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CHEMOTHERAPY OF PSEUDOMONAS SEPTICAEMIA IN BURNED MICE: SYSTEMIC THERAPY WITH STREPTOMYCIN AND SULPHONAMIDES

BY

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The high incidence of pseudomonas septicaemia in severely burned humans was reported by Markley, Gurmendi, Mori-Chavez & Bazan (1957) from the Peru project, and its importance as a cause of death has been generally established. The importance of the burn wound as a portal of entry was shown by Walker, Mason & Raulston (1964), by McRipley & Garrison (1964) and by Jones, Jackson & Lowbury (1966); their studies revealed that a much smaller number of bacteria were required to produce fatal septicaemia if inoculated into the burn wound as compared with parenteral injection. The success of local therapy in the prophylaxis of septicaemia in burns is established (Moyer, Brentano, Gravens, Margraf & Monafo, 1965; Lindberg, Montcrief, Switzer, Order & Mills, 1965). In a recent publication (Rosenthal, 1967) we have standardized a simple method for producing fatal pseudomonas septicaemia in mice by immersion of the burned tail in a culture of these organisms of sufficient virulence; a procedure was also developed to permit application of local therapy by enclosing the tails in rubber tubes stapled to the skin at the base of the tail, and the effects of local chemotherapy were reported. With systemic administration the antibiotics usually used were found to have low therapeutic activity when employed in non-toxic doses. Streptomycin and sulphadiazine, administered systematically 6 hr after challenge, however, were highly effective against those strains that were sensitive to these drugs *in vitro*.

Previous work by Fust & Böhni (1962) and by DeLorenzo & Schnitzer (1959) showed that sulphadiazine was superior to several of the newer sulphonamide drugs in preventing death in mice from pseudomonas, where virulence was enhanced by the use of gastric mucin. A similar relation to *in vitro* sensitivity was shown by Neipp, Sacksmann & Tripod (1961), who emphasized, however, the lack of reliability of *in vitro* results as an index of therapeutic activity. This work has been reviewed by Schnitzer & Hawking (1964).

METHODS

Pseudomonas aeruginosa strain 180 (ATCC No. 19660) was used for most of these experiments, because it is sensitive *in vitro* to streptomycin and sulphadiazine and because it has maintained its virulence to mice for several years without animal passage.

Under ether anaesthesia the tails of mice (18–22 g) were burned by immersion in water at 70° C for 5 sec, and challenged a few hours later by dipping in a brain-heart broth culture of pseudomonas diluted with equal parts of sterile saline solution. A 6 hr culture accelerated by shaking was used. Other details have been previously reported (Rosenthal, 1967). *In vitro* sensitivity tests were made by the tube dilution technique in 5 ml. of brain-heart infusion broth (Difco), with an inoculum of approximately 10,000 organisms; readings were taken after incubation for 20 hr at 37° C, using almost complete inhibition as the endpoint.

The sulphonamide drugs were administered thoroughly mixed in the diet of pulverized pellets (Ralston). Therapy was begun 18 hr before the burn, in order to secure constancy of blood levels (Litchfield, White & Marshall, 1939), because the trauma may temporarily interfere with dietary intake; it was continued for 10 days after the challenge. Where individual doses were employed, streptomycin was administered subcutaneously and the sulphonamides orally by gastric tube. The animals were observed for 3–4 weeks; autopsies were carried out on all mice which died, and in those infected renal abscesses were found in nearly all mice which died later than the second day.

The drugs were either obtained from the manufacturers or purchased locally. Fifteen available sulphonamides were selected on a basis of representing various categories of chemical structure or pharmacological behaviour.

RESULTS

Comparison of various sulphonamides

Fifteen sulphonamides were given for 10 days in 0.1% concentration in the diet, to mice challenged with pseudomonas 180 by local inoculation of the burned tail.

In Fig. 1 the results of individual experiments are shown; in Table 1 the drugs are arranged in order of decreasing effectiveness. The order of activity ranged from complete protection to total inactivity; sulphadiazine was the most effective, with no mortality among fifty mice.

