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TOXICOKINETICS OF SULFASALAZINE (SALICYLAZOSULFAPYRIDINE) AND ITS METABOLITES IN B6C3F$_1$ MICE

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ABSTRACT:

The toxicokinetics of salicylazosulfapyridine (SASP) and its metabolites were investigated in male and female B6C3F$_1$ mice either following single intravenous (5 mg/kg) or oral (67.5, 675, 1350, and 2700 mg/kg) doses, or following three consecutive daily oral doses (675, 1350, and 2700 mg/kg). Plasma concentrations of SASP and its metabolites were quantified by HPLC. Upon intravenous administration, SASP rapidly disappeared from blood with a mean residence time of 0.45–0.78 hr. The only metabolite of SASP found in plasma after an intravenous dose was sulfapyridine (SP). In both sexes, the absolute oral bioavailability of SASP ranged between 16.6–18.2% at a dose of 67.5 mg/kg, and between 2.6–8.7% at doses of 675–2700 mg/kg. Following oral administration of SASP, both SP and AcSP were identified in plasma. The area under the plasma concentration-time curves (AUC) of SP at all four oral doses were ~21- to 32-fold or 5- to 25-fold greater than those of SASP in male or female mice, respectively. The acetylated form of SP and AcSP, produced AUC values higher than SASP but much less than SP. Multiple oral doses with SASP did not alter the temporal patterns of SASP absorption and elimination in comparison to a single dose. However, SP accumulated in both sexes following multiple oral doses. A gender-dependent difference in toxicokinetic profiles for SASP and SP was also observed. Female mice displayed a higher C_{max} of SASP and SP than did male mice. Although the volume of distribution of SASP was similar in both sexes, the systemic clearance of SASP in males was about twice that observed in females. The results indicated that after SASP administration, the metabolites SP and AcSP displayed higher and more prolonged plasma concentration-time profiles than parent SASP. Therefore, SP may accumulate in the body following repeated dosing and contribute to the genotoxicity observed following administration of high doses of SASP.

SASP[1] is the drug most widely prescribed for the treatment of inflammatory bowel diseases, such as ulcerative colitis, mild cases of regional enteritis, and granulomatous colitis (1–3). The drug is also being used as an alternative approach to treatment of rheumatoid arthritis (4–6). SASP is extensively metabolized by reductive cleavage of the azo-linkage in the gut to SP and 5-ASA (3). SP is well-absorbed and eventually excreted in the urine, but 5-ASA is poorly absorbed and mainly excreted in the feces (3). Although there has been extensive studies of the pharmacokinetics of SASP in humans, including patients with inflammatory bowel disease or rheumatoid arthritis (7–12), there have been few toxicokinetic studies performed in laboratory animals (13). The lack of such data limits the interpretation of the results of a variety of toxicological studies conducted in those species.

The possibility that SASP may induce DNA damage and chromosome aberrations in humans was indicated by cytogenetic studies of patients undergoing SASP therapy for inflammatory bowel diseases (14, 15, 17). In these studies, peripheral blood lymphocytes from SASP-treated individuals exhibited an increase in frequencies of both SCE and MN, compared with healthy, untreated controls (14, 15). Recent *in vivo* studies in B6C3F$_1$ mice indicate that SASP significantly increases the frequencies of MN observed in erythrocytes obtained from peripheral blood or bone marrow (16). Through *in vitro* studies, it has been demonstrated that SASP itself can induce both SCE and MN. However, its metabolites, SP and acetylated SP, induce only SCE (17). It has been suggested that the *in vivo* metabolite responsible for SASP cytotoxicity is SASP hydroxylamine, which may account for these *in vivo vs. in vitro* differences (18).

Knowing the temporal patterns of the parent compound and its metabolites in the blood would help better define the mechanism(s) by which SASP and/or its metabolites induce mutagenic damage. However, there have been no toxicokinetic studies or identification of plasma metabolites in B6C3F$_1$ mice, the animal model in which the extensive mutagenicity test data were obtained. Therefore, in this study, we have investigated the toxicokinetics of SASP and its metabolites following intravenous and oral administration of SASP to B6C3F$_1$ mice. An important aspect of this study was determination of the time-dependency of the plasma concentrations of SASP and its metabolites at doses associated with the genotoxicity endpoint as studied by the National Toxicology Program (16).

