where AUC and AUMC are area under the plasma concentration-time curve from time zero to infinity and area under the first moment of the plasma concentration-time curve from time zero to infinity, respectively. K, α and β are apparent first-order rate constants; K for overall elimination process of TP, α and β for alpha and beta phase of elimination processes of the metabolites. They were obtained by fitting the plasma concentration data to a conventional one- (for K) or two-compartment model (for α and β) using the program MULTI (Yamaoka *et al.*, 1981). AUC and AUMC from time zero to the last sampling point were claculated by the trapezoidal method and extrapolated from the last sampling point to infinity using the respective elimination rate constant (K or α and β).

The apparent first-order rate constant for metabolite formation (K_f) was calculated by the relationship (Kaplan *et al.*, 1973).

$$K_f = K[AUC_m]_p/[AUC_m]_m \quad (6)$$

where $[AUC_m]_p$ and $[AUC_m]_m$ represent the total area under each metabolite concentration-time curve after administration of the parent drug (6 mg/kg) and the metabolite (6 mg/kg), respectively.

Renal clearances (CL_r) of TP, MU, DMU and inulin were calculated by dividing the mean urinary excretion rate during urine collection intervals by their interpolated plasma concentrations at the midpoints of the urine-collection intervals. Renal clearance of inulin was considered GFR.

Statistical Analysis

One-way analysis of variation (ANOVA) with Turkey's multiple range comparison procedure was used to compare the pharmacokinetic parameters and plasma concentrations of each compound, and a p value of 0.05 or less was considered to be significant.

RESULTS

Plasma profiles of TP, MU and DMU after Respective iv Injection

Fig. 1 shows the plasma concentration-time curves of TP, MU and DMU following *iv* administration of each to rats. Plasma concentrations of TP showed monoexponential elimination curve, while those of MU and DMU showed biexponential elimination curve. MU and DMU were eliminated much faster than TP. Time-averaged pharmacokinetic parameters of them are summarized in Table 1. MU and DMU showed significantly shorter half-lives, smaller Vd_{ss} and larger

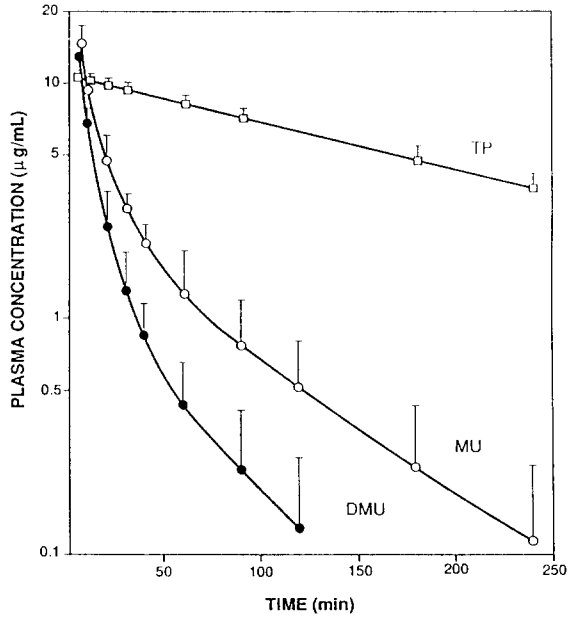

Fig. 1. Plasma concentration-time profiles of TP (n=8), MU (n=4) and DMU (n=4) following respective iv administration (6 mg/kg each) to the rat. Data were fitted to 1-compartment open model for TP and 2-compartment model for MU and DMU. Each point and bar in the figure represents the mean± SE.

