POSSIBLE ACTIVE TUBULAR SECRETION OF SULFAMONOMETHOXINE AND ITS METABOLITES IN PIGS

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The effect of probenecid on the plasma kinetics of sulfamonomethoxine (SMM) was examined in conscious pigs. A linear kinetic dose of SMM (10 mg/kg) was injected with or without probenecid. Probenecid (25 mg/kg, *i.v.*) was injected immediately before SMM injection and again at 1, 2, 3, 4 and 6 h later. The *AUC*s of SMM and of the acetylated compound of SMM (AcSMM) in probenecid injected animals were significantly larger when compared with those of non-injected animals ($p < 0.05$). Next, possible active secretion from the renal tubule of SMM and its metabolites was examined using probenecid and pyrazinoate. Ten commercial pigs with ureter cannulae were used under anesthesia. The plasma concentration of SMM and AcSMM was maintained at a steady state by a priming dose of SMM (5 mg/kg, *i.v.*) followed by sustaining infusion (4 μ g/kg/min). Renal clearance of AcSMM was suppressed by bolus injection of probenecid (25 mg/kg), but that of SMM was not. The renal excretion of a water soluble metabolite was suppressed by probenecid. No marked changes in renal excretory kinetics were found by pyrazinoate injection (12.5 mg/kg, *i.v.*). Consequently, the saturation in the active tubular secretion of AcSMM may play an important role in the nonlinear pharmacokinetics of SMM in pigs.

Keywords — sulfamonomethoxine; *N*⁴-acetylsulfamonomethoxine; active tubular secretion; pig; nonlinear pharmacokinetics; probenecid; pyrazinoate

INTRODUCTION

Nonlinear pharmacokinetics with a capacity limited elimination of sulfamonomethoxine (SMM) has been reported in pigs.¹⁾ The saturation in the renal excretion of *N*⁴-acetylsulfamonomethoxine (AcSMM) was speculated as a causal factor for the nonlinear pharmacokinetics of SMM from the pharmacokinetic analysis. AcSMM was the main metabolite of SMM in pigs.²⁾

The renal excretion of drugs by active tubular secretion can be involved in the capacity limited elimination. The active secretion of sulfa-drugs and their metabolites has been demonstrated in man and in some experimental animals.^{3–9)} No reports were found on the active tubular secretion of SMM and its metabolites, and of sulfa-drugs in pigs. In the present experiment, possible active tubular secretion of SMM and its metabolites was examined by means of blocking agents for the renal tubular secretion, probenecid and pyrazinoate in anesthetized pigs. The effect of the blocking agent on the plasma kinetics of SMM and AcSMM after SMM intravenous injection was also examined in conscious pigs.

MATERIALS AND METHODS

Five Goettingen miniature pigs (male), weighing 13–44 kg, were used for experiment 1. Ten commercial pigs (female) with ureter cannulae, weighing 20–31 kg, were used for experiment 2. They were used after withholding feed for 24 h.

Experiment 1 — The effect of probenecid on the plasma kinetics of SMM was examined in the intact pigs. They were injected SMM (10 mg/kg, *i.v.*) or SMM with probenecid. Probenecid (25 mg/kg, *i.v.*) was injected immediately before SMM injection and again 1, 2, 3, 4 and 6 h later. A 4 ml sample of blood was taken at 0.5, 1, 2, 3 and 4 h and at 2 h intervals for 12 h after injection with SMM alone, and up to 16 h after injection with SMM and probenecid. The intravenous injection of the drugs was done through the right anterior vena cava and blood sampling from the left.

Experiment 2 — The effect of probenecid and pyrazinoate on urinary excretion of SMM and its metabolites was examined. The experiment was done under pentobarbital anesthesia. Pentobarbital-Na was intravenously injected

with 10 mg/kg initially and subsequently with 2 mg/kg at 15 to 30 min intervals. The plasma concentrations of inulin, SMM and AcSMM were maintained at steady states by intravenous priming doses and sustaining infusions of inulin and SMM. The priming doses were 100 mg/kg for inulin and 5 mg/kg for SMM. The sustaining infusions were 1 mg/kg/min for inulin and 4 μ g/kg/min for SMM. They were infused at 1 ml/min as solutions prepared in 0.9% NaCl. After an initial equilibration period for 2 h, urine sampling for 10 min was done every 10 min up to 1 h, and a 4 ml of blood was sampled from anterior vena cava at the midpoint of the urine collection. Probenecid (25 mg/kg) or pyrazinoate (12.5 mg/kg) in NaOH solution (final pH 8.0) was injected intravenously. The urine sampling for 10 min was done every 10 min up to 1 h with further sampling every 20 min up to 2 h after the injection of blocking agents. The blood was sampled at the midpoint of the urine collection period. The room temperature and humidity were maintained during the experiment at about 30 °C and 80%, respectively.

