Disposition of Sulfamethazine and *N*-Acetylsulfamethazine in the Rat

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INTRODUCTION

Quantitative assessment of xenobiotic acetylation can be performed *in vivo* by administration of a substrate which is biotransformed by acetylation with subsequent assessment of partial clearance values. To assess accurately acetylation via this means, however, the acetylated metabolite must be excreted without further biotransformation and quantitatively recovered. Incomplete recovery or subsequent biotransformation of the acetylated metabolite will result in an underestimate of the acetylation rate when using partial clearance methods.

One of the most widely used substrates for assessing acetylation is sulfamethazine (SMZ). In the rabbit and human, SMZ is largely recovered in the urine as unchanged drug and its acetylated metabolite (1,2). Though the rat is a widely used model for assessing xenobiotic disposition, little information is available on the disposition of SMZ in this species. When examining the influence of nongenetic factors on drug acetylation, the rat would appear to be an attractive model since this species displays a monomorphic pattern of acetylation for those substrates studied to date (3). Thus, the objective of the present investigation was to examine the potential of using partial clearance estimates of SMZ in the rat to assess the rate of xenobiotic acetylation *in vivo*.

MATERIALS AND METHODS

Materials. SMZ and sodium SMZ were purchased from Sigma Chemical Company (St. Louis, Mo.). Acetonitrile, ethyl acetate, and methanol were purchased from Curtin–Matheson Scientific and used as received. *N*-Acetylsulfamethazine (NASMZ) was synthesized by incubating 1 g of SMZ in 100 ml of acetic anhydride for 24 hr at room temperature while stirring. The NASMZ was recrystallized from 95% ethanol:water (1:1, v:v). Identification of NASMZ was confirmed by melting-point determination, nuclear magnetic resonance spectroscopy, and high-performance liquid chromatography (HPLC).

Animals. Male Sprague–Dawley rats (200–300 g) were purchased from Charles River and allowed to acclimate for at least 1 week in our animal facility. Animals were allowed water and rat chow ad libitum during this period.

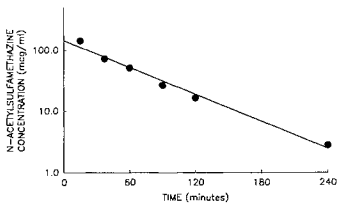

Fig. 1. Plasma concentration versus time profile for NASMZ in a representative rat after intravenous administration of 25 mg/kg.

Pharmacokinetic Studies. Animals had an indwelling cannula implanted in the right jugular vein while under light ether anesthesia 1 day prior to drug administration. On the morning of the study, animals were placed in individual plastic metabolism cages while food and water were withdrawn. Each animal was administered a dose of SMZ (50 mg/kg as the sodium salt in saline) or NASMZ (25 mg/kg dissolved in saline:propylene glycol, 60:40) through the jugular cannula. An aliquot of the dosing solution was stored at −20°C until assayed for drug content. Serial blood samples (0.25 ml) were obtained through the cannula prior to and at timed intervals after drug administration. Urine was collected through 12 or 48 hr and combined with three washes of the metabolism cage using either water or methanol. The combined solutions were diluted to a final volume of 100 ml, an aliquot centrifuged to pellet solid materials (i.e., rodent hair), and an aliquot of the supernate stored at −20°C until analyzed for drug content. SMZ and NASMZ content in plasma and urine were determined using the extraction and HPLC method of Reeves *et al.* (4), with minor modifications. Noncompartmental pharmacokinetic parameters were determined using standard methods (5).

RESULTS AND DISCUSSION

NASMZ concentrations in plasma declined in a monoexponential fashion (Fig. 1). The pharmacokinetic parameters of this compound (Table I) indicate that it is rapidly eliminated in this species (half-life averaged 33 min). As shown in Table II, the recovery of NASMZ was far from complete. This suggests that NASMZ is eliminated by a nonrenal route or is indicative of technical problems in urinary recovery. Since SMZ was not detected in either plasma or

Table I. Pharmacokinetics of NASMZ in the Rat[a]

Parameter	NASMZ[b]
CL (ml/min/kg)	4.36 (1.82)[c]
V_{ss} (ml/kg)	189 (43)
MRT (min)	47 (14)
$t_{1/2}$ (min)	33 (10)

[a] CL, total plasma clearance; V_{ss}, steady-state volume of distribution; MRT, mean residence time; $t_{1/2}$, half-life.
[b] Parameters following intravenous administration of NASMZ 25 mg/kg (n = 6).
[c] Data presented as mean (±SD).

