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Thiazol Derivatives of Sulfanilamide and Experimental Beta-Hemolytic Streptococcal and Pneumococcal Infections in Mice.

O. W. Barlow and E. Homburger.

From the Research Laboratories of the Winthrop Chemical Company, Inc., Rensselaer, New York.

In a previous paper[1] the specific chemotherapeutic action of 2-sulfanilamidothiazol (sulfathiazol) and 2-sulfanilamidomethylthiazol (sulfamethylthiazol) in experimental *Staphylococcus aureus* infections has been described. This communication presents a comparison of the efficiency of sulfathiazol, sulfamethylthiazol and 2-sulfanilamidophenylthiazol (sulfaphenylthiazol) with that of sulfanilamide

TABLE I.
Chemotherapy: Beta-hemolytic Streptococcus (Mice).

No. of mice	Preparation	Dosage* Single, mg/kg	Dosage* Total, g/kg	Survival in hrs Range	Survival in hrs Median	Survival 10 days % of series
60	None (controls)			10-25	14.25	0
40	Sulfanilamide	25	0.35	64-240+	152	30
40	"	100	1.40		240+	100
40	"	250	3.5		240+	100
40	Sulfapyridine	10	0.14	31-240+	64	30
50	"	25	0.35	20-240+	240+	80
50	"	100	1.40		240+	100
50	"	250	3.5		240+	100
40	Sulfathiazol*	14.8	0.192	30-47	44	20
40	"	37.0	0.481	54-114	240+	80
40	"	148.0	1.92		240+	100
40	"	370.0	4.81	120	240+	90
40	Sulfamethylthiazol	10	0.14	40-240	91	30
50	"	25	0.35	68-240	240+	88
50	"	100	1.40		240+	100
50	"	250	3.5		240+	100
40	Sulfaphenylthiazol	10	0.14	69-240	87	40
50	"	25	0.35	90-240	240+	90
50	"	100	1.4		240+	100
50	"	250	3.5		240+	100

* All of the dosage figures represent the actual amounts of each compound. The doses of sulfathiazol in Table I, and of sulfathiazol and sulfaphenylthiazol in Tables II and III, were calculated for comparative purposes on the basis of their sulfanilamide equivalents.

[1] Barlow, O. W., and Homburger, E., Proc. Soc. Exp. Biol. and Med., 1939, 42, 792.

and sulfapyridine in experimental beta-hemolytic streptococcus and pneumococcus Types I, II, and III infections.

Method. Male albino mice of uniform age from a standard strain weighing between 19 and 21 g were inoculated intraperitoneally with 0.3 cc of a saline suspension of an 18-hour culture of beta-hemolytic streptococcus (Lancefield Group A) C-203 of proven high mouse virulence representing from 0.75 to 1.0 million organisms. In a test of the virulence of this culture, 100 organisms proved fatal to all of a group of 20 mice. The dosage, therefore, represented from 7,500 to 10,000 lethal doses. Treatment was carried out 1½, 7, 12, 24, 32, and 48 hours after inoculation, and once daily thereafter until death occurred or up to and including the tenth day. The preparations were administered as a suspension in ¼ to ½ cc of milk (per single dose) by means of the stomach tube.

Results. The effects of sulfanilamide and various derivatives on beta-hemolytic streptococcus infections in mice are illustrated in Table I.

Tables II, III, and IV indicate the effectiveness of the thiazol compounds in the treatment of experimental pneumococcal infections of various types in albino mice.

In the tests summarized in these tables, all mice were inoculated with 0.3 cc of a saline suspension of 24-hour mouse virulent serum dextrose culture of pneumococci. Typed strains were obtained from

TABLE II.
Chemotherapy: Pneumococcus Type I (Mice).

