Bioavailability of Aminosalicylic Acid and Its Various Salts in Humans IV: Comparison of Four Brands of the Sodium Salt

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Abstract □ The relative bioavailability of four commercially available brands of the sodium salt of aminosalicylic acid was investigated in a crossover study with healthy volunteers. The estimation of bioavailability was based on comparison of areas under the plasma drug concentration–time curves and on cumulative excretion of aminosalicylate as well as its metabolites in urine, following a single dose of the drug. The total absorption of all products was similar, 86–90% of the dose being recovered in the urine during 24 hr. The fraction of dose available as the biologically active form of nonmetabolized aminosalicylate was significantly higher for one product. This difference, however, was probably due to the higher dose of this brand studied compared to the other brands (4.0 and 2.9 g as the free acid, respectively), since the main metabolic route, conjugation to *N*-acetyl-*p*-aminosalicylic acid, is known to be dose dependent and capacity limited. There were no significant differences in the bioavailability of the other three brands studied.

Keyphrases □ Sodium aminosalicylate tablets—bioavailability of four commercially available products compared □ Aminosalicylic acid, sodium salt, tablets—bioavailability of four commercially available products compared □ Bioavailability—comparison of four different commercially available sodium aminosalicylate tablets

Although its use is decreasing, aminosalicylic acid is still widely used in the treatment of tuberculosis as a supplement to other tuberculostatic agents. This drug is used in several chemical forms and pharmaceutical formulations developed to reduce its GI side effects. Blood levels of aminosalicylic acid obtained with different products vary considerably (1–4). The sodium salt is one of the most commonly used forms since it is relatively well tolerated and gives higher blood levels of aminosalicylate than other forms of the compound (5, 6). Although several brands of the sodium salt are commercially available, no reported studies have systematically compared the bioavailability of these products. The recent findings of inequivalencies between commercially available products of several drugs have emphasized the importance of evaluation of bioavailability of chemically equivalent products.

Several factors complicate the bioavailability studies of aminosalicylic acid. The interpatient variation in GI absorption of the compound is large (4), necessitating the use of relatively large groups of subjects. Crossover design and statistical methods help distinguish the differences in products from other variables. Since aminosalicylic acid is largely inactivated by metabolism, in part during the first pass, the measurement of unchanged drug only gives information about the fraction that is available in biologically active form but does not necessarily reflect total absorption. The latter can be better estimated by measuring the excretion of both unchanged drug and its metabolites in urine. In addition, the nonlinear pharmacokinetics of aminosalicylic acid (7) complicate the interpretation of results of previous absorption studies.

The bioavailability of four commonly used brands of the sodium salt was compared by measuring both unchanged drug and its main metabolite in plasma and urine in this crossover study in healthy individuals.

MATERIALS AND METHODS

Products—Four brands of sodium aminosalicylate from different manufacturers were tested: Product A[1], 0.5 g; Product B[2], 0.5 g; Product C[3], 0.5 g; and Product D[4], 0.57 g. All products were purchased by the Food and Drug Administration and met USP specifications for purity and content uniformity[5].

Subjects—Twelve healthy volunteers between 20 and 44 years of age, 11 men and one woman, weighing between 62.7 and 88.6 kg, participated. Before the study, in addition to written informed consent, a history, physical examination, and laboratory tests (complete blood count, serum alkaline phosphatase, serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, serum creatinine, and urinalysis) were obtained. The results of these examinations were within normal limits for all volunteers.

Drug Administration—Each subject received a single dose of 4 g of the sodium salt (equivalent to 2.9 g of free acid) of Products A, B, and C and a dose of 6.8 g (equivalent to 4 g of free acid) of Product D in a random order on four separate occasions. These doses were dictated by the potency of the different products. The drug was administered as eight tablets with 250 ml of water at 8 am after an overnight fast. Fasting was continued for the first 5 hr after drug administration. The use of other drugs was not allowed for at least 2 weeks and alcohol was not allowed for at least 3 days prior to as well as during the study. One week elapsed between the study of each product in each volunteer.

