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Studies on Metabolism of Drugs. XIII.[1] Quantitative Separation of Metabolites in Human Urine after Oral Administration of Sulfisomezole and Sulfaphenazole

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Sulfisomezole, N^1-(5-methyl-3-isoxazolyl)sulfanilamide, was synthesized by Kano and others,[3] and is widely used as a long-lasting sulfa drug. The metabolites of this drug have been separately determined by Nishimura and others[4] as the free and acetylated compound. We also examined the metabolites of sulfisomezole and found sulfisomezole N^1-glucuronide,[5] sulfisomezole 2′-glucuronide,[6] and hydroxymethyl-sulfisomezole[7] in addition to sulfisomezole and its N^4-acetylated compound.

Another sulfa drug, sulfaphenazole, 5-sulfanilamino-1-phenyl-pyrazole was synthesized by Schmidt and others,[8] is also used widely and its metabolites have been reported merely as a free and acetylated compounds.[9] Schmid and others[10] examined the determination of sulfaphenazole metabolites in rabbits and reported that colorimetric determination of the metabolites 0—24 hr after its ingestion gave a considerably lower value than that by the isotope method. Half-life of sulfaphenazole is variously reported as 24 hr[11] and 10 hr.[12] We reported previously that the metabolites of sulfaphenazole in human urine were sulfaphenazole 2′-glucuronide[13] and N^4-acetylsulfaphenazole 2′-glucuronide.[14]

In the present series of work, therefore, methods for the determination of acetylated sulfaphenazole were examined. Separatory determination for metabolites of either of sulfisomezole and sulfaphenazole was carried out in order to clarify the quantitative relation of these metabolites with passage of time.

Experimental

Reagents and Apparatus——These were the same as those reported in the preceding paper.[1]

Method for Separation——In the case of sulfisomezole, 0.3 ml of the test urine collected after its ingestion was spotted on Toyo Roshi filter paper No. 50 (40×26 cm) and developed ascendingly using a solvent

1) Part of this work was presented at the 32th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Kanazawa, June 1971. Part XII: M. Ueda, Y. Tsurui, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2042 (1972).
2) Location: *Gofuku-3190, Toyama.*
3) H. Kano and K. Ogata, *Shionogi Kenkyusho Nenpo*, **7**, 1 (1957).
4) H. Nishimura, K. Nakajima, S. Okamoto, N. Shimaoka, and K. Sasaki, *Shionogi Kenkyusho Nenpo*, **8**, 5 (1958).
5) M. Ueda and K. Kuribayashi, *Yakugaku Zasshi*, **84**, 1104 (1964).
6) M. Ueda, Y. Nakagawa, and N. Murakami, *Yakugaku Zasshi*, **87**, 451 (1967).
7) M. Ueda, I. Takegoshi, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2041 (1971).
8) P. Schmidt and J. Druey, *Helv. Chim. Acta*, **41**, 306 (1958).
9) Y. Tochino, Y. Hamanaka, and K. Inui, *Journal New Remedies & Clinics*, **9**, 677 (1960).
10) K. Schmid, J. Tripod, and F. Gross, *Klin. Wochenschrift*, **38**, 862 (1960); W. Padowetz, K. Schmid, and J. Druey, *Helv. Chim. Acta*, **44**, 89 (1961); W. Riess, K. Schmid, and H. Keberle, *Klin. Wochenschrift*, **43**, 740 (1965).
11) A.M. Walter, *Chemotherapia*, **6**, 161 (1963).
12) E. Kruger-Thiemer and P. Bunger, *Proceedings of the European Society for the Study of Drug Toxity*, **6**, 185 (1965).
13) M. Ueda, N. Murakami, and Y. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 345 (1968).
14) M. Ueda, K. Orita, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2046 (1971).

system of BuOH–MeOH–H_2O (4:1:1), at 28° for 16 hr, in the same manner as reported previously.[1] The corresponding sections of the test urine were cut out with reference to the *Rf* of colored spots of the control urine. The sections cut out centered around *Rf* 0.88 for sulfisomezole and acetylsulfisomezole, *Rf* 0.72 for hydroxymethyl-sulfisomezole, *Rf* 0.28 for sulfisomezole N^1-glucuronide, and *Rf* 0.15 for sulfisomezole 2′-glucuronide. Sulfisomezole and the acetylated compound were eluted together with 0.2 N Na_2CO_3 and eluate was received in a conical graduate. The hydroxymethyl compound and the two glucuronides were eluted with 0.02 N Na_2CO_3 and eluate was received in a measuring flask. These solutions were made up to a definite volume and determined in the same way as reported.[1]

In the case of sulfaphenazole, the test urine was diluted and the amount determined was taken as sulfaphenazole 2′-glucuronide and that colored after hydrolysis was taken as N^4-acetylsulfaphenazole 2′-glucuronide.