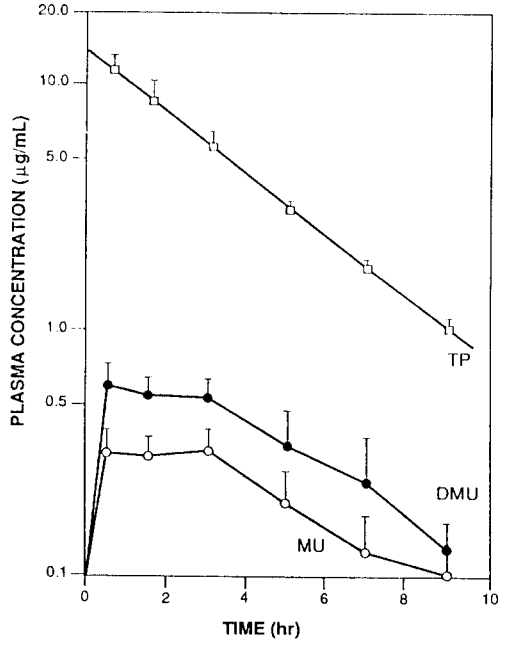

Fig. 2. Plasma concentration-time profiles of TP, MU and DMU following iv injection of TP (6 mg/kg) to rats receiving iv infusion of 3% (w/v) mannitol-inulin-saline. Each point and bar in the figure represents the mean± SE of four experiments.

Table 1. Time-Averaged Pharmacokinetic Parameters of TP, MU and DMU in Rats (6 mg/kg)[a]

Parameters	TP (n=8)	MU (n=4)	DMU (n=4)
$t_{1/2}$ (min)	181.0± 26.4	–	–
$t_{1/2\alpha}$ (min)	–	7.2± 0.5	5.5± 0.7
$t_{1/2\beta}$ (min)	–	45.8± 1.8	32.4± 7.4
CL_t (ml/min/kg)	4.2± 0.5	15.2± 3.6	24.9± 3.0
Vd_{ss} (ml/kg)	961.1± 23.6	584.0± 64.0	477.1± 57.0
K_f (min^{-1})	–	3.2	14.0

[a] All the parameters except K_f were obtained from Eqs. 1-5 after *iv* administration of each compound at 6 mg/kg dose, and were expressed as the mean± SD. K_f was calculated from Eq. 6 using mean values of K, $[AUC_m]_p$ and $[AUC_m]_m$.

CL_t than TP.

Plasma Profiles and Renal Clearances of TP, MU and DMU Following iv Injection of TP to Rats Receiving Mannitol Infusion

The UFR of each rat fluctuated but could be maintained in certain ranges (1.31± 0.44 ml/hr/body) by the mannitol infusion. Fig. 2 shows the plasma profiles of TP and its two metabolites following iv administration of TP (6 mg/kg) to these rats. MU and DMU appeared immediately in the plasma after TP administration: They showed their plasma peaks in 30 min after the administration. From the data in Figs. 1 and 2, the apparent formation rate constants of MU and DMU from TP (K_f) were calculated (Table 1): DMU formation was much more rapid (>fourfold) than MU formation. The plasma profiles of the two metabolites at terminal phase were almost parallel with that of TP (Fig. 2), but their slopes were smaller than those after *iv* administration of TP (Fig. 1).

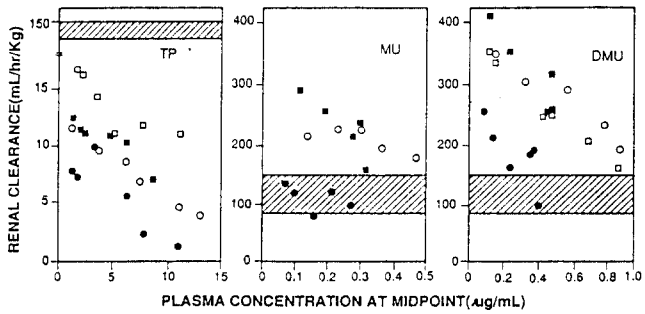

Fig. 3. Relationship between the renal clearances and plasma concentrations of TP, MU and DMU after iv injection of TP (6 mg/kg) to rats (n=4) receiving iv infusion of 3% (w/v) mannitol-inulin-saline. Each symbol represents a particular rat. Hatched areas in the figures indicate the mean± SD range of GFR variations in each rat. The CL_r of MU was shown in the figure only for three rats, since the plasma concentrations of MU in one rat were below the detection limit (0.1 μg/ml).

