Chemotherapeutic Activity of Some Sulfapyridine-1-Oxides. (23830)

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Sulfapyridine-l-oxide, and several alkyl substituted derivatives prepared in our laboratories(1), were found to be more acidic than the corresponding sulfapyridines. It was assumed that these compounds might not induce urolithiasis and for that reason their potential as chemotherapeutic agents was investigated in laboratory animals.

Methods. Chemotherapeutic activity of the drugs was determined(2) in *E. coli*-infected mice as follows. Eighteen-hour cultures of *E. coli* (A.T.C.C. No. 26) in dextrose peptone broth were diluted 1 x 10^4 fold, and 1 ml of the diluted culture was mixed with 9 ml of 5% gastric mucin. The dose used for intraperitoneal infection, 0.5 ml of the 1 x 10^5 dilution, contained an average of 6000 cells or 5 to 10 thousand lethal doses. Virulence of the culture was maintained by frequent mouse passage. Test compounds and reference drugs were suspended in 10% gum acacia, and 0.5 ml of the suspension was given by stomach tube. The first dose was given 30 to 45 minutes before infection; the second was given about 6 hours later. The drugs were then given at a rate of 2 doses per day for 2 days, and in some cases, as noted, for 4 days. Control mice were treated in a parallel manner with acacia solution. Groups of 10 or 15 male albino Carworth Farms CF_1 mice were used at each dose level. Some experiments were run in duplicate. In these instances, the data were combined for presentation in Table I. All animals were observed daily for 10 days. To conserve space survivals are recorded in Table I only for the first, second and tenth days. The pKa's of the sulfapyridine-l-oxides and reference drugs as well as their acetylated derivatives were approximated by titrating 0.1% solutions of the drugs in water at 25°C with one-half an equivalent of 0.1 N sodium hydroxide. The data are recorded in Tables I and II for comparative purposes. Using procedure first described by Antopol(3) in his studies of sulfonamide urolithiasis, precipitation within the kidney was sought following intravenous injection of sodium salts of 6 acetylsulfonamides. Groups of 6 Sprague Dawley rats were used at each dose level. Solutions of the sodium salts were prepared in concentrations of 5 and 10% by the addition of approximately 1 equivalent of dilute sodium hydroxide. The pH's were 8 $\pm$ 0.5 except in the case of acetylsulfapyridine where pH was 10.5. Solutions were injected into tail vein at 1 ml/30 seconds. Twenty-four, 48 and 96 hours after administration of the drug, kidneys were removed, sectioned, and examined under dissecting microscope. Urolithiasis, if it occurred at all, appeared within 24 hours. Consequently the data summarized in Table II include only observations recorded after 24 hours. Hypoglycemic activity was assayed according to procedure of Mirsky *et al.*(4). The drugs were dissolved to the extent of 500 mg % in 0.5% sodium bicarbonate solution. Sprague Dawley rats which had been fasted overnight were given either 2 or 5 ml of solution by stomach tube/100 g body weight. Thus, each rat received either 100 or 250 mg of the individual drugs/kg body weight. Groups of 3 to 6 rats were used for each drug. Samples of tail blood were taken shortly before administration of drugs and at 1, 2 and 4 hours thereafter. Blood glucose determinations were performed according to Nelson(5). Averaged data are recorded in Table III.

Results. As shown in Table I, the 6'-methyl derivative is the most active of the sulfapyridine-l-oxides. Shifting the methyl group to any other position in the pyridine ring results in a considerable loss of chemotherapeutic activity. Similarly, increasing the chain length from methyl to ethyl results in a diminution of activity. Under the conditions of these experiments, sulfamethoxypyridazine is clearly the most active of the reference drugs. Sulfamethylthiadiazole and sulfadimethoxytriazine are relatively weak and sulfisoxazole is of intermediate activity. Calculated according to the method of Litchfield

and Wilcoxon(6) the ED_{50}'s for 6′-methylsulfapyridine-l-oxide and sulfisoxazole are 0.54 and 0.65 mg/mouse, respectively. 6′-Methylsulfapyridine-l-oxide appears to be more potent than sulfisoxazole, but the difference is not significant at the 5% level.

In an attempt to decide qualitatively which drugs would and which would not form uroliths, solutions of the sodium salts of various acetylated sulfonamides were rapidly injected intravenously in the rat. In this way, absorption, and detoxication are largely by-passed and a maximum blood concentration of the drug is available for distribution and renal excretion. If the drugs are cleared in the kidney by simple glomerular filtration, tubular reabsorption of water will cause the drugs to precipitate only if their solubilities in the tubular urine are exceeded. The solubilities of these acetylsulfonamides are determined primarily by the extent to which they undergo salt formation at the pH of the plasma filtrate. As shown in Table II, acetylsulfisoxazole, acetylsulfamethylthiadiazole, and 6′-methylsulfapyridine-l-oxide, which are 50% ionized at pH levels of 4.4, 5.2 and 5.4

TABLE I. Activity of Sulfapyridine-l-oxides and Reference Drugs against *E. coli* Infections in Mice.