No. of mice	Preparation	Dosage		Survival in hrs		Survival 10 days % of series
		Single, mg/kg	Total, g/kg	Range	Median	
40	None (controls)			19-75	30.1	0
50	Sulfanilamide	1000	14.0	42-190	84	0
40	Sulfapyridine	250	3.5	64-240+	82	20
40	"	500	7.0	86-240+	97	30
50	"	1000	14.0	89-240+	240+	50
30	Sulfathiazol	370	5.18	34-240+	47	10
40	"	740	10.36	48-240+	62	20
40	"	1480	20.72	27-240+	139	40
40	Sulfamethylthiazol	250	3.5	69-240+	84	20
40	"	500	7.0	82-240+	129	30
40	"	1000	14.0	90-240+	240+	52
40	Sulfaphenylthiazol	480	6.7	35-190	47	0
40	"	960	13.4	39-240+	67	10
50	"	1920	26.88	40-240+	69	20

TABLE III.
Chemotherapy: Pneumococcus Type II (Mice).

No. of mice	Preparation	Dosage Single, mg/kg	Dosage Total, g/kg	Survival in hrs Range	Survival in hrs Median	Survival 10 days % of series
60	None (controls)			29-56	37	0
50	Sulfanilamide	250	3.5	44-111	52	0
50	"	500	7.0	62-133	82	0
50	"	1000	14.0	82-235	124	0
40	Sulfapyridine	250	3.5	50-220	78.5	0
40	"	500	7.0	48-240+	87	12.5
40	"	750	10.5	79-240+	109	22.5
50	"	1000	14.0	75-240+	132	30.0
30	"	1500	21.0	92-240+	168	23.3*
20	Sulfathiazol	370	5.18	52-240+	69	10
20	"	740	10.36	78-240+	90	25
20	"	1480	20.72	69-240+	136	30
40	Sulfamethylthiazol	250	3.5	65-186	70	0
40	"	500	7.0	74-240+	97	15
40	"	750	10.5	72-240+	104	20
40	"	1000	14.0	75-240+	154	27.5
40	"	1500	21.0	110-240+	182	30
30	Sulfaphenylthiazol	480	6.72	57-142	74	0
30	"	960	13.44	60-240+	93	10
30	"	1920	26.88	70-240+	205	26.6

* Animals showed evidence of toxic effects from sulfapyridine.

the Maryland State Health Department, Baltimore, Maryland. Treatment was carried out by stomach tube at the time intervals indicated for the antistreptococcus tests.

The antistreptococcal actions of the thiazol derivatives of sulfanilamide in experimental beta-hemolytic streptococcus infections in mice, as based on the percentage of survivals in the lower dosage group of animals, were 2 to 3 times as effective as sulfanilamide and equal to those of sulfapyridine.

The therapeutic efficiency of sulfathiazol, sulfamethylthiazol or sulfapyridine on oral administration by tube to mice infected experimentally with L 100 as well as L 70 suspensions of pneumococcus Types I, II, III was essentially the same as indicated by the incidence of survivals. All 3 compounds were definitely (2 to 7.5 times) superior to sulfanilamide in the treatment of experimental pneumococcic infections. Sulfaphenylthiazol was superior to sulfanilamide, but appeared to be somewhat inferior to sulfapyridine or to either the unsubstituted thiazol or the methyl substituted thiazol derivatives.

In order to determine the blood concentration range required for

TABLE IV.
Chemotherapy: Pneumococcus Type III (Mice).*

No. of mice	Preparation	Dosage Single, mg/kg	Dosage Total, g/kg	Survival in hrs Range	Survival in hrs Median	Survival 10 days % of series
20	None (controls)			21-39	27	0
20	Sulfapyridine	250	1.75- 3.5	59-240+	89.5	5
20	"	500	3.5 - 5.0	88-142	121	0
10	"	750	5.25- 7.5	80-140	110	0
35	"	1000	7.0 -14.0	82-240+	183	20
10	Sulfathiazol	250	1.25- 1.75	36-86	48	0
10	"	1000	7.0 -14.0	79-240+	142	20
10	Sulfamethylthiazol	250	1.5 - 2.25	54-120	87.5	0
10	"	750	5.25- 7.5	85-152	127	0
15	"	1000	5.0 -14.0	95-240+	132.5	6.7
10	Sulfaphenylthiazol	1000	7.0 -14.0	80-240+	89	10
20	None (controls)			35-240+	36.5	30
50	Sulfanilamide	1000	14.0	48-240+	87	8
10	Sulfapyridine	250	1.5 - 3.5	50-240+	132	40
20	"	500	3.0 - 7.0	56-240+	240+	55
25	"	1000	8.0 -14.0	90-240+	240+	52
10	Sulfathiazol	250	1.25- 3.5	32-240+	90	40
10	"	1000	5.0 -14.0	40-240+	240+	70
10	Sulfamethylthiazol	250	1.25- 3.5	65-240+	240+	80
10	"	500	4.0 - 7.0	96-240+	240+	80
15	"	1000	8.0 -14.0	100-240+	240+	60