Fig. 3 shows the relationship of the CL_r versus the mean plasma concentrations of TP, MU and DMU

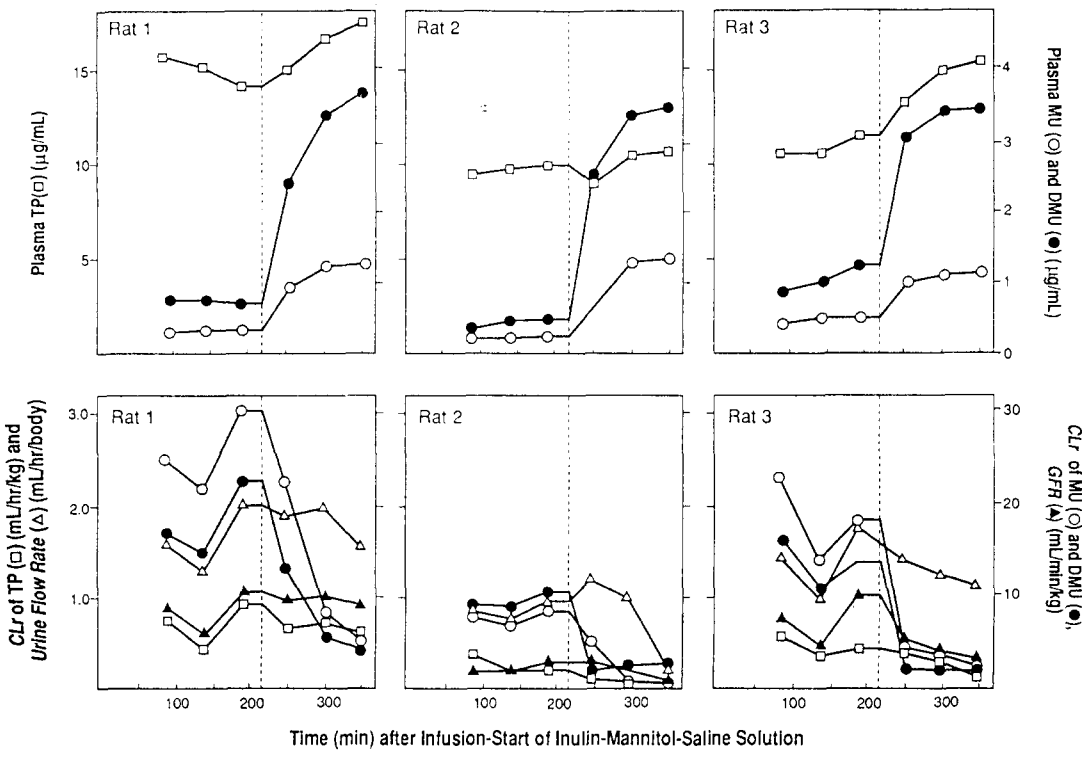

Fig. 4. Effect of probenecid treatment on the steady-state plasma concentrations of TP and its metabolites (upper row), and on the UFR, GFR and CL_r of TP and its metabolites (lower row). TP was administered iv bolusly (8.6 mg/kg) at 60 min after the start of infusion of 3% (w/v) mannitol-inulin-saline and followed by constant infusion (720 μg/hr/rat). At 220 min after the start of the mannitol infusion, probenecid (142.7 mg/kg) was injected intraperitoneally. Times in the figure indicate midpoints of urine collection intervals.

following iv injection of TP (6 mg/kg) in the rats (n=4) receiving mannitol infusion. Although there were great intersubject variations, the CL_r of TP showed a distinct plasma concentration-dependency: The CL_r of TP increased as the mean plasma TP concentration decreased in all the rats tested, but they were always much smaller than the mean GFR of the rats. The CL_r of MU was shown only for the three rats in the figure since the plasma concentrations of MU in one rat were lower than the detection limit (0.1 μg/ml). In one rat (symbol ■), the CL_r of MU increased as its plasma concentrations decreased, while the CL_r of the other two rats did not. However, all the rats except one (symbol ●) showed much larger CL_r than the mean GFR over the entire plasma concentration range examined.

The CL_r of DMU also increased as its plasma concentrations decreased. They were larger than the mean GFR especially in its low plasma concentration range.