Compound	pKa	No. of mice	Dose (mg/mouse)	Survivals in days (%) 1	2	10
Sulfapyridine-l-oxide	5.2	10*	10.	100	100	100
		10*	5.	100	40	20
		10	2.5	50	0	
3′-Methylsulfapyridine-l-oxide	6.1	10	5.0	10	0	
		10	1.0	0		
		10	.5	0		
4′-Methylsulfapyridine-l-oxide	5.5	15	5.0	93	80	80
		15	1.25	33	0	
5′-Methylsulfapyridine-l-oxide	5.7	10	2.5	90	0	
6′-Methylsulfapyridine-l-oxide	5.9	30	1.25	100	100	100
		30	1.0	100	93	93
		40	.5	98	60	45
		10	.4	90	30	20
		10	.3	100	10	0
6′-Ethylsulfapyridine-l-oxide	6.1	10	2.5	70	60	50
		10	1.0	90	80	70
		10	.5	40	10	0
4′, 6′-Dimethylsulfapyridine-l-oxide	6.2	15	5.0	100	87	87
		15	1.25	33	0	
Sulfadimethoxytriazine	5.0	15	2.5	7	0	
		15	1.0	0		
		15	.5	0		
Sulfamethylthiadiazole	5.4	10	1.25	30	0	
		10	.9	30	10	10
		20	.6	25	10	10
		10	.4	30	0	
Sulfisoxazole	5.0	35	1.25	100	94	94
		20	1.0	100	80	80
		35	.6	100	37	34
		10	.4	90	20	20
		10	.3	80	0	0
Sulfamethoxypyridazine	7.2	15	2.5	100	100	100
		15	1.0	100	100	100
		15	.5	100	100	100
Controls	10^{-5}	80		6	1	1
	10^{-6}	80		23	9	9
	10^{-7}	80		31	8	6
	10^{-8}	85		54	14	14
	10^{-9}	85		85	56	56

* Animals were treated for 4 days. In all other instances treatment was limited to 2 days.

TABLE II. Urolithiasis following Intravenous Acetylated Sulfonamides in the Rat.

Drug*	pKa	Dose† (mg/kg)	No. dead	No. of animals showing uroliths
Sulfapyridine	8.2	400	1	6
		200	0	6
		100	0	3
Sulfamethoxypyridazine	6.9	1000	1	0
		400	0	0
Sulfadiazine	6.1	400	0	6
		200	0	6
		100	0	2
6′-Methylsulfapyridine-l-oxide	5.4	500	1	0
		400	0	0
Sulfamethylthiadiazole	5.2	1000	0	0
		400	0	0
Sulfisoxazole	4.4	1000	0	0
		400	0	0

* All were acetylated.
† 6 rats were used at each dose level.

respectively, did not precipitate within the tubules. In contrast, acetylsulfapyridine, which undergoes little dissociation in acidic urine, produced uroliths in a high proportion of the animals at doses of 100 mg/kg and above. Acetylsulfadiazine (pKa = 6.1) produced a high incidence of kidney stones; acetylsulfamethoxypyridazine, though a weaker acid (pKa = 6.9), did not. Perhaps in this particular instance, the slow rate of renal excretion of the pyridazine, as noted by others(7), is determinant. Although all the implications of this intravenous procedure have admittedly not been developed, it is an interesting approach to the sulfonamide-urolith problem, and it appears to afford useful information.

TABLE III. Blood Sugar Levels in Rats following Sulfapyridine-l-oxides or Sulfonylureas.

Substituent or drug	Dose (mg/kg)	Mean blood sugar given in % of initial conc. 1 hr	2 hr	4 hr
4′-Methyl	250	85	96	85
5′ "	250	73	93	104
6′ "	100	114	104	
6′ "	250	105	74	80
4′,6′-Dimethyl	250	93	99	99
Tolbutamide	250	73	47	39
Carbutamide	100	79	63	

In the course of evaluation of the sulfapyridine-l-oxides their potential hypoglycemic activity was assayed in the rat. As shown in Table III none produced a significant hypoglycemia.

Summary. 6′-Methylsulfapyridine-l-oxide was found to be a potent chemotherapeutic agent in the treatment of *E. coli* infections in mice. It did not cause hypoglycemia in the rat. Intravenous administration of the sodium salt of its acetylated derivative did not lead to urolithiasis.

1. Childress, S. J., Scudi, J. V., *J. Org. Chem.*, 1958, v23, 67.
2. Scudi, J. V., Childress, S. J., Plekss, O. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 571.
3. Antopol, W., *Arch. Pathol.*, 1940, v30, 985; *J. Urol.*, 1940, v43, 589; Lehr, D., Antopol, W., *Urol. Cut. Rev.*, 1941, v45, 3.
4. Mirsky, I. A,. *Metabolism*, 1956, v4, 156.
5. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.
6. Litchfield, J. T., Wilcoxon, F., *J. Pharmacol.*, 1949, v96, 99.
7. Jones, W. F., Finland, M., *Ann. N. Y. Acad. Sci.*, 1957, v69, 473.

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