Materials and Methods

Chemicals. Chemicals were obtained from the following sources: SASP (2-hydroxy-5-[[4-[(2-pyridyl-amino)sulfonyl]phenyl]azo]benzoic acid, CAS 599-79-1) from NTP Chemical Repository, Radian Corporation (Research Triangle Park, NC); SP from Sigma Chemical Company (St.

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[1] Abbreviations used are: SASP, salicylazosulfapyridine; SP, sulfapyridine; 5-ASA, 5-aminosalicylic acid; SCE, sister-chromatid exchange; MN, micronuclei; SPOH, 5-hydroxy-sulfapyridine; AcSPOH, *N*-acetyl-5-hydroxy-sulfapyridine; AcSP, *N*-acetylsulfapyridine; C_{max}, maximum plasma concentration; T_{max}, time at which C_{max} is achieved; K, first-order disposition rate constant; AUC, area under the plasma concentration-time curve; AUMC, total area under the first-moment curve; MRT, mean residence time; MAT, mean absorption (input) time; CL_s, total body clearance; F, absolute oral bioavailability; V_β apparent volume of distribution.

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Louis, MO); SPOH and AcSPOH from Kabi Pharmacia Therapeutics (Uppsala, Sweden); sodium phosphate (dibasic or monobasic) and methanol from Fisher Scientific Company (Fair Lawn, NJ). AcSP standard was synthesized by acetylation of SP with acetic anhydride and purified by recrystallization (19). The structure was confirmed by mass spectral analysis. All reagents used in this experiment were of analytical grade, HPLC grade, or the best available pharmaceutical grade.

Animals. Male and female B6C3F$_1$ mice (24 ± 3 g) were supplied by the National Institute of Environmental Health Sciences (Research Triangle Park, NC). At the time of use the mice were 8–10 weeks old. Upon arrival at the University of Arizona, the mice were kept in a temperature-controlled, 12-hr light/dark cycle facility, and acclimated for 1 week prior to experimentation. They were allowed tap water and food (Teklad mouse diet) *ad libitum*. Animals were fasted for 12 hr prior to administration of SASP.

Administration of SASP. SASP in corn oil was administered orally by gavage to groups of mice at doses of 67.5, 675, 1,350 and 2,700 mg/kg. For the multiple-dose study, mice were given daily oral doses (675, 1,350, and 2,700 mg/kg) at the same time of the day for three consecutive days. In an intravenous dose study, SASP was dissolved in distilled-deionized water (pH 9–10, adjusted with 5 N NaOH) and administered *via* the tail vein at a dose of 5.0 mg/kg (0.2–0.3 ml/mouse) over ~5–10 sec.

Collection of Blood Samples. At the appropriate times, the mice were subjected to euthanasia with CO_2. Blood samples were collected from the inferior vena cava into heparinized syringes prior to (0 hr) and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hr following intravenous SASP administration and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hr following oral administration. The experimental design allowed for 4–7 mice at each time. The blood was centrifuged at 5,000*g* for 5 min, and the plasma was transferred to an Eppendorf tube and stored at −20°C until analyzed. The samples were usually processed within 3 days.

Sample Analysis. The plasma samples (0.15 ml) were mixed with 2 volumes of methanol and allowed to stand in an ice bath for 15 min to precipitate proteins. After centrifugation at 13,000*g* at 4°C for 15 min, an aliquot (20 μl) of the supernatant was analyzed by HPLC. SASP and its metabolites were separated and quantified on a 25 cm × 4.6 mm (i.d.) reversed-phase column (Ultracarb, C_{18}, 7 μm) purchased from Phenomenex (Torrance, CA). Mobile system 1, used for SASP analysis, consisted of methanol and 25 mM (pH 2.5) phosphate buffer in a ratio of 64:36 (v:v) (20). Separations were performed at room temperature at a rate of 1 ml/min. Mobile system 2, used for analysis of SP, AcSP, SPOH, and AcSPOH, was composed of methanol and 20 mM (pH 7.0) phosphate buffer (20:80, v:v). The flow rate was 2 ml/min. A Spectra-Physics model 100 UV detector was used for detection of SASP and its metabolites. Wavelengths of 360 and 254 nm were used for detection of SASP and the metabolites, respectively. A typical HPLC chromatogram is illustrated in fig. 1. The detection limit in plasma for SASP was 0.32 nmol/ml; for SP, 0.5 nmol/ml; and for AcSP, 1.0 nmol/ml. For all compounds at 50 nmol/ml of plasma, the coefficient of variation was less than ±5% for intraday analyses and less than ±10% for interday analyses.