Effect of Probenecid Injection on Renal Clearances of TP, MU and DMU Following iv Infusion of TP

The upper row of Fig.4 shows the plasma profiles of TP, MU and DMU following iv bolus administration (8.6 mg/kg) and successive infusion (720 μg/hr/body) of TP, together with the effect of *ip* administration of probenecid (142.7 mg/kg) on the profiles. Plasma levels of TP and its metabolites reached their steady-state within 30 min after the infusion start of TP, i.e. within 90 min after the infusion-start of mannitol, in two out of three rats. Probenecid injection, which was indicated by the vertical dots in the figure, affected the plasma levels of TP and its metabolites differently. Plasma levels of TP, MU and DMU were increased abruptly by the probenecid injection. The increase was most significant for DMU and least significant for TP.

The lower row of Fig. 4 shows the correspondent time-courses of the CL_r of TP and its metabolites compared with those of GFR and UFR. The CL_r of TP was much smaller than those of MU and DMU in all cases. The CL_r, GFR and UFR fluctuated considerably, especially in rat 1 and 3 even under the steady-state plasma concentrations of TP and its metabolites. The fluctuations of CL_r , GFR and UFR before the probenecid treatment were almost parallel with each other. The CL_r of the metabolites were decreased significantly by probenecid, while the CL_r of TP, GFR and UFR were not.

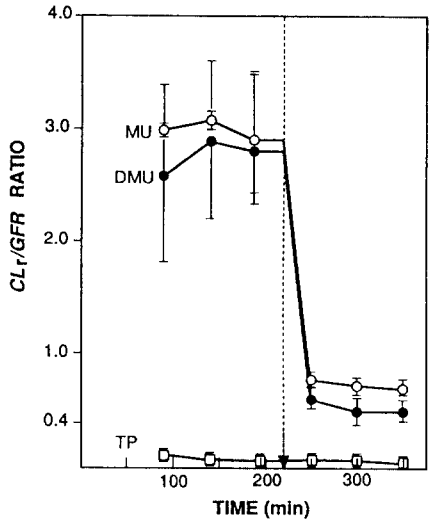

Fig. 5. Effect of probenecid treatment at 220 min on the clearance ratio of TP, MU and DMU to GFR. The ratio was recalculated from the data in Fig. 4 and expressed as the mean± SD of three rats. Times in the figure indicate midpoints of urine collection intervals.

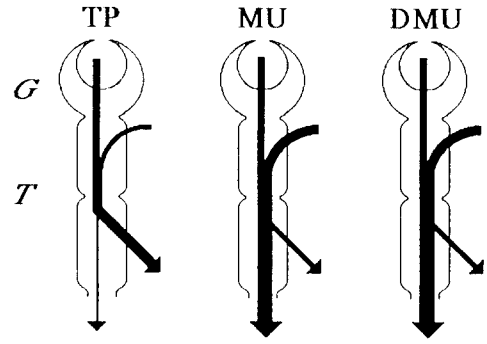

Fig. 6. Schematic illustration of the most probable mechanism of renal excretion of TP, MU and DMU. The width of each arrow represents approximately the contribution of each process, i.e., glomerular filtration, tubular secretion and reabsorption, to the overall CL_r. *G* and *T* in the figure indicate glomerulus and renal tubule of the nephron, respectively.

Fig. 5 was redrawn from the data in Fig. 4 to show the effect of probenecid treatment on the GFR-normalized CL_r (CL_r/GFR ratio) of each compound. Although the CL_r and GFR values fluctuated considerably in each rat, the GFR-normalized clearance ratio of the metabolites were much larger than unity, while that of TP was much smaller than unity. The clearance ratio of the metabolites was decreased significantly ($P<0.001$) by the treatment, while that of TP did not.

DISCUSSION

The renal excretion kinetics of TP and its metabolites has been investigated extensively in literature. However, data available in the literature regarding the renal elimination of TP are not always reproducible. For example, a negative relationship between CL_r and plasma concentration of TP has been found (Agbaba *et al.*, 1990; Kuh *et al.*, 1991), suggesting involvement of a nonlinear renal elimination mechanism(s). In contrast, positive relationship between plasma concentration of TP and its renal clearance has also been reported (Tang-Liu *et al.*, 1982; Gundert-Remy *et al.*, 1983; Bonnacker *et al.*, 1989). Because of these conflicting results, the underlying mechanism of renal excretion kinetics of TP is currently unknown.