Method of Determination——Metabolites of Sulfisomezole and Sulfaphenazole 2′-glucuronide: These were effected by the diazotized method reported earlier,[1] and is measured with the spectrophotometer at 555 mμ.

For the determination of N^4-acetylsulfaphenazole 2′-glucuronide, 0.3 ml of 4 N NaOH was added to 1 ml of the test solution, heated in a water bath for 1 hr, and allowed to cool in a water tray of 16—18°. To this mixture, 0.6 ml of 4 N HCl was added, followed by 0.5 ml of 0.2% $NaNO_2$ solution, allowed to stand for 10 min, and 0.5 ml of 0.5% ammonium sulfamate was added. After standing for 5 min, 0.5 ml of EtOH and 1 ml of Tsuda reagent were added and allowed to stand for 10 min. This mixture was diluted to 10 ml with water and from the absorbance value of this solution, the value of sulfaphenazole 2′-glucuronide was subtracted to obtain the amount of N^4-acetylsulfaphenazole 2′-glucuronide.

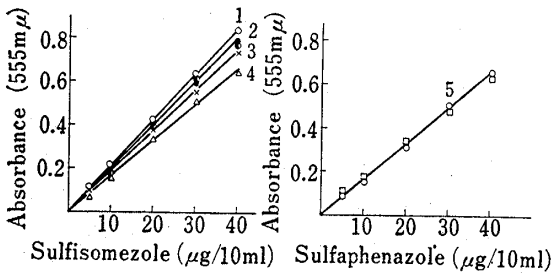

Fig. 1. Calibration Curves for Metabolites of Sulfisomezole and Sulfaphenazole

1: —○—: sulfisomezole 2′-glucuronide
2: —●—: sulfisomezole
—◐—: N^4-acetylsulfisomezole
3: —×—: sulfisomezole N^1-glucuronide
4: —△—: hydroxymethyl-sulfisomezole
5: —⊙—: sulfaphenazole 2′-glucuronide
—□—: N^4-acetylsulfaphenazole 2′-glucuronide

Calibration Curve——Authentic samples used were commercial product after recrystallization, or that extracted or synthesized. A solution of 100 mg of sulfisomezole or 116.5 mg of N^4-acetylsulfisomezole dissolved in 8 ml of 0.2 N NH_4OH was diluted with normal urine to make 200 ml. In the case of hydroxymethyl compound, N^1-glucuronide, and 2′-glucuronide, 3.517, 6.062, or 6.065 mg, respectively, was dissolved in normal urine to make to 20 ml. In the case of sulfaphenazole, 5.627 mg of sulfaphenazole 2′-glucuronide or 6.400 mg of N^4-acetylsulfaphenazole 2′-glucuronide was dissolved in normal urine to make to 20 ml. These standard solution were suitably diluted and submitted to the procedures of separation and determination as described above to prepare calibration curves (Fig. 1).

Result and Discussion

Sulfaphenazole 2′-glucuronide is soluble in water and contains a free aromatic amine so that it can be determined directly with the diluted urine. In the case of acetylsulfaphenazole 2′-glucuronide, its calibration curve obtained by diazotization and coloration with the Tsuda reagent came out much lower than that of sulfaphenazole 2′-glucuronide. The reason for the lowering was examined. When acetylsulfaphenazole 2′-glucuronide is hydrolyzed by heating with HCl on a water bath, glucuronic acid is liberated together with the acetyl group to form sulfaphenazole. This compound has a benzene ring attached to a heterocycle, has a large molecule, so that it tends to precipitate out when its aqua solution or dissolved in normal urine when coupled with the Tsuda reagent. For this reason, when its solution is brought to 10 ml with water, the compound will precipitate out above the concentration of 10 μg/10 ml and this will result in the lowering of the absorbance.

It was then found that the addition of EtOH before that of the Tsuda reagent increased the solubility of the large coupled molecule. Based on this knowledge and because the Tsuda reagent is an ethanolic solution, 0.5 ml of EtOH was added before additing 1.0 ml (double the usual amount) of Tsuda reagent, and the mixture was allowed to react for 10 min. By this measns, the calibration became a straight line up to 40 μg/10 ml.