Toxicokinetic Analysis. Plasma concentration-time data were analyzed by noncompartmental methods. Values of C_{max} and T_{max} were obtained directly from plasma concentration-time profiles. The apparent K was estimated by linear least squares regression of the data in the terminal phase. From these values, the half-lives were calculated ($t_{½} = 0.693/K$). AUC and AUMC were calculated using the linear trapezoidal rule and extrapolating to time infinity. For multiple doses, the steady-state AUC (0–24 hr) was used. MRT and MAT were calculated as follows:

$$MRT_{SASP,iv} = \left[\frac{AUMC}{AUC}\right]_{iv} \quad (1)$$

$$MAT_{SASP,po} = \left[\frac{AUMC}{AUC}\right]_{SASP,po} - MRT_{SASP,iv} \quad (2)$$

$$MRT_{SP,iv} = \left[\frac{AUMC}{AUC}\right]_{SP,\ from\ iv\ SASP} - \left[\frac{AUMC}{AUC}\right]_{SASP,iv}. \quad (3)$$

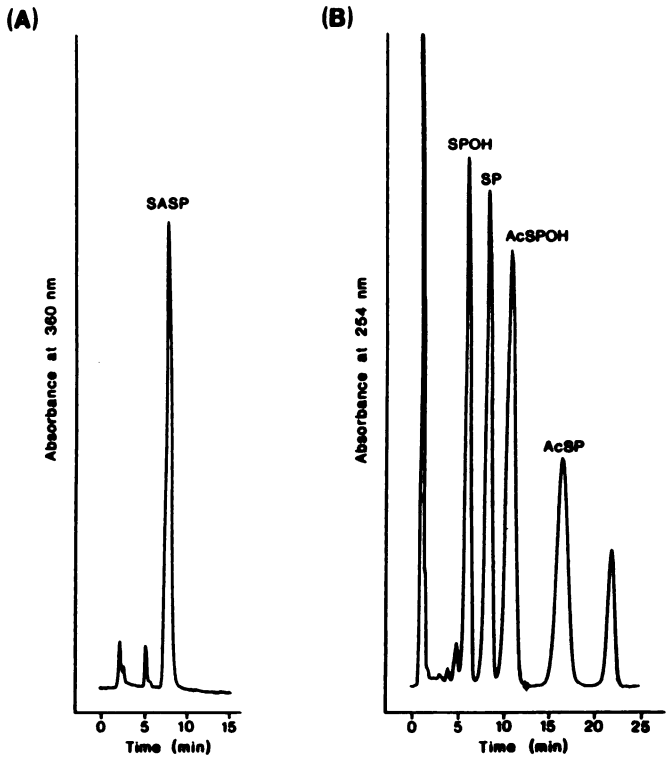

FIG. 1. *HPLC chromatogram of SASP and its metabolites in B6C3F$_1$ mouse plasma.*

(*A*) HPLC system 1: 25 mM (pH 2.5) phosphate buffer:methanol (36:64), λ_{360nm}; SASP, 20 μM. (*B*) HPLC system 2: 20 mM (pH 7.0) phosphate buffer:methanol (80:20), λ_{250nm}; SPOH, 100 μM, SP, 100 μM, AcSPOH, 100 μM, and AcSP, 100 μM.

Equation 3 provides an estimate of the MRT of SP that is formed following the administration of its precursor, SASP (21). The systemic CL_s was computed by dividing the intravenous dose by AUC. F for SASP was estimated from the ratio of AUC_{po}/AUC_{iv} and corrected for differences in doses. V_β was calculated from the following relationship,

$$V_\beta = \frac{CL_s}{K}. \quad (4)$$

All data are presented as mean ± SE. Statistical analysis for comparison of two means was performed using unpaired Student's t test.