Sampling—Venous blood samples were drawn in tubes containing sodium heparin through a Teflon cannula inserted in an antecubital vein just before drug administration and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, 8, and 10 hr thereafter. Urine was collected prior to ingestion of the drug and then continuously in 2-hr periods for 10 hr and finally from 10 to 24 hr. Plasma was separated by centrifugation, and the plasma and aliquots of each urine collection were kept frozen until analyzed.

Analysis of Aminosalicylic Acid—A modified Marshall method was used for the assay of aminosalicylic acid and *N*-acetyl-*p*-aminosalicylic acid as described earlier (7). With this modification, drug concentrations as low as 1 μg/ml could be measured accurately.

Analysis of Results—The plasma concentrations of aminosalicylic acid were corrected to a 70-kg body weight. In addition, a correction to a 2.9-g dose was made for Product D. Areas under the plasma drug concentration–time curve *(AUC)* were calculated by the trapezoidal rule. The half-lives of elimination of aminosalicylic acid and the acetyl conjugate were calculated[6] from a one-com-

[1] Pamisyl Sodium, Lot MB501, Parke, Davis & Co., Detroit, Mich.
[2] Parasal Sodium, Lot 73204, Panray Division, Englewood, N.Y.
[3] Pasdium, Lot 3727121, Kasar Laboratories, Niles, Ill.
[4] PAS Sodium, Lot X8646, Hellwig, Inc., Chicago, Ill.
[5] Analyses performed by FDA.
[6] Using the BMDX 85 program on an IBM 1130 computer.

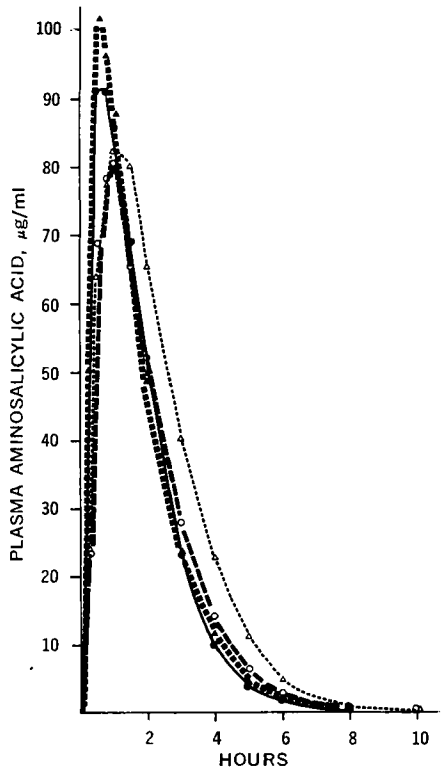

Figure 1—*Mean plasma concentration of aminosalicylic acid from 12 subjects following administration of four different brands of sodium aminosalicylate. Data corrected to 70 kg body weight and to a dose equivalent to 2.9 g aminosalicylic acid. Key: ●, Product A; ○, Product B; ▲, Product C; and △, Product D.*

partment open model. The cumulative excretion of total drug, non-acetylated drug, and acetylated drug in urine was calculated as a percent of the dose. The *AUC's*, the peak plasma concentrations of aminosalicylic acid independent of sampling time, the times to reach the peak level, and the urine data were analyzed by analysis of variance for crossover experiments. This method permitted the fractioning of the total variance into variance between the products, individuals, and periods or sequence of administration. Whenever significant differences ($p < 0.05$) between the products were found, Duncan's multiple-range test was applied to find the source of the difference (9).

RESULTS

Analysis of Plasma Data—The absorption of aminosalicylate from all products studied was rapid, as reflected by the relatively short time to attain the peak plasma drug level (Fig. 1 and Table I). The peak level after Product D was reached markedly later than that with the other products.