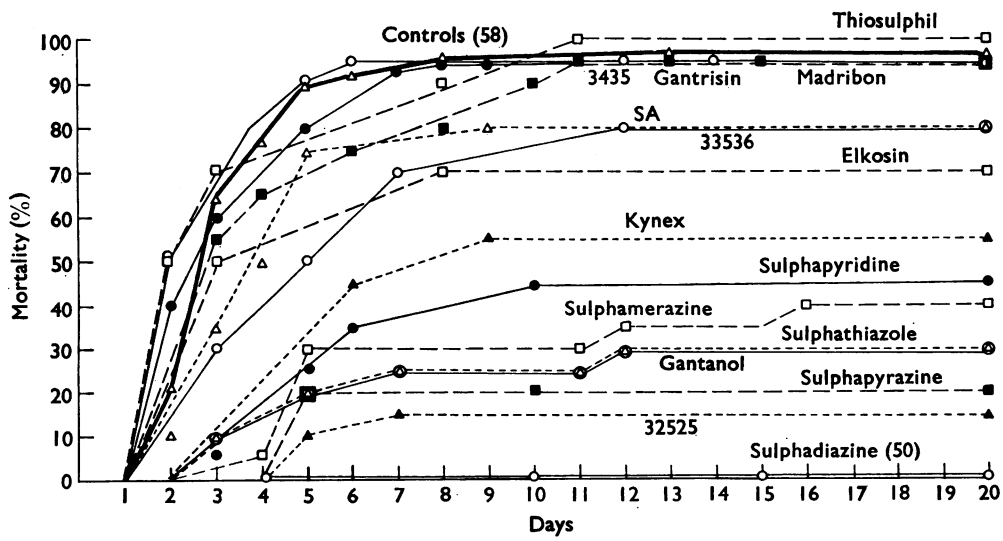


Fig. 1. Comparison of fifteen sulphonamide drugs prophylactically in pseudomonas septicaemia in mice. The drugs were administered in the diet at concentrations of 0.1%, begun the day before challenge and continued for 10 days. Twenty mice were used for each drug except where indicated by numbers in parentheses.

TABLE 1

RELATIVE EFFECTIVENESS OF FIFTEEN SULPHONAMIDE DRUGS ADMINISTERED IN THE DIET IN PSEUDOMONAS SEPTICAEMIA IN MICE, WITH A COMPARISON OF SURVIVAL *IN VIVO* AND LETHAL CONCENTRATIONS *IN VITRO*

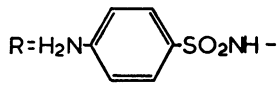
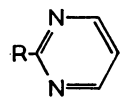
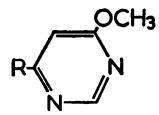
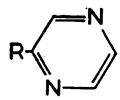
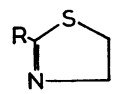
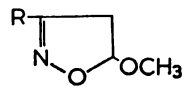
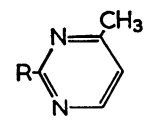
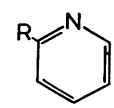
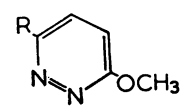
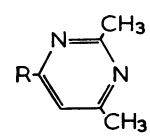
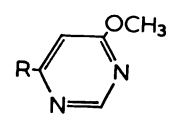
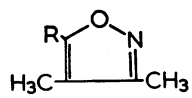
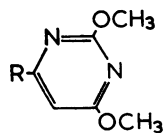
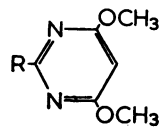
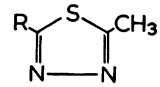
Sulphonamide	Structure	*In vivo* Survival (%)	*In vitro* (μg/ml.)
			
1. 2-Sulphanilamidopyrimidine. Sulphadiazine		100	25–50
2. 4-Sulphanilamido-6-methoxypyrimidine. I.C.I. 32525, Sulphamonomethoxine		80	250
3. *N'*-2-Pynazinylsulphanilamide. Sulphapyrazine		80	50
4. *N'*-2-Thiazolylsulphanilamide. Sulphathiazole		70	200
5. *N'*-(5 Methyl-3-isoxazolyl) Sulphanilamide. Gantanol		70	50
6. *N'*-(4-Methyl-2-pyrimidyl) Sulphanilamide. Sulphamerazine		60	75
7. *N'*-2-Pyridylsulphanilamide. Sulphapyridine		55	100
8. *N'*-(6-Methoxy-3-pyridazinyl) Sulphanilamide. Kynex, Midicil		45	250
9. *N'*(2,6-Dimethyl-4-pyrimidyl) Sulphanilamide. Elkosin		30	150
10. 4-Sulphanilamido-6-ethoxypyrimidine. I.C.I. 33536		20	1,000