Results

Intravenous Dose. After an intravenous bolus injection, the plasma concentration-time profiles of SASP in both male and female mice were similar and the data, although limited, could be described by a multiexponential equation (fig. 2). SASP was rapidly eliminated from the plasma with an elimination $t_{½}$ of 0.5 hr in males and 1.2 hr in females. The male mice also had a greater CL_s in comparison with the females (table 1). By 6 hr, no detectable concentrations of parent SASP were found in plasma of mice of either sex (fig. 2). Following intravenous administration of SASP, its metabolite, SP, appeared in plasma and reached a C_{max} at 3 hr (fig. 2). SP was eliminated from plasma more slowly than parent SASP in both sexes ($t_{½}$ = 2–5 hr). The AUC of SP was ~2- to 4-fold higher than that of SASP (table 1). Neither AcSP nor hydroxylated SP metabolites were detected in plasma after intravenous administration of SASP.

Single Oral Dose. Following single oral doses of SASP, detectable concentrations of parent SASP appeared rapidly in plasma at all four doses (fig. 3, 675 mg/kg dose illustrated). The C_{max}

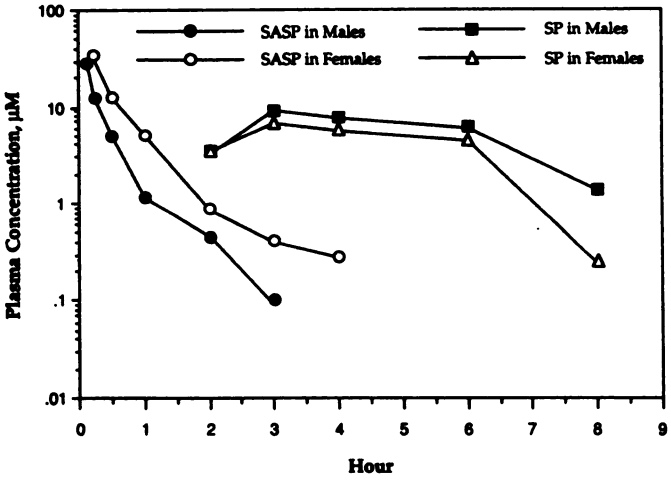

FIG. 2. *Plasma concentration-time profiles of SASP and its major metabolite, SP, following intravenous administration of SASP (5.0 mg/kg).*

Each *point* represents the mean of 4–6 animals.

values were achieved within 0.5–2 hr of dosing. Thereafter, the concentrations declined rapidly with an elimination $t_{1/2}$ of 0.9–2.3 hr in both sexes (table 2). *F* for SASP was 16.6–18.2% in both sexes at a dose of 67.5 mg/kg. At doses of 675, 1,350, and 2,700 mg/kg, *F* varied between 2.6–7.8% in males and 6.7–8.7% in females (table 2). At these three higher doses, *F* tended to decrease with an increase in the dose of SASP (table 2, correlation coefficient, −0.982). Determinations of *F* and MAT were made with reference to the single intravenous dose experiment that used only one dose (5 mg/kg, iv).

After oral administration of SASP, SP and AcSP were identified in mouse plasma at all four doses (table 2, fig. 3). Both SP and AcSP showed a delayed appearance in plasma (T_{max} occurred ~3–7 hr after the T_{max} for SASP). SP was present in much higher concentrations (highest C_{max} and AUC). In male mice, the AUC of SP was 21- to 32-fold greater than that of SASP at corresponding doses (table 2). However, the AUC of SP did not increase with increasing doses of SASP at doses exceeding 675 mg/kg. AcSP, an acetylated metabolite of SP, had AUC values 1.9- to 2.5-fold higher than those of SASP, but 10- to 13-fold lower than those of SP in male mice (table 2). The apparent *K*s of SP or AcSP were 2.5- to 6.4-fold lower than those of SASP (table 2). These results indicate that the metabolites were eliminated more slowly than the parent compound (fig. 3). The toxicokinetic properties of SASP, SP, and AcSP in female mice were similar to those obtained in males. It is clear that after a single oral dose of SASP, the metabolites, SP and AcSP, achieved greater plasma concentrations that were more prolonged than SASP. It is interesting to note that the AUC values of SASP and SP in females, in general, were consistently higher than those seen in males at all four doses (table 2).