Schmid and others[10] reported that the measured value at 24 hr after ingestion of sulfaphenazole was lower than that determined by the isotope method and that reason for it was unknown. The same tendency was found in the present work when the urine was hydrolyzed with HCl. The amount of sulfaphenazole 2′-glucuronide did not vary even when the test urine was allowed to stand for some time. It was assumed from the decrease in absorbance of sulfaphenazole 2′-glucuronide and acetylsulfaphenazole 2′-glucuronide, that sulfaphenazole differs from other sulfa drugs, and a substance formed *in vivo* after its ingestion which, when hydrolyzed with HCl, interferes with the aromatic amine to lower the absorbance. Based on this assumption, hydrolysis was carried out with NaOH to effect deacetyl-

Table I. Amount of Substances excreted in Human Urine after Ingestion of Sulfisomezole (Calculated as Sulfisomezole (mg))

Subject and dose (g)	Substance[a]	Time of urine collection (hr after ingestion) 2	4	8	12	24	36	48	60	72	% of total excreted	% of dose excreted
A 1	SI	—	6.3	36.4	60.7	25.5	10.0	7.2	4.5	0.2	16.3	93
	ASI	0.2	5.9	67.6	53.4	233.3	124.4	56.8	48.1	7.2	64.1	
	SIOH	0.2	1.6	8.4	5.4	16.1	6.1	3.5	2.7	0.4	4.8	
	SIN^{1}G	0.1	2.6	15.1	18.8	42.1	21.7	8.9	4.5	1.3	12.4	
	SI2′G	—	0.4	3.0	2.5	7.8	3.2	1.0	0.8	0.4	2.1	
B 1	SI	0.1	4.7	56.8	54.8	35.0	35.9	3.1	1.7	—	22.1	87
	ASI	0.1	2.3	27.6	84.5	191.0	131.9	22.2	14.9	4.2	55.0	
	SIOH	0.2	2.0	4.2	7.6	19.3	4.0	3.6	3.1	—	5.4	
	SIN^{1}G	0.1	3.1	50.2	22.4	44.0	12.4	4.6	3.5	1.5	15.7	
	SI2′G	0.1	0.3	2.1	3.1	5.0	4.2	1.0	0.7	—	1.8	
A 2	SI	0.8	36.3	119.2	99.6	53.5	39.7	16.6	0.7	—	21.9	84
	ASI	1.0	40.6	138.4	161.1	320.1	192.8	58.9	44.6	14.7	58.1	
	SIOH	0.2	2.1	10.2	16.0	16.8	15.0	5.8	1.9	0.8	4.1	
	SIN^{1}G	0.8	6.2	49.0	48.6	69.9	41.3	8.4	7.6	3.4	14.0	
	SI2′G	—	0.8	2.0	7.4	11.3	6.3	1.8	1.0	0.4	1.9	
B 2	SI	1.7	5.8	109.3	48.5	30.2	14.0	11.0	10.3	1.5	14.2	82
	ASI	2.7	22.8	228.3	155.4	400.6	199.5	71.2	24.4	9.0	68.2	
	SIOH	1.3	2.4	5.5	16.6	34.7	32.2	5.8	2.7	1.6	6.3	
	SIN^{1}G	1.1	7.2	41.9	36.5	26.3	25.5	8.0	4.5	1.1	9.3	
	SI2′G	0.2	1.0	5.9	7.0	6.8	6.6	2.0	2.3	0.5	2.0	

a) SI: sulfisomezole ASI: N^4-acetylsulfisomezole SIOH: hydroxymethyl-sulfisomezole SIN^{1}G: sulfisomezole N^1-glucuronide SI2′G: sulfisomezole 2′-glucuronide

Table II. Amount of Substances excreted in Human Urine after Ingestion of Sulfaphenazole (Calculated as Sulfaphenazole (mg))

Subject and dose (g)	Substance[a]	Time of urine collection (hr after ingestion) 2	4	8	12	24	36	48	60	72	% of total excreted	% of dose excreted
A 1	SPG	2.2	55.7	106.1	84.5	154.3	74.8	30.3	12.7	9.5	74.9	71
	ASPG	0.7	7.5	9.3	25.8	51.1	35.4	32.3	6.7	8.6	25.1	
B 1	SPG	2.4	97.5	162.3	95.6	110.8	50.6	28.4	25.0	17.9	78.4	75
	ASPG	0.6	8.1	10.5	33.7	83.1	12.7	8.0	3.4	2.5	21.6	
C 1	SPG	3.6	43.2	113.8	85.2	178.4	75.2	64.8	37.3	19.3	76.6	81
	ASPG	0.9	5.0	16.5	23.4	75.2	30.6	15.4	12.8	10.3	23.4	
A 2	SPG	1.3	29.5	281.4	318.7	425.3	125.7	39.1	25.7	12.2	75.5	83
	ASPG	0.4	8.2	52.7	98.4	130.4	62.3	32.5	19.2	4.7	24.5	
B 2	SPG	4.5	47.3	244.6	203.1	235.7	104.6	51.7	22.6	12.2	66.9	69
	ASPG	1.7	3.9	62.5	77.3	192.0	58.9	27.2	23.1	12.5	33.1	
C 2	SPG	2.4	28.6	393.1	309.6	280.5	96.0	63.3	33.6	25.1	77.9	79
	ASPG	1.4	7.9	49.7	90.7	96.4	47.6	19.9	21.7	15.0	33.1	

a) SPG: sulfaphenazole 2′-glucuronide ASPG: N^4-acetylsulfaphenazole 2′-glucuronide