The peak plasma level attained following administration of Product C was significantly higher than that following Product B or D. Also, the peak level with Product A was significantly higher than with Product D, whereas no statistically significant difference was observed between Products B and D (Table I).

Table I—Peak Plasma Concentration of Aminosalicylic Acid and Time to Reach Peak Concentration

Product	Peak Concentration, μg/ml[a,b]	Peak Time, hr[a]
A	100.0 ± 6.0[c]	0.69 ± 0.06
B	89.0 ± 6.6	0.81 ± 0.09
C	106.6 ± 6.6[d]	0.65 ± 0.06
D	84.8 ± 7.3	1.19 ± 0.10

[a] Values are mean ± standard error ($n = 12$). [b] Results are corrected to 70 kg body weight and to a dose equivalent to 2.9 g free acid. [c] Significantly higher than Product D. [d] Significantly higher than Product B or D.

Table II—Areas under the Aminosalicylic Acid Plasma Concentration–Time Curves (*AUC*) and Their Statistical Analysis following Administration of Four Brands of Sodium Aminosalicylate

Product	*AUC*, μg hr ml^{-1}, mean ± *SE*
A	199.5 ± 11.2
B	199.9 ± 12.1
C	207.1 ± 9.5
D	245.4 ± 10.7

Analysis of Variance

Source of Variation	*df*	*ss*	*ms*	*F*
Between individuals	11	31707	2882	3.30
Between products	3	17113	57504	6.54
Between periods	3	5218	1739	1.99
Error	30	26181	872	
Total	47	80220		

SE of varietal mean = 8.528, $F_{0.05}$ (3.30) = 2.92, $F_{0.05}$ (11.30) = 2.12

Multiple-Range Analysis

	p	(2)	(3)	(4)
Shortest significant ranges		24.6	25.9	26.6
Product	A	B	C	D
AUC[a]	199.5	199.9	207.1	245.4

D − A = 45.9 > 26.6; significant
D − B = 45.5 > 25.9; significant
D − C = 38.0 > 24.6; significant
C − A = 7.6 < 25.9; not significant
C − B = 7.2 < 24.6; not significant
B − A = 0.4 < 24.6; not significant

[a] Any two or more means not underscored by the same line are significantly different.

The corrected *AUC* of aminosalicylic acid was significantly larger for Product D than for the other products (Table II). No statistically significant difference was found between the *AUC's* after Product A, B, or C. Significant interpatient variation was obvious, but there was no significant difference between periods.

The rate constant for elimination of aminosalicylic acid was the same for all products; the mean half-lives calculated for Products A, B, C, and D were 0.94, 1.03, 1.00, and 0.98 hr, respectively.

The peak plasma levels of the acetyl conjugate were obtained approximately 2 hr later than those of aminosalicylate (Fig. 2). The rate of elimination of the acetyl conjugate was slower than that of unchanged drug. The mean half-lives of elimination of the acetyl conjugate were 1.66, 1.40, 1.19, and 1.61 hr for Products A, B, C, and D, respectively.

Analysis of Urine Data—The cumulative excretion of total drug, nonacetylated drug, and acetylated drug in urine following administration of different products is illustrated in Figs. 3–5. The 24-hr values and their statistical analyses are presented in Table III.

The fraction of dose excreted in urine as aminosalicylate and its metabolites averaged 86–90%, and there were no significant differences between the products. The fraction excreted as nonacetylated compound during 24 hr was 47% of the dose of Product D and was significantly higher than the corresponding figures after Product A, B, or C (38–41%). There was a similar relationship between the products for the *AUC's* of aminosalicylic acid. A significant interpatient variation was found in the urinary excretion of nonacetylated drug, but the fraction of the dose excreted as the acetyl conjugate in urine during 24 hr was similar (43–49% of dose) for all products. However, the ratio of nonacetylated drug to acetylated drug was greater than 1 only in the case of Product D.