Multiple Oral Doses. In the multiple-dose study, animals received daily doses of SASP for three consecutive days. As in the single-dose studies, SASP, SP, and AcSP were found in plasma of both sexes. Multiple dosing in male or female mice did not cause any change in the plasma concentration-time patterns of SASP relative to that seen after a single oral dose. Nor did multiple dosing (at any dose) alter significantly the values of SASP kinetic parameters such as C_{max}, T_{max}, $t_{1/2}$, AUC, MAT, and *F*.

Multiple dosing with SASP, however, did have a significant impact on the toxicokinetics of SP. For example, at a dose of 1,350 mg/kg, multiple-dose treatment significantly increased the plasma C_{max} of SP in male mice as compared with the value achieved after a single oral dose (fig. 4*A*). Similar increases in the plasma C_{max} of SP following multiple dosing were also observed at the other dose levels (675 and 2,700 mg/kg, data not shown), as well as in female mice (fig. 4*A*). The AUC values of SP were in general greater in the multiple-dose study than in the single-dose study (fig. 4*B*), although the data did not allow for statistical comparison. Multiple-dose treatment of SASP with all three doses did not increase the plasma C_{max} and AUC of AcSP compared with the corresponding single doses, although an increase in those parameters of its precursor, SP, was observed in these same animals (fig. 4). The other potential hydroxylated and/or acetylated metabolite(s) of SP were not detected in plasma of either male or female mice following multiple dosing of SASP.

Gender Differences. A gender-related difference in the toxicokinetic properties of SASP, as well as its metabolite, SP, was observed in this study with both single and multiple doses at all dose levels investigated (figs. 4 and 5). For example, female mice had a higher C_{max} of SASP and SP than did male mice when both groups of animals were administered orally 2,700 mg/kg of SASP daily for 3 days (fig. 5). Further analysis of the MAT in both sexes revealed that the MAT in males (4.4 hr) was similar to that in females (3.5 hr), following repeated dosing of 2,700 mg/kg of SASP. Therefore, the absorption of SASP by both sexes seemed to occur at a similar rate. Because the female mice had a smaller CL_s (table 1), the higher C_{max} and AUC of SASP in female mice might be due to the slower elimination of SASP in this sex. Although the plasma AUC values of SP in female mice were higher than those obtained in males (table 2, fig. 4), the AUC of AcSP in both sexes was approximately the same in either single- or multiple-dose studies at all three doses (table 2, fig. 4).

TABLE 1

Pharmacokinetic parameters of SASP and SP in B6C3F$_1$ mice following intravenous administration of SASP

	MRT	$t_{1/2}$	*K*	AUC	CL_s	V_β
	hr	*hr*	hr^{-1}	$\mu M \cdot hr^{-1}$	$liters/hr \cdot kg^{-1}$	*liters/kg*
Male						
SASP	0.45	0.54	1.278	9.21	1.36	1.07
SP	4.60	1.90	0.364	42.20		
Female						
SASP	0.78	1.19	0.581	21.39	0.59	1.01
SP	8.67	4.80	0.144	52.65		

Mice (22 ± 2 g) were administered an intravenous bolus dose of SASP (5.0 mg/kg). Parameters were computed from AUCs, where each point represents the mean of 4–6 animals.

TABLE 2

Pharmacokinetic parameters of SASP, SP, and AcSP following single oral doses of SASP in B6C3F$_1$ mice

SASP Dose	Compound Analyzed	$t_{1/2}$	K	AUC	MAT	F
mg/kg		*hr*	*hr^{-1}*	*μM·hr^{-1}*	*hr*	*%*
			Male			
67.5	SASP	0.9	0.802	21	0.43	16.61
	SP	2.2	0.318	430		
	AcSP	1.6	0.440	44		
675	SASP	1.7	0.402	97	1.95	7.82
	SP	11.1	0.063	3134		
	AcSP	5.8	0.119	239		
1,350	SASP	2.3	0.304	135	2.79	5.44
	SP	7.4	0.094	2995		
	AcSP	6.2	0.112	260		
2,700	SASP	1.8	0.379	127	2.40	2.56
	SP	7.3	0.094	2811		
	AcSP	10.1	0.068	271		
			Female			
67.5	SASP	1.3	0.520	52	0.73	18.16
	SP	3.3	0.210	540		
	AcSP	3.4	0.206	17		
675	SASP	1.0	0.672	250	0.72	8.66
	SP	7.9	0.088	6261		
	AcSP	10.2	0.068	268		
1,350	SASP	1.4	0.483	385	1.20	6.67
	SP	8.3	0.084	3464		
	AcSP	8.7	0.080	234		
2,700	SASP	1.7	0.401	845	2.03	7.32
	SP	9.8	0.071	4082		
	AcSP	8.3	0.083	224		

Mice (5–7/time point) received SASP in corn oil by oral gavage.