The ureter cannula was equipped as follows. Each animal was anesthetized by the intramuscular injection of azapelon 8 mg/kg (Stresnil; Jensen Co., Ltd., Belgy) followed by inhalation of halothane. The abdominal cavity was opened through a midline incision. After both ureters were exposed at a site several centimeters from the bladder, silicon tubes (2.5 mm $\times$ 4 mm of inner and outer diameter) were inserted and advanced to the ureter cranially 3 or 4 cm. After ligation was in place the tubes were fixed on *Musculus psoas minor* and on *Musculus transversus abdominis*. The distal ends of the 2 cannulae were separately exteriorized through the peritoneum, abdominal musculature and skin and fixed separately on the skin just in front of the groin.

The plasma concentration of free and total SMM was determined by a fluorometric method.¹⁰⁾ The acid hydrolysis of the sample with HCl at 95 °C for 25 min was performed for total SMM determination. The concentration of acetyl derivative (AcSMM) was estimated by subtraction of free SMM concentration from total SMM. The urinary products after SMM administration were determined as follows. McIlvaine buffer solution (pH 5.6, 1 ml) and ethyl acetate (10 ml) were added to diluted urine and

shaken for 5 min. After standing for 30 min, the upper layer (ethyl acetate layer) was analyzed for SMM and AcSMM. The lower layer was analyzed for a water soluble metabolite, after a washing with ethyl acetate. The concentrations of SMM and a water soluble metabolite were determined by a fluorometric method¹⁰⁾ using SMM as the standard.

The concentration of inulin in plasma and in urine was determined by the method of Heyrovsky.¹¹⁾ After deproteinization by a trichloroacetic acid (TCA) solution, 0.1 ml of 3-indoleacetic acid and 4 ml of concentrated HCl were added to the diluted sample (plasma or urine), the mixture was incubated for 70 min at 37 °C and then analyzed spectrophotometrically. All the samples were held at -20 °C until they were analyzed.

The *AUC* value was calculated by the trapezoidal rule. The renal clearance values were calculated by dividing the mean excretion rate of the substance during urine collection period by the correspondent plasma concentration.

Statistical analysis was performed using paired Student's *t* test.

RESULTS

Experiment 1

Effect of Probenecid on Plasma Kinetics of

FIG. 1. *Effect of Probenecid on the Plasma Kinetics after intravenous injection of 10 mg/kg of SMM in Goettingen Miniature Pigs*

■, □, SMM and AcSMM after SMM with probenecid, respectively; ●, ○, SMM and AcSMM after SMM alone, respectively.

Each point and vertical bar represent the mean $\pm$ S.E. ($n=5$).

SMM in Conscious Animals — The time course of plasma SMM and AcSMM concentrations after intravenous injection of SMM and SMM with probenecid is shown in Fig. 1.

The elimination of plasma SMM was slower after SMM with probenecid injection than after SMM alone. A considerable amount of AcSMM was found after SMM with probenecid. The AUC value (0–12 h) after SMM with probenecid was significantly larger when compared with that after SMM alone (Table I).

Experiment 2

Effect of Probenecid and Pyrazinoate on Renal Excretion of SMM and Its Metabolites — The effect of probenecid and pyrazinoate on the urinary pH and flow rate, plasma concentration of SMM and AcSMM, and urinary excretion rate of SMM, AcSMM and a water soluble metabolite was examined in the anesthetized pig (Fig. 2). The urinary pH and flow rate were almost constant during the experimental period. The rate

of urinary excretion of AcSMM and the water soluble metabolite was suppressed by probenecid, but that of SMM was not. That of SMM, AcSMM and the water soluble metabolite were not suppressed by pyrazinoate. An occasional statistical significance was occasionally observed in the urinary flow rate and in the plasma concentration of SMM and AcSMM which may have been due to spontaneous changes.

The effect of probenecid and pyrazinoate on renal clearance of SMM, AcSMM and inulin is shown in Fig. 3. The clearance of AcSMM was suppressed by probenecid, whereas that of SMM and inulin was not. The clearance of AcSMM, SMM and inulin was not suppressed by pyrazinoate.