DISCUSSION

The importance of measuring both the unchanged drug and its metabolites in the bioavailability studies of aminosalicylic acid has been stressed (10). In humans, the drug is metabolized mainly to *N*-acetyl-*p*-aminosalicylic acid and to a lesser degree to its glycine conjugate, *p*-aminosalicyluric acid. These metabolites together ac-

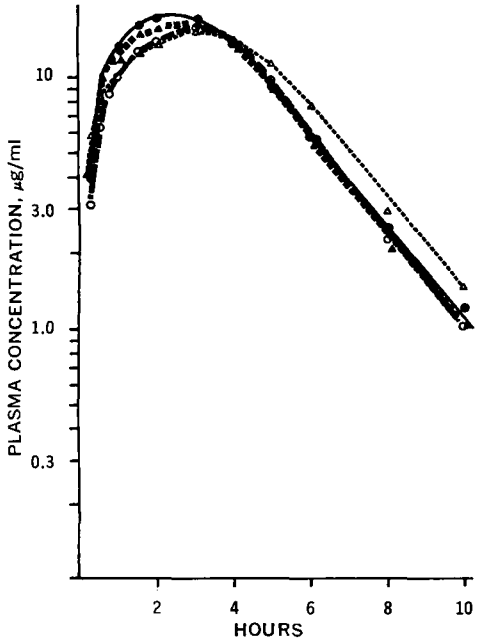

Figure 2—*Mean plasma levels of* N-*acetyl*-p-*aminosalicylic acid from 12 subjects following administration of four different brands of sodium aminosalicylate. Data corrected to 70 kg body weight and to a dose equivalent to 2.9 g free acid. Key:* ●, *Product A;* ○, *Product B;* ▲, *Product C; and* △, *Product D.*

count for more than 90% of the metabolites in urine (6, 11). Lauener *et al.* (7) showed that unchanged drug is the biologically active form, with the metabolites having virtually no bacteriostatic action. Thus, the biologically active fraction available from the dose can be estimated on the basis of the *AUC* and by measuring the excretion of unchanged drug in urine, whereas the cumulative excretion of total drug in urine better reflects the total absorption of the product.

The method used in the present study does not differentiate be-

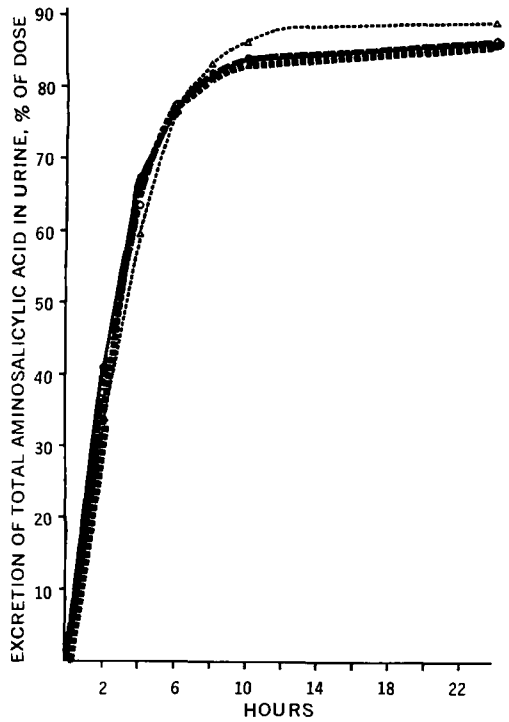

Figure 3—*Mean cumulative excretion of total drug in urine from 12 subjects following administration of four different brands of sodium aminosalicylate. Key:* ●, *Product A;* ○, *Product B;* ▲, *Product C; and* △, *Product D.*

