Acta Paediatrica Academiae Scientiarum Hungaricae, Vol. 13 (3), pp. 221—224 (1972)

Antibacterial Activity and Absorption of Sulphenazone

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(Received April 15, 1972)

Absorption and antibacterial activity of sulphenazone and of sulphamethoxypyridazine have been compared. Blood concentrations were determined by the reaction of Bratton and Marshall. The levels were approximately the same after oral dosage but sulphenazone showed a significantly higher concentration after rectal administration. Antibacterial activity was investigated by the disk method and by the tube dilution test. The minimum inhibitory concentration of sulphenazone for most of the strains tested was significantly lower than with sulphamethoxypyridazine, especially against *E. coli*, *St. aureus* and *Ps. aeruginosa*.

The synthesis of new sulphonamides and the study of their activity [4] has led to more profound knowledge of the multiple pharmacological aspects. The activity of these drugs in bacterial infections is quantitatively different and the same is true for their fate in the organism, their absorption, distribution, excretion and bacteriological activity. We have studied some of these aspects (absorption and antibacterial activity) of sulphenazone.

Sulphenazone originated from the necessity of introducing into therapy agents rapidly absorbed and slowly excreted, offering the advantage of maintaining an effective plasma concentration by modest dosage and in this way a reduction of possible side effects.

Materials and Methods

Sulphenazone is a clear yellow microcrystalline powder obtained by reaction in an equimolecular ratio with sulphamethoxypyridazine and the sodium salt of hydroxysulphonic acid derived from 4-formalphenazone and sodium bisulphate. Sulphenazone has a very good water solubility and the solution has a neutral reaction.

Blood sulphenazone levels were determined by the classical reaction of Bratton and Marshall [2]: the acetylated form of the drug is hydrolyzed to obtain free sulphonamide and the difference between total and free drug yields the acetylated form.

Tests in vivo

The absorption curve of sulphenazone and sulphamethoxypyridazine was studied in rabbits for a period of 24 hours after oral and rectal administration.

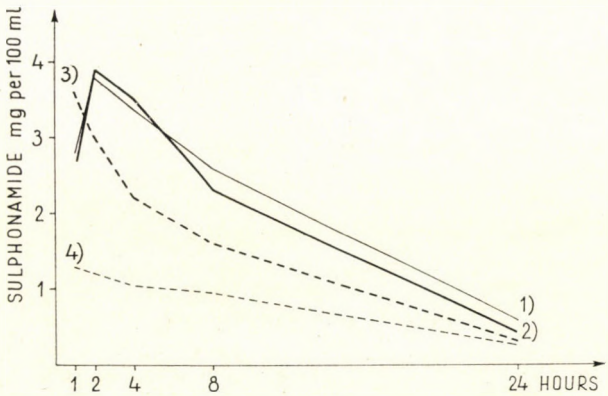
The oral dose was 100 mg/kg, the rectal one 150 mg/kg. Blood samples were taken at 1, 2, 4, 8, and 24 hours. For comparison, sulphamethoxypyridazine was given in equimolecular doses orally and in a double dose rectally.

Tests in vitro

Antibacterial activity was tested by the tube dilution method. Serial dilutions of the drugs were made from the original standard concentration. To 1.0 ml of each dilution of the drug 1.0 ml of inoculated medium containing 500.000 viable cells/ml was added. In order to maintain a constant inoculum, the original bacterial suspension was diluted to a constant optical density (Spectronic 20 Bausch—Lomb at 620 μm). Tubes were incubated at 37°C for 24 hours and read for M. I. C. recorded as the highest dilution inhibiting bacterial growth.

Antibacterial activity was tested also on paper disks 7 mm in diameter impregnated with the drugs at dilutions ranging from 50 to 1000 μg exactly distributed with microsyringe.

Penassay agar plates were seeded with 1.0 ml of bacterial suspension obtained by diluting 1 : 10 (one to ten) a standard prepared by centrifugation of a 18-hour broth culture, eliminating the supernatant and suspending the sediment in physiological saline to a constant absorption value.