The CL_r of TP is significantly smaller than GFR, suggesting that tubular reabsorption of TP is involved in the elimination process. However, a diuretic effect of TP poses difficulty in estimating the extent of reabsorption of TP and the true renal clearance of TP since the diuretic effect is likely to be proportional to plasma concentration of TP. In this study, we could achieve mean urine flow of 1.31± 0.44 ml/hr/body by the mannitol inufison. Therefore, under our experimental condition, the diuretic effect exerted by TP could be neglected.

Under the urine flow controlled condition, the renal clearance of TP was negatively related to its plasma concentrations. This observation supports the involvement of non-linear pharmacokinetics in the renal clearance mechanism of TP. In this study, individual process(es) responsible for the nonlinearity was not directly investigated. However, our data are consistent with the view that active secretion process(es) of TP exists in the rats. In this study, we could not reproduce reported observations of positive relationship between CL_r of TP and the plasma concentration. The discrepancy may be partly due to the differencies in experimental design(s) (e.g., urine flow rate control and/or doses given) and/or other variables (e.g., species differencies).

Despite the suggested nonlinear kinetics in the renal excretion of TP, plasma TP concentration was declined monoexponentially. It is possible that CL_r of TP was substantially small compare to total clearance of TP and, thus, nonlinearity in the renal elimination of TP may not be apparent in the overall elimination. Tang-Liu *et al.* (1982) proposed that the metabolic clearance and urinary clearance of TP compensate each other since the higher plasma concentration of TP may induce more pronounced diuretic effect, and increase the renal clearance mechanism of TP. However, this mechanism is not likely to explain the mono-exponential decline in this study because the urinary clearance of TP was consistently less than 20 ml/hr/kg while total body clearance was approximately 240 ml/hr/kg. Furthermore, diuretic effect induced by TP was negligible compared to overall diuretic effect in this study. Also, minor fluctuation of CL_r did not affect the plasma

concentration of TP, supporting the view that the renal clearance of TP contributes not significantly in the overall pharmacokinetics.

The renal clearances of TP and its metabolites fluctuated in parallel to UFR and GFR (Fig. 4 before probenicid administration), similar to those reported previously (Levy and Koysooko, 1976; Agbaba *et al.*, 1990). Since the renal clearance of metabolites of TP exhibited UFR dependency, the data are consistent with the hypothesis that the passive reabsorption in the renal tubule for TP may occur. The CL_r/GFR ratios for MU and DMU were significantly larger than unity before the probenicid administration, suggesting that an active secretion is also involved in the renal elimination of these compounds. Upon the administration of probenicid, however, the ratios drastically decreased less than unity. Collectively, these observations suggested that the metabolites are secreted via a transport system and share the process with probenicid. In contrast, CL_r/GFR ratio for TP was essentially not affected by the probenicid and remained less than unity (Fig. 4), indicating that TP and its metabolites do not share the same secretion mechanism(s).

Plasma protein binding is not likely to explain the nonlinear pharmacokinetics we found in this study. In literature, it is well documented that plasma protein binding of TP and its metabolites were found to be linear in the concentration upto 25 μg/ml (Gundert-Reny and Hilderbrandt, 1983). Therefore, in the concentration range we studied, the linear protein binding is likely to be prevalent.

Fig. 6 summarizes the most probable conclusion of this study on renal excretion of each compound: much less significant secretion than reabsorption for TP, and much more significant secretion than reabsorption for MU and DMU. The width of the arrows in the figure represents approximately the relative contribution of each process to the overall CL_r.

In conclusion, under the urine flow rate control, the CL_r of TP was negatively correlated with plasma concentration of TP. This observation is most likely due to saturable secretion process(es). Also, CL_r of two major metabolites of TP, but not TP, was decreased by the administration of probenicid, suggesting a common secretion mechanism in the renal elimination process. However, the active secretion pathway of the metabolites appear to be distinct from TP.

ACKNOWLEDGMENT

This work was partly supported by Korean Health Foundation (1989).

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