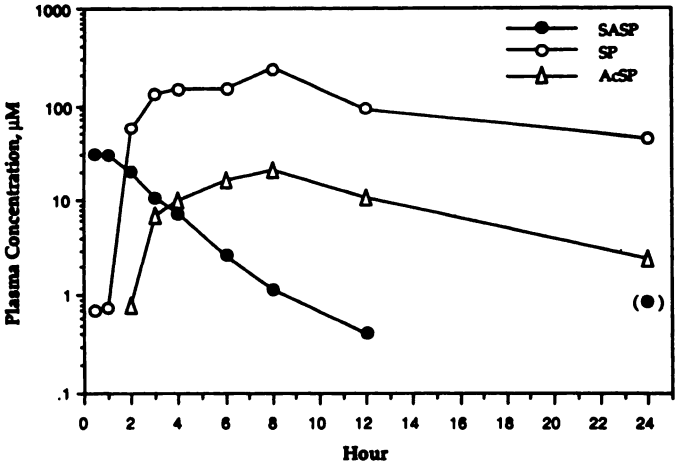

FIG. 3. *Concentration-time profiles of SASP and its major metabolites, SP and AcSP, in plasma of male mice, following a single oral dose of SASP (675 mg/kg by gavage in corn oil).*

Each *point* represents the mean of 5–7 animals. Value in parentheses was not used in data analysis.

Discussion

SASP-induced genotoxicities have been observed in both humans and experimental animals (14–17). Although SP is identified (22) and thus suggested as a major metabolite pertinent to the genotoxicity in humans with SASP therapy (14, 15), no information is available on metabolism and toxicokinetics of SASP and its metabolite(s) in B6C3F$_1$ mice. Therefore, in an attempt to interpret further the mechanism by which SASP induces mutagenic damage, the toxicokinetics and metabolism of SASP were studied in male and female B6C3F$_1$ mice.

Following intravenous bolus administration of SASP to mice, the plasma concentrations of SASP declined rapidly in both sexes (table 1). SP was a major metabolite in mouse plasma (fig. 2). The work conducted by Zapp *et al.* (25) indicates that the liver does not reduce SASP to a great extent after SASP enters the systemic circulation. Hence, the appearance of SP in mouse plasma following an intravenous dose of SASP suggests that the parent SASP must undergo substantial biliary excretion prior to gastrointestinal azo reduction and subsequent absorption of SP. The delayed appearance of SP in the plasma supports this conclusion.

F for SASP following a single oral dose of 67.5 mg/kg varied between 15–18% in both sexes. These values fall in the range of those obtained in humans (F = 10–20% of the oral dose) who received therapeutic doses (2–4 g/day) of SASP [*i.e.* 40–70 mg/kg of body weight/day (2, 3, 6)]. In contrast, at doses that exceed therapeutic doses, F for SASP appears to decrease (table 2). This dose-related decrease of F probably reflects the very limited aqueous solubility of SASP in the gastrointestinal fluids and the subsequent decrease in absorption efficiency with increasing dose. Hence, the lower F for SASP in mice could be attributed largely to the limited solubility of the compound at the higher doses.

After a single oral dose of SASP, two major metabolites (SP and AcSP) were identified in mouse plasma of both sexes (fig. 3), which is consistent with results from humans (7, 8) and rats (14). The toxicokinetic profile of SP indicates the following characteristics: (*1*) a delayed T_{max} (*i.e.* C_{max} appeared 4–8 hr after