* Results of two separate tests are shown in order to illustrate the relation between virulence and therapeutic efficiency of the compounds.

effective therapy, the following study of absorption of the thiazol compounds was undertaken.*

Twenty-five mice, weighing from 20 to 24 g each, were medicated by stomach tube with 1 cc of a milk suspension of sulfapyridine in a dosage of 100 mg per kg body weight. A second group of 25 animals was medicated similarly with a dosage of 1000 mg per kg. These experiments were duplicated one or more times at each dosage level with each of the following compounds: sulfathiazol, sulfamethylthiazol, and sulfaphenylthiazol. Five mice from each dosage level for each compound were killed at 2, 4, 6, 24, and 48 hours after medication. The blood of the 5 mice was pooled at each of the above

* A complete report on the absorption, excretion and toxicity of the thiazol derivatives of sulfanilamide in laboratory animals and human beings will be published elsewhere.

time intervals, and the amount of the free drug and total drug (free and combined) was determined by a modification of the Marshall method,[2, 3] using a new coupling reagent: N(1-naphthyl) ethylene diamine dihydrochloride.

The observed blood values at the above time intervals post medication are indicated in Table V.

In the dosage range tested, these data do not indicate the minimally effective antistreptococcal blood concentrations of these compounds. However, the effective concentrations of the several preparations are probably less than those indicated in Table V after medication with the single 100 mg/kg dosage. Under similar conditions much higher blood concentrations (3.8 to 15 mg %) were found optimal for effective therapy of experimental pneumococcal infections.

It is of interest to note that the degree of conjugation which occurs in the mouse in order from greatest to least is: sulfaphenylthiazol, sulfapyridine, sulfathiazol or sulfamethylthiazol.

Sulfaphenylthiazol is excreted by mice and other animals (cats,

TABLE V.
Blood Concentration and Degree of Conjugation (Mice).

Preparation	Sulfa-pyridine		Sulfa-thiazol		Sulfamethyl-thiazol		Sulfaphenyl-thiazol	
Oral dose, g/kg	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0
No. of mice	25	25	25	25	50	75	25	25
2 hrs after medication								
Free mg %	4.6	11.8	2.5	12.5	8.8	12.2	6.7	14.3
Total mg %	4.7	14.9	2.9	13.8	9.15	13.5	7.8	16.7
% of total conjugated	2.1	20.8	13.8	9.4	3.8	9.6	14.1	14.3
4 hrs after medication								
Free mg %	3.2	15.2	2.3	9.5	5.1	11.0	5.4	14.6
Total mg %	4.2	18.0	2.7	10.5	5.8	12.6	8.1	17.4
% of total conjugated	23.8	15.5	14.8	9.5	12.0	12.7	33.3	16.09
6 hrs after medication								
Free mg %	1.4	15.0	2.0	8.6	4.0	10.3	5.1	9.3
Total mg %	1.8	18.2	2.3	10.2	4.5	11.9	7.3	13.3
% of total conjugated	22.2	17.5	13.0	15.7	11.1	13.4	30.1	30.0
24 hrs after medication								
Free mg %	trace	3.3	—	4.1	—	2.5	0.6-0.7	3.3
Total mg %	trace	3.8	0.2	6.0	trace	4.0	2.2	6.4
% of total conjugated	—	13.1	—	31.6	—	37.5	70.0	48.4
48 hrs after medication								
Free mg %	—	—	—	—	—	trace	—	trace
Total mg %	—	0.4	—	—	—	0.8	0.9	1.2
% of total conjugated	—	—	—	—	—	—	—	—

[2] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

[3] Bratton, A. L., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

dogs, man—unpublished) more slowly than the other 3 compounds so that cumulation tends to occur after frequently repeated doses.