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Table II. Urinary Recovery of NASMZ in the Rat[a]

Subject No.	% of dose recovered in urine as NASMZ	Wash solution
1	61.4	Water
2	51.9	Water
3	39.3	Water
4	40.6	Water
5	16.0	Methanol
6	21.5	Methanol
7	12.6	Methanol

[a] Data for the animals shown in Table I plus urine data from an additional rat whose blood sampling was incomplete due to cannula failure. Urine was collected for 12 hr postdose.

urine after NASMZ administration, it appears that deacetylation is not a significant pathway of elimination.

To ascertain that solubility problems did not contribute to the poor recovery, metabolism cages were washed with water for some animals and methanol for others. As shown in Table II, the recovery after water wash was substantially greater than that after methanol wash.

To examine the possibility that incomplete recovery was due to residual drug remaining on the metabolism cages (i.e., washing did not remove drug which was excreted), we performed a simulation experiment in which the contents of a solution of NASMZ in water were "squirted" onto the metabolism cage by means of a syringe. After allowing the solution retained on the cage to dry, the cage was then rinsed with water in the same manner as cages housing experimental animals. In this simulation, quantitative recovery of NASMZ was observed (data not shown).

Figure 2 illustrates a representative plasma concentration versus time profile for SMZ and NASMZ after SMZ administration. The profiles for SMZ and NASMZ exhibited a spike at approximately 90 min in all but one animal, making assessment of kinetic parameters questionable. In particular, the discontinuity in the plasma concentration–time profile precluded the use of the area ratio method to estimate the fraction of SMZ which is acetylated. It is apparent from the data presented in Table III that the urinary excretion of SMZ and NASMZ account for only a small fraction (9 to 25%) of the drug eliminated in this species.

These results indicate that the acetylated metabolite of SMZ is not quantitatively recovered in urine after administration of SMZ or NASMZ. The incomplete recovery of NASMZ suggests that this compound is either subsequently metabolized in this species or eliminated via another route

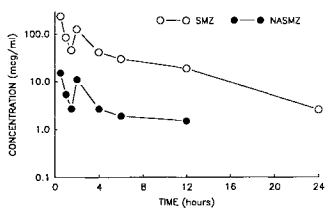

Fig. 2. Plasma concentration versus time profile for SMZ and NASMZ in a representative rat after intravenous administration of SMZ 50 mg/kg.

Table III. Urinary Recovery of SMZ in the Rat[a]

Subject No.	% of dose recovered in urine		
	SMZ	NASMZ	Total
8	12.7	11.8	24.5
9	8.1	1.4	9.5
10	9.5	9.1	18.6
11	11.4	13.2	24.6
12	8.3	5.8	14.2
13	7.9	11.2	19.1

[a] Recovery following intravenous administration of SMZ 50 mg/kg with collection of urine for 48 hr.

(e.g., biliary excretion). The insolubility of many sulfonamides, including NASMZ, indicates that precipitation of the drug in the renal tubules is a possible explanation for the low recovery. However, determination of the renal content of NASMZ in two animals sacrificed after a dose of NASMZ indicated that less than 1% of the dose remained in the kidneys, despite a urinary recovery of only 40% (data not shown).

Our data are in agreement with a recent report by Lindsay and Baty (6), who found that only 13–15% of an oral dose of SMZ administered to male Sprague–Dawley rats was recovered in urine as SMZ and NASMZ. While the poor urinary recovery in their study may have been due to incomplete oral bioavailability of SMZ, the present investigation documents poor recovery after intravenous administration. To our knowledge, the present study is the first reported attempt to quantitatively assess the disposition of NASMZ in the rat. The results indicate that partial clearance values for SMZ to NASMZ cannot be accurately calculated in the rat due to incomplete recovery of the acetylated metabolite. This paradigm is, therefore, an inadequate method of assessing factors affecting drug acetylation.

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