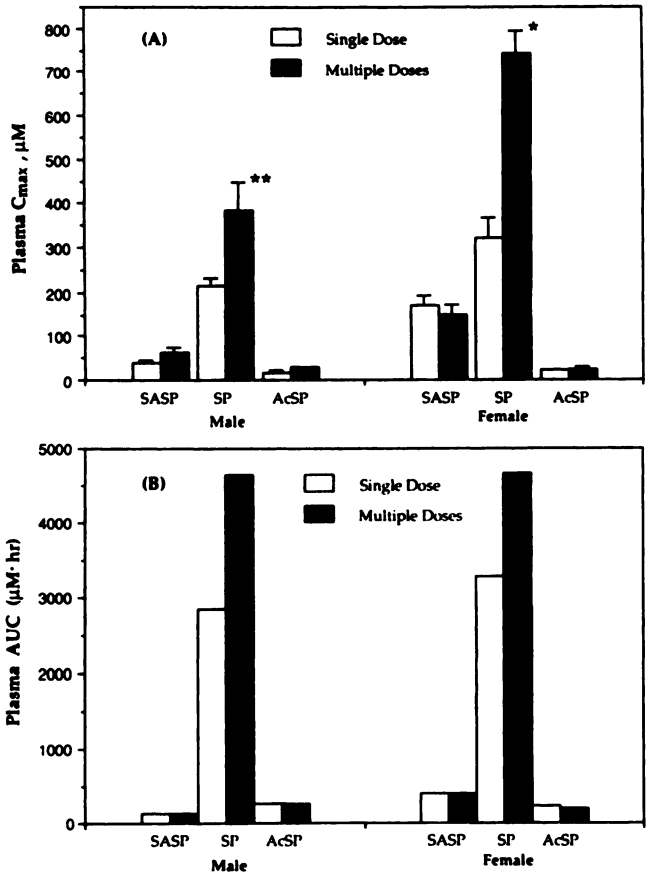

FIG. 4. *Effect of multiple oral dosing with SASP. on the plasma C_{max} and AUC of SASP and its major metabolites in mice.*

Mice (both sexes) received either a single oral dose or three daily consecutive doses of SASP at the dose of 1,350 mg/kg. $^{*}p < 0.001$; $^{**}p < 0.05$, $N = 4$–6.

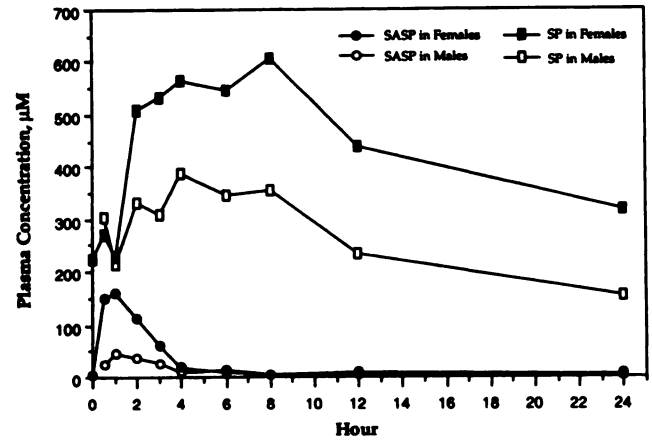

FIG. 5. *Plasma concentration-time profiles of SASP and SP in male and female mice who received oral doses of SASP (2,700 mg/kg) once daily for 3 days.*

Plasma samples were obtained at the indicated times after the last dose. Each *point* represents the mean of 5–7 animals.

an oral dose of SASP) (fig. 3); (*2*) ratios much greater than 1 for AUC_{SP} to AUC_{SASP} and AUC_{SP} to AUC_{AcSP} (table 2); (*3*) a longer elimination $t_{½}$ than parent SASP (figs. 2, 3, and 5). Oral administration of SP to male B6C3F$_1$ mice revealed that SP is rapidly absorbed into the systemic circulation with a T_{max} of 1 hr and an F of 75% (26). In healthy human volunteers, SP is detected in the blood 3–5 hr after ingestion of SASP, a time consistent with the transit time of the parent drug to sites of absorption (colon) and where azo bond reduction takes place (22). Therefore, the delayed appearance of SP in B6C3F$_1$ mice following oral administration of SASP is probably related to the movement of the parent compound down the gastrointestinal tract. The appearance of SP in blood could also be affected by, among other factors, the capacity of intestinal bacteria to reduce the azo linkage and the further metabolism of SP.