TABLE 1—*continued*

Sulphonamide	Structure	*In vivo* Survival	*In vitro*
11. 4-Aminobenzene-sulphonamide Sulphanilamide		20	250
12. *N'*-(3,4-Dimethyl-5-isoxazolyl)-Sulphanilamide. Gantrisin		5	250
13. *N'*-(2,6-Dimethoxy-4-pyrimidyl)-Sulphanilamide. Madribon		5	>1,000
14. 2-Sulphanilamido-4,6-dimethoxypyrimidine. I.C.I. 3435		5	>1,000
15. *N'*(5 Methyl-1,3,4-thiodiazol-zyl)-Sulphanilamide. Thiosulphil		0	350
Controls (untreated)		3·4	

The relation of chemical structure to therapeutic action (Table 1) indicated in general that with the active compounds, introduction of additional methyl or methoxy groups was deleterious. The parent sulphanilamide showed low activity, which was improved by the introduction of a pyridine (sulphapyridine), a thiazole (sulphathiazole), or oxazole (gantanol), a pyrazine (sulphapyrozine), or a pyrimidine (sulphadiazine) radical.

A comparison of *in vitro* and *in vivo* activity was consistent only in that two compounds (Nos. 13 and 14) to which the organisms were highly resistant *in vitro* (greater than 1,000 μg/ml.) were devoid of activity. Among the active compounds, however, the correlation was poor; the marked superiority of sulphadiazine could not be predicted from its *in vitro* activity.

TABLE 2

COMPARISON OF THE ACTIVITY IN PSEUDOMONAS SEPTICAEMIA OF THE FOUR MOST ACTIVE SULPHONAMIDES, EMPLOYING DECREASING CONCENTRATIONS IN THE DIET

Sulphonamide	Concentration in diet (%)	No. of mice	20 day mortality (%)
Sulphadiazine	0·1	20	0
	0·05	20	0
	0·025	20	10
	0·01	20	30
I.C.I. 32525	0·1	20	15
	0·05	20	35
Sulphapyrazine	0·1	20	20
	0·05	10	80
Gantanol	0·1	20	30
	0·05	10	80
Controls	—	20	85

Relative potency of sulphonamides

The therapeutic activity of four of the more active compounds was compared with decreasing dose levels (Table 2). Sulphadiazine proved to be approximately 5 times as effective as the next most active drug, sulphamonomethoxine (I.C.I. 32525), and 10 times as active as sulphapyrazine or gantanol. The molecular weights of these compounds are sufficiently close that adjustment to a molar basis would not appreciably affect the results.

Delayed systemic therapy

The curative action of streptomycin and sulphadiazine given 1 or 2 days after challenge, when systemic invasion had presumably occurred, was tested. Streptomycin sulphate was administered subcutaneously, and sulphadiazine by stomach tube, each in doses of 0.1 g/kg, and repeated once daily for a total of five doses.

When therapy was started 24 hr after challenge, streptomycin and sulphadiazine each brought about 80% survival, while no deaths occurred with the combination of both drugs (Table 3). Started after 48 hr, streptomycin produced 61% survival, sulphadiazine 17%, and the combination 84%, while less than 7% of control animals survived.

TABLE 3

MORTALITY WHEN THERAPY WAS DELAYED FOR 1 OR 2 DAYS AFTER CHALLENGE WITH PSEUDOMONAS No. 180

Sulphadiazine was administered orally and streptomycin subcutaneously in doses of 0·1 g/kg and repeated once daily for five doses.

* 24% of the mice died before onset of therapy (or within 4 hr after). These were excluded from the experiments.

	No. of mice	Deaths in day					Total mortality (%)
		1–3	4–7	8–11	12–15	16–20	
Begun 24 hr after challenge							
Sulphadiazine	10			2			20
Streptomycin	10	1	1				20
S.D.+streptomycin	20						0
Controls	15	7	7				93·3
Begun 45 hr after challenge*							
Sulphadiazine	18	2		7	5	1	83·3
Streptomycin	18	2	1	2	2		39
S.D.+streptomycin	19				3		16·6
Controls	18	12	4			1	94·4

The additive effect obtained with combination therapy was also shown *in vitro*, where streptomycin was inhibitory at 10 μg/ml., sulphadiazine at 25–50 μg/ml. and the combination at 5 μg/ml. each.