Tolerance of Single Doses. Single oral doses of 25 g/kg of sulfapyridine or of the thiazol derivatives of sulfanilamide were tolerated by groups of 25 young adult albino mice. No symptoms of any kind were noted, other than some depression due to handling and to dilatation of the stomach. This high tolerance is largely due to the relatively low absorption of these compounds. The major portions of such large doses are passed in the feces.

Sulfapyridine sodium in large oral doses to mice produces a marked increase in reflex excitability, muscular tone, opisthotonus or even severe convulsions within 3 to 10 minutes after medication. These effects persist for from 45 to 90 minutes and are terminated by death or followed by a progressive depression and an irregular labored respiration to the 2nd or 3rd hour with gradual recovery to normal by the 7th hour, as described by Marshall and Long.[4]

On the other hand, intolerance to oral doses of the sodium salts of the 3 thiazol derivatives of sulfanilamide was indicated by a marked general depression and irregular respiration corresponding to the secondary effects of sulfapyridine sodium.

Table VI permits a comparison of the single M.L.D. (60% kills) and absolute lethal oral doses of the soluble salts of the several compounds for mice. The dosages were calculated on the basis of the

TABLE VI.
Oral Toxicity of Sulfapyridine and Thiazol (as Sodium Salts) for Mice.

Dose, g/kg	Aqueous concen. used %	Sulfapyridine		Sulfaphenylthiazol		Sulfamethylthiazol		Sulfathiazol	
		No. mice	% kills	No. mice	% kills	No. mice	% kills	No. mice	% kills
1.0	10	11	0	11	0	11	0	11	0
1.5	10	11	9.09	11	0	11	0	11	0
1.75	10	11	63.6	11	9.09	11	0	11	0
2.0	10	11	72.7	11	18.18	11	0	11	0
2.25	10	11	90.9	11	36.36				
2.5	10	11	90.9	11	63.6	11	0	11	0
2.75	10	11	90.9	11	72.7				
3.0	10	11	100.0	11	81.8	11	0	11	0
3.5	10	11	100.0	11	100.0	11	0	11	9.09
4.0	10			11	100.0	11	0	11	18.18
4.5	10			11	100.0	11	0	11	18.18
5.0	10					11	9.09	11	18.18
5.5	10					11	36.36		
6.0	10					11	63.63	11	27.27
7.0	10					11	90.9	11	54.54
8.0	10					11	100.0	11	81.81
10.0	10					11	100.0	11	100.0

[4] Marshall, E. K., Jr., and Long, P. H., *J. A. M. A.*, 1939, **112**, 1671.

compounds *per se* and the maximal volume by tube per mouse did not exceed ½ cc. These data indicate that sulfapyridine sodium orally to mice is 1.4, 3.4 and 4.57 times more toxic than the sodium salts of sulfaphenylthiazol, sulfamethylthiazol and sulfathiazol respectively.

Tolerance—Chronic Medication. Sulfapyridine, or the thiazol derivatives, is tolerated by groups of 50 or more mice in single daily oral doses up to 1.5 g/kg over a 15-day period or 2 g/kg daily over a 10-day period without ill effects as judged by weight maintenance and growth. Necropsy of the sacrificed animals at the 30th post-medication day likewise indicated no gross abnormalities.

However, single daily doses of 5 g/kg by stomach tube to groups of 30 mice over a 10-day period resulted in a cumulative toxicity. This maximal dosage represented the M.L.D. (60% kills) of sulfapyridine, but killed only 10% of animals receiving sulfathiazol, 20% of animals to which sulfaphenylthiazol was administered and 30% of the mice which were medicated with sulfamethylthiazol. The majority of all deaths occurred after discontinuance of medication up to the 19th day. Necropsy of such animals showed parenchymatous changes of greater or less degree in the kidney. The necropsy of the surviving animals of each group sacrificed after 30 days was essentially negative.

Conclusions. The chemotherapeutic effects of 3 thiazol derivatives of sulfanilamide under conditions of experimental streptococcal or pneumococcal infections are definitely superior to those of the parent substance and compare very favorably with those of sulfapyridine. On the basis of the superior margin of safety of sulfathiazol and particularly sulfamethylthiazol, as compared with sulfanilamide and sulfapyridine, these new compounds appear quite promising.