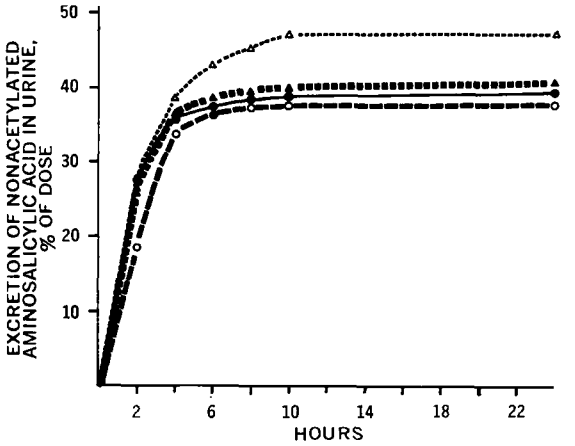

Figure 4—*Mean cumulative excretion of nonacetylated drug in urine from 12 subjects following administration of four different brands of sodium aminosalicylate. Key:* ●, *Product A;* ○, *Product B;* ▲, *Product C; and* △, *Product D.*

tween unchanged drug and the glycine conjugate; therefore, the term nonacetylated drug was used for the sum of these compounds in the urine. The overestimation of aminosalicylic acid in plasma as a result of the nonspecificity of the assay employed is small since the nonacetylated fraction in plasma consists of 95% unchanged drug and only 5% glycine conjugate (12). This ratio has been shown to be constant at different plasma levels of aminosalicylate (7). The ratio of these compounds is also constant in urine, although the fraction of glycine conjugate in urine is higher (20–25%) than in plasma (7). A specific method for aminosalicylate has been described but is impractical for a large-scale study (10). Considering the short half-lives of aminosalicylic acid (approximately 1 hr) and the acetyl conjugate (approximately 1.5 hr), the 24-hr collection period was long enough for virtually all excreted drug to be recovered and should satisfactorily reflect total absorption.

The sodium salt is rapidly and completely absorbed from a solution as shown earlier (8). The tablet formulation somewhat decreases the rate and the extent of absorption of the sodium salt, but the recovery of 86–90% of the dose in urine still indicates very good GI absorption of the sodium aminosalicylate tablets studied. As demonstrated earlier, the total absorption of sodium, potassium, and calcium salts in tablet form is essentially similar and markedly faster and more complete than the absorption of tablets of the free acid (13).

The four brands of the sodium salt compared in the present study did not differ in the total absorption of the compound. How-

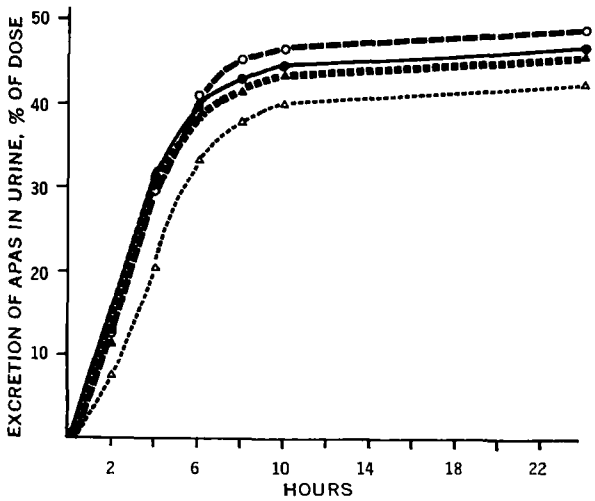

Figure 5—*Mean cumulative excretion of* N-*acetyl*-p-*aminosalicylic acid (APAS) in urine from 12 subjects following administration of four different brands of sodium aminosalicylate. Key:* ●, *Product A;* ○, *Product B;* ▲, *Product C; and* △, *Product D.*

Table III—Cumulative Excretion of Total Drug, Nonacetylated Drug, and Acetylated Drug in Urine during 24 hr following Administration of Four Brands of Sodium Aminosalicylate