Amputation of the tail was performed in groups of ten mice at intervals after infection, to obtain evidence of the time of systemic invasion. If the tail was amputated 24 hr hr after challenge with pseudomonas 80% survived, while none survived if amputation was done at 48 hr. This experiment also demonstrates that growth of the bacteria in the burn wound plays an essential part in the development of septicaemia.

Thus a combination of streptomycin and sulphadiazine can save most animals from an established pseudomonas septicaemia when the organism is sensitive to these drugs. Combination therapy, however, was found to be ineffective on pseudomonas strain 181, which has been previously shown to be resistant *in vitro* and *in vivo* to streptomycin and to sulphadiazine, when tested separately (Rosenthal, 1967).

DISCUSSION

Some earlier studies on the prophylactic use of streptomycin and sulphadiazine in human burns have been reported (Altemeier & MacMillan, 1962), but at that time the importance of pseudomonas septicaemia was not realized, nor was the frequent emergence of resistant strains known to be a consequence of continued therapy. With the development of polymyxin B and colistin, which possess high *in vitro* bacteriostatic activity (0.2 to 1 μg/ml.), these drugs largely replaced other agents, because satisfactory methods of assay in laboratory animals were not available at that time. By *in vivo* methods of assay, however, polymyxin, colistin, and gentamycin in experimental pseudomonas septicaemias are only effective when toxic doses are used (Millican, 1963 ; Rosenthal, 1967).

In the previous studies of experimental pseudomonas infections in mice, where gastric mucin was used to enhance virulence (Fust & Böhni, 1962 ; DeLorenzo & Schnitzer, 1959), four of the same sulphonamides used in our experiments showed similar activity. For sulphadiazine, the 50% curative dose was 5 mg/kg/day, for gantanol 12 mg, for kynex 39 mg, and for gantrisin 151 mg.

In comparing the activity of the various sulphonamides by the drug-diet method, no attempt has been made to establish the 50% curative dose, because the relative activity obtained in this survey was considered adequate. The dietary intake of the ground pellet diet, however, has been established as 3.77 ± 0.79 g over 24 hr for a mouse weighing 20 g (Litchfield, White & Marshall, 1939). On this basis, the daily dosage for drug concentrations of 0.1–0.01% would be 188–18.8 mg/kg. It is thus observed (Tables 1 and 2) that our results with sulphadiazine fell within the same range as those reported (Fust & Böhni, 1962 ; DeLorenzo & Schnitzer, 1959), while the other drugs were less effective. Millican (1963) using the mucin technique with the same strain (180) of pseudomonas as we used, found results with polymyxin similar to ours. He reported some effect from tetracycline, however, which was inactive in our experiments. Because the animals die within 12–24 hr after challenge when mucin is used, this technique may not be a valid replica of clinical infections.

No systematic survey of the activity of a large number of sulphonamide drugs in experimental pseudomonas septicaemia has previously been reported, and the present study, with a method more closely related to clinical infections, demonstrates the wide variations in activity that are encountered, as well as the superiority of sulphadiazine over all other sulphonamides tested. The present report also demonstrates that systemic treatment with a combination of streptomycin and sulphadiazine is capable of bringing about survival in well advanced pseudomonas septicaemia, if the organisms are sensitive to these drugs. It was previously suggested (Rosenthal, 1967) that when blood cultures are taken for suspected septicaemia in humans, an aliquot be placed in a tube containing streptomycin (50 μg/ml.) and another in a tube with sulphadiazine (50 μg/ml.), in order to test sensitivity to these agents as early as possible.

SUMMARY

1. An assay has been made of the therapeutic activity of fifteen sulphonamide drugs in experimental pseudomonas septicaemia in mice, produced by local challenge to the burned tail.

2. The activity with 0.1% of the drug in the diet ranged from complete protection to total inactivity. Sulphadiazine was approximately 5 times as effective as the next most active drug.

3. *In vitro* sensitivity showed a poor correlation with therapeutic activity.

4. Systemic therapy with a combination of streptomycin and sulphadiazine was shown to bring about survival of most of the animals, even when therapy was delayed until an established septicaemia was present.

5. This therapy offers promise of clinical effectiveness in those cases of pseudomonas septicaemia in which the organisms are sensitive to these drugs; however, it is without effect where there is a high degree of resistance.

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