DISCUSSION

Arita *et al.* demonstrated active renal tubular secretion of sulfisoxazole⁴⁾ and Owada *et al.* of sulfamethazole⁷⁾ in anesthetized rabbits using io-

TABLE I. Effect of Probenecid on AUC ($\mu\text{g} \cdot \text{h}/\text{ml}$)^{a)} of SMM and AcSMM after 10 mg/kg of SMM

Treatment	SMM	AcSMM
SMM	123 ± 23.3	21.0 ± 3.47
SMM with probenecid	192 ± 18.2	58.0 ± 7.34 ^{b)}

Each value represents mean ± S.E. (n=5).

b) Significant difference, $p < 0.05$.

a) AUC from 0 to 12 h after the drug injection.

FIG. 2. Effects of Probenecid and Pyrazinoate on Urinary pH, Flow Rate, Plasma Concentration of SMM and AcSMM, and Urinary Excretion Rate of SMM, AcSMM and Water Soluble Metabolite

●, SMM; ○, AcSMM; □, water soluble metabolite; FL, urinary flow rate (ml/min); PC, plasma concentration ($\mu\text{g}/\text{ml}$); ER, urinary excretion rate ($\mu\text{g}/\text{min}$). Each point and vertical bar represent mean ± S.E. (n=5). a) Significant difference compared with the mean value at time 0 ($p < 0.05$).

FIG. 3. Effects of Probenecid and Pyrazinoate on the Renal Clearance of SMM, AcSMM and Inulin

●, SMM; ○, AcSMM; ■, inulin.

Each point and vertical bar represent mean $\pm$ S.E. ($n=5$). a) Significant difference compared with the mean value at time 0 ($p < 0.05$).

dopyracet. They injected a bolus intravenous iodopyracet on the steady state plasma concentration of the drugs and found a decrease of the renal clearance of the drugs. We also performed similar experiments. The renal excretion of SMM and its metabolites in anesthetized pigs was examined using blocking agents of active tubular secretion. Because probenecid suppressed the renal clearance of AcSMM but not of SMM, we concluded that AcSMM was subject to the active tubular secretion but not SMM. Moreover, the value of the renal clearance of AcSMM was about 3 times larger than that of GFR (inulin clearance). These findings coincided with the theory by Despopoulos *et al.*³⁾ and Vree *et al.*⁹⁾ They demonstrated in man, dog and rabbit that most acetylated compounds of sulfa-drugs were excreted *via* the renal active tubular secretion. On the other hand, the sulfa-drugs which have a pyrimidine ring in the side chain, including SMM, were not.

Pyrazinoate is a potent inhibitor of urate tubular secretion in man,¹²⁾ chimpanzee,¹³⁾ rat,¹⁴⁾ dog and guinea-pig.¹⁵⁾ It did not suppress the active tubular secretion of AcSMM in pigs in the present experiment. This result coincided with the finding of Roch-Ramel *et al.*¹⁶⁾ They described in their paper that both urate and *p*-aminohippurate were secreted by the same transport pathway in pigs, and the pathway was not suppressed by pyrazinoate.

The plasma protein binding of SMM and AcSMM in pigs was both dose-independent and the fraction was not very high (60–70%).²⁾ It is thought that the excretory kinetics of SMM or AcSMM is not affected by the effect of probenecid on the plasma protein binding of SMM or AcSMM.

In a previous paper we reported the nonlinear plasma kinetics with capacity limited elimination of SMM in pigs.¹⁾ The normalized *AUC* of SMM after 100 mg/kg (nonlinear dose) of SMM injection was significantly larger than that after 10 mg/kg (linear dose) of SMM. In the present experiment, we demonstrated the significant increase of *AUC* of SMM by the co-medication of probenecid after a linear dose of SMM. It had been reported that the acetylation of SMM in pigs was not saturated in the nonlinear dose of SMM injection.²⁾ Therefore, this result may suggest that the saturation of renal excretion of AcSMM elicits nonlinear plasma kinetics of SMM.

The possible mechanisms, which increase the *AUC* of the parent compound due to the inhibition of renal excretion of acetylated compound, was then examined in relation to the deacetylative reaction. The deacetylative reaction of *N*⁴-acetylated sulfa-drug had been demonstrated *in vivo* in man,⁹⁾ dog¹⁷⁾ and calf.¹⁸⁾ We observed large amounts of deacetylative compound (SMM) in plasma after AcSMM intravenous injection in pig; the *AUC* ratio of SMM to AcSMM was about 3.¹⁹⁾

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