1 = oral dosage SF 100 mg/kg in rabbits
2 = oral dosage SMP 50 mg/kg in rabbits
3 = rectal dosage SF 150 mg/kg in rabbits
4 = rectal dosage SMP 150 mg/kg in rabbits
SF = sulphenazone
SMP = sulphamethoxypyridazine

			Free sulphonamide in blood, mg per 100 ml					Total sulphonamide in blood mg per 100 ml				
Time after administration, hours			1	2	4	8	24	1	2	4	8	24
Administration	oral	SF 100 mg/kg	2.75	3.80	3.40	2.60	0.60	6.0	9.1	7.8	6.2	1.1
		SMP 50 mg/kg	2.70	3.90	3.50	2.30	0.40	5.6	9.6	8.4	5.6	0.75
	rectal	SF 150 mg/kg	3.60	3.00	2.20	1.60	0.30	8.7	7.1	5.8	3.8	0.8
		SMP 150 mg/kg	1.30	1.20	1.05	0.95	0.25	3.4	3.0	2.5	2.1	0.9

Fig. 1. Blood sulphenazone and sulphamethoxypyridazine levels

The plates contained $1—3 \times 10^6$ viable cells of the test organisms. After incubation at 37°C for 24 hours, the diameter of the inhibition zones was read to the nearest 0.5 mm.

In the first experiments Penassay medium was used, because its antibacterial activity seemed to be better than that of other media (BHIB, Mueller II, etc.). However, in our experience sulphonamide sensitivity may be different regardless of the medium used, and attempts are made to find for these tests a wholly adequate medium. Recently, in order to eliminate the antagonism of medium components against sulphonamide activity, we have used triptic soy broth plus 7% haemolyzed horse blood. The MIC obtained with this medium is much lower than with the other media.

Results and Conclusions

In the case of oral administration, the blood samples showed no significant difference in absorption between sulphenazone and sulphamethoxypyridazine. With both drugs the concentration rose rapidly, reached the maximum at the 2nd hour and then descended slowly, up to the 24th hour.

On the other hand, after rectal administration a significant difference was observed in that the blood sulphenazone concentration was clearly higher than that of sulphamethoxypyridazine. The total amount of sul-

Table I

SF and SMP sensitivity of bacteria by the disk method

	SF μg					
	50	100	300	500	700	1000
E. coli B	16	20	24	26	27	30
P. mirabilis	R	R	R	R	R	R
St. aureus	16	18	25	25	25	27
Pseudomonas sp.	—	—	11	14	15	16
P. morganii	R	R	R	R	R	R
A. aerogenes	10	11	13	16	18	19
P. vulgaris	14	19	25	27	28	29
Kl. pneumoniae	—	—	13	15	16	18
	SMP μg					
E. coli B	18	20	27	27	27	29
P. mirabilis	R	R	R	R	R	R
St. aureus	18	23	25	26	28	30
Pseudomonas sp.	—	—	12	14	15	18
P. morganii	R	R	R	R	R	R
A. aerogenes	11	12	17	17	19	22
P. vulgaris	17	20	27	27	29	30
Kl. pneumoniae	—	—	13	14	15	18

TABLE II

Minimum inhibitory concentrations of SF and SMP

	M. I. C. μg/ml	
	SF	SMP
E. coli	4.500	6.000
P. mirabilis	>5.000	>5.000
St. aureus	2.400	>5.000
P. morganii	>5.000	>5.000
A. aerogenes	5.000	>6.000
Ps. aeruginosa	4.000	>5.000
P. vulgaris	5.000	>6.000
Kl. pneumoniae	>3.500	>3.500

phenazone in the blood reached 8.7 mg per 100 ml at 1 hour, against 3.4 mg per 100 ml of sulphamethoxypyridazine given in a double dose. The difference persisted during the whole day (see Fig. 1).

As to antibacterial activity, in the disk test there was no significant difference between the two substances (Table I) while in the tube dilution test a better activity of sulphenazone was evident (Table II).

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According to the above observations, sulphenazone is easily absorbed, especially after rectal administration when its blood level is significantly higher than that of sulphamethoxypyridazine. This means that the drug belongs to the group of low-dose long-active sulphonamides and has its due place among the antibacterial substances.

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