In the present study, SP had the highest C_{max} and AUC of the compounds measured. The substantially higher plasma concentrations of SP compared with those of SASP following SASP oral dosing has been reported for humans and other experimental animals (1, 2, 8). Because the elimination rate constant of SP in mice was smaller than that of SASP (table 2) and because SP is better absorbed from the gut than SASP (26), it is reasonable to conclude that the higher plasma concentrations of SP in B6C3F$_1$ mice after a single oral dose of SASP is due to the more efficient absorption and slower clearance of SP in both sexes compared with those processes for SASP.

AcSP appeared in the plasma at times later than SASP. The AUC values of AcSP were much lower than those of SP. This low ratio of AcSP *vs.* SP might suggest a slow rate of acetylation. The polymorphic acetylation of SP has been verified in humans (22, 27, 28). In those who are classified as slow acetylators, the SP plasma concentrations are significantly higher than those of fast acetylators. The reverse is also true for AcSP; the fast acetylators have higher plasma concentrations of AcSP than do the slow acetylators (29, 30). A study conducted by Astbury and Taggart (29) indicated that the steady-state serum concentrations of unchanged SP were about twice that of AcSP in human subjects with a slow acetylator status. It has also been reported that the incidence of side effects to SASP treatment is higher in slow acetylators than in fast acetylators (30–32). Results of the present study in both male and female mice following a single oral dose of SASP at three doses indicated that the AUC values of SP were ~10- to 23-fold greater than those of AcSP (fig. 4, table 2). It is possible, therefore, that B6C3F$_1$ mice might acetylate SP at a slower rate than humans. Other factors, however, must also be considered when comparing metabolite concentrations such as metabolite elimination clearance.

Repeated dosing of mice with SASP for 3 days did not greatly change the plasma concentration-time profiles of SASP and AcSP in comparison to the single-dose profile. Because SASP was rapidly eliminated from the plasma (table 2, fig. 5), the lack of effect of multiple dosing on the plasma profile of SASP was to be expected and suggests that SASP will not accumulate in the body. In contrast, multiple dosing did affect the SP plasma concentrations. For compounds that accumulate in the body, it is possible to achieve a higher plasma C_{max} following repeated dosing than following single dosing. In the current study, the plasma C_{max} of SP after multiple dosing was significantly higher than that after a single dose (fig. 4). Therefore, following multiple dosing with SASP, SP would tend to accumulate in the body.

A distinct and significant gender-dependent difference in the toxicokinetic profiles of both SASP and SP was also observed in the current study. Female mice had higher plasma concentrations of SASP and SP than did the males (fig. 5). Although MATs in males were similar to those in females following oral dosing of SASP (table 2), the CL_s of SASP in males was about twice that in females (table 1). Hence, a slower clearance of SASP in females

may account for the higher plasma SASP concentrations in this sex. Because we did not observe any difference in AcSP plasma profiles between the two sexes, the higher SP plasma concentrations in females does not seem to be related to a difference in the rate of acetylation of SP.

SP has been regarded as a candidate responsible for the toxicities associated with SASP therapy. Examples of these toxicities include bone marrow depression and anemia (3, 28). Strong evidence indicates that the increases in the frequency of SCE and MN in patients taking SASP for inflammatory bowel disease are caused by the drug *per se* and not by the disease (14, 15). The dose of SASP and the length of treatment period determine, to a large extent, the magnitude of these indicators of genotoxicity. In addition, patients of slow acetylator status appear to have a greater SCE than fast acetylators (14, 15). Taken together, these results from human studies suggest that the chromosome-damaging effects of SASP therapy may be partially mediated through SP and/or some other metabolites. Although the exact molecular form(s) responsible for the genotoxicity caused by SASP treatment is still unknown at present, the high SP plasma concentrations resulting from SASP administration in B6C3F$_1$ mice, supports the assumption that SP and/or its metabolite(s) may be related to the mutagenicity observed in B6C3F$_1$ mice and in humans as well.

In conclusion, SASP was rapidly but poorly absorbed after oral dosing. The percentage of the dose absorbed was <10% at doses of 675 mg/kg and above in both sexes. Following oral administration of SASP, SP and AcSP were identified in mouse plasma. The efficient absorption and slow elimination of SP by both sexes contribute to its higher plasma concentration-time profiles. The elimination of the parent SASP appears gender-dependent. The low plasma concentrations of AcSP and a large ratio of AUC_{SP} to AUC_{AcSP} may suggest a slow acetylation of SP by B6C3F$_1$ mice.

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