Product	Total Excretion, % of Dose	Nonacetylated Drug, % of Dose	Acetylated Drug, % of Dose
A	86.0 (71.3–103.3)[a]	39.4 (27.6–48.9)	46.6 (35.2–60.9)
B	86.4 (69.1–121.9)	37.9 (19.3–55.4)	48.5 (33.5–70.9)
C	86.2 (57.2–96.2)	40.8 (26.1–54.5)	45.4 (31.2–62.9)
D	89.7 (65.9–101.4)	47.1 (31.7–63.7)	42.6 (23.8–55.4)

Analysis of Variance for Nonacetylated Drug

Source of Variation	*df*	*ss*	*ms*	*F*
Between individuals	11	2540	223	8.74
Between products	3	628	210	8.22
Between periods	3	193	65	2.54
Error	30	764	26	
Total	47	4037		

SE of varietal mean = 1.457, $F_{0.05}$ (3.30) = 2.92, $F_{0.05}$ (11.30) = 2.12

Multiple-Range Analysis

p	(2)	(3)	(4)
Shortest significant ranges	4.21	4.43	4.55
Product	B	A	C	D
Mean percent of dose excreted[b]	37.9	39.4	40.8	47.1

D − B = 9.20 > 4.55; significant
D − A = 7.70 > 4.43; significant
D − C = 6.30 > 4.21; significant
C − B = 2.90 < 4.43; not significant
C − A = 1.40 < 4.21; not significant
A − B = 1.50 < 4.21; not significant

[a] Means and ranges are given. [b] Any two or more means not underscored by the same line are significantly different.

ever, a significantly higher fraction of dose was available as nonmetabolized, therapeutically active product from Product D than from the other three brands, whether determined on the basis of the *AUC* of unchanged drug or the excretion of nonacetylated drug in urine. This finding can be at least partly explained on the basis of the higher dose of Product D (4 g) than A, B, or C (2.9 g), even though the results were normalized for dose and weight. The acetylation of aminosalicylic acid in liver and intestinal mucosa has been shown to be concentration dependent and capacity limited in humans (7). Near saturation of the enzyme is already achieved following a 1-g dose of drug (14). Thus, the larger the dose the larger will be the fraction of the dose that reaches the systemic circulation and is excreted in urine in the nonmetabolized form. The plasma elimination rate constant for aminosalicylic acid was the same following administration of all four products, suggesting that the dose-dependent effects on acetylation occur during the first pass through the intestinal wall and liver.

In our opinion, the nonacetylated fraction from the dose would be the same for all products studied if studied in equivalent doses. A different tablet size in the case of Product D complicated the dosing. The doses used in this study correspond to the recommendations of the manufacturers for single doses for tuberculosis treatment. The dose-dependent acetylation of aminosalicylic acid, with the consequent increase in the fraction of dose available as the biologically active form, supports the concept that single daily large doses may be preferred to the smaller multiple doses suggested (15). This would depend on the minimum inhibitory concentration *in vivo*, which is not known; however, the single dose would be preferred if intermittent high levels are required. The rapid and nearly complete absorption of sodium aminosalicylate tablets and the relatively good patient tolerance of this drug favor the choice of this form of aminosalicylic acid in the management of patients with tuberculosis. Moreover, a higher fraction of dose is available in a biologically active form from the sodium salt than from the free acid or as its potassium or calcium salts (13). According to the present results, the variation in total absorption of different brands of the sodium salt is small, further favoring the use of this form of aminosalicylic acid.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 4, 1974, from the *Clinical Pharmacology–Toxicology Center, Departments of Medicine and Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103*

Accepted for publication May 10, 1974.

The work upon which this publication is based was performed pursuant to Contract No. FDA 72-333 with the U.S. Public Health Service, Food and Drug Administration, Department of Health, Education, and Welfare.

The authors thank Dr. Khatab Hassanein for assistance with computer programs for the statistical analyses and Anthony Miella, Glenn Tucker, and Nathan Wick for their excellent technical assistance.

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