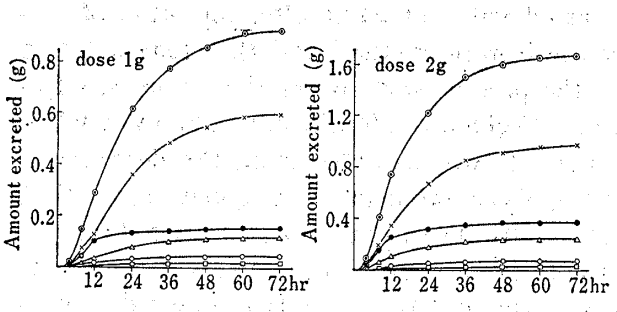

Fig. 2. Cumulative Excretion Curves of Sulfisomezole Metabolites in Human Urine

—●—: sulfisomezole
—×—: N⁴-acetylsulfisomezole
—△—: sulfisomezole N¹-glucuronide
—○—: hydroxymethyl-sulfisomezole
—□—: sulfisomezole 2′-glucuronide
—⊙—: total sulfisomezole (calculated as sulfisomezole)

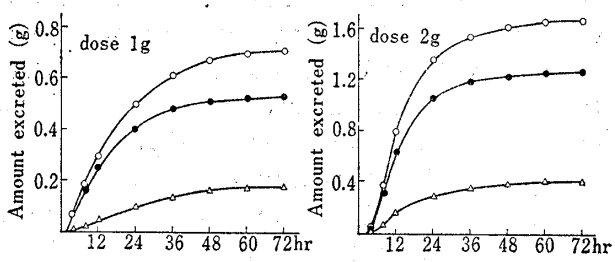

Fig. 3. Cumulative Excretion Curves of Sulfaphenazole in Human Urine

—●—: sulfaphenazole 2′-glucuronide
—△—: N⁴-acetylsulfaphenazole 2′-glucuronide
—⊙—: total sulfaphenazole (calculated as sulfaphenazole)

ation, diazotized in HCl acidity, and colored with Tsuda reagent after addition of EtOH by which there was no lowering of absorbance, and a calibration curve agree with N⁴-acetylsulfaphenazole 2′-glucuronide and sulfaphenazole 2′-glucuronide was obtained.

Since the determination of sulfaphenazole has become more reliable, majority of the drug ingested was found to be excreted into urine in 0—24 hr. Determination with the urine showed that one-half of 2 g ingested was excreted within 12 hr, which is considerably shorter than the 24-hr half-life reported in literature.[11]

The amount of each metabolite formed after ingestion of sulfisomezole and sulfaphenazole is given in Tables I and II, and in Fig. 2 and 3. Viewed from the time of total amount and half total amount excreted into urine after ingestion of 2 g (Figs. 2 and 3), half-life of sulfisomezole is 13 hr and that of sulfaphenazole is 12 hr.

In order to calculate the recovery of the metsbolites, authentic metabolites dissolved in normal human urine as shown ingredients (with nearly above measured amount) of Table III. The separatory determination as above carried out, and were calculated the mean values and standard errors (Table III).

Table III. Recovery Test of Metabolites on Sulfisomezole and Sulfaphenazole from the Known Human Urine

Substance	Recovery (%)			$n^{a)}$	Standard error (%)
	Mean	Max.	Min.		
Sulfisomezole	99.9	102.7	97.9	5	0.89
N⁴-acetylsulfisomezole	100.2	102.6	98.5	5	0.78
Hydroxymethyl-sulfisomezole	101.4	104.5	97.6	5	1.34
Sulfisomezole N¹-glucuronide	100.9	102.6	98.1	5	0.91
Sulfisomezole 2′-glucuronide	98.9	103.3	97.4	5	1.18
Sulfaphenazole 2′-glucuronide	100.2	102.4	97.3	5	0.96
N⁴-acetylsulfaphenazole 2′-glucuronide	99.9	103.2	96.8	5	1.10

a) number of determinations

ingredients

SI	2.147 mg	SPG	7.528 mg
ASI	5.820	ASPG	2.536
SIOH	0.411	normal urine ad 10 ml	
SIN¹G	1.519		
SI2′G	0.228		
normal urine ad 10 ml			

The percentage of metabolites after ingestion of 1 g and 2 g was similar in both cases. When the subject drank more water and the volume of urine became larger, the amount excreted became relatively large.