Local and Systemic Therapy of Pseudomonas Septicemia in Burned Mice

SANFORD M. ROSENTHAL, M.D.

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

IN PREVIOUS WORK from this laboratory mice were rendered susceptible to *Pseudomonas aeruginosa* infection by pretreatment with cortisone,[6] by severe scalding [10] and by injection of the organisms in gastric mucin.[7] Recently it was demonstrated by Walker *et al.*,[14] McRipley and Garrison [4] and by Lowbury,[1] that burned animals were more susceptible to systemic invasion when pseudomonas was inoculated in the burned area than when the organisms were injected.

We have standardized the production of fatal septicemia in mice by local inoculation, and have developed a simple technic for the application of local therapy. With these procedures a preliminary study has been carried out on the comparative effectiveness of certain agents used for local and systemic treatment.

Recent reports indicate the effectiveness of local treatment of severe burns by the application of p-aminomethlybenzene sulfonamide (homosulfanilamide) ointment,[2] by wet dressings with 0.5% silver nitrate [8] and by gentamycin sulfate.[12]

Methods

A strain (NIH) of albino mice of 18 to 22-Gm. weight was used. No difference in response was noted between males and females. Burns were produced by immersion of the etherized animals in water at 70° C. Inoculation with *P. aeruginosa* was made by dipping the burned area in a diluted 18-hour broth culture of the bacteria. The culture was adjusted with 0.85% NaCl solution at wave lengths of 660 mμ on a Coleman spectrophotometer to an approximate optical density of 0.7, from which dilutions in 0.85% NaCl were made. Comparisons between washed bacteria and the broth culture showed essentially no difference in virulence, and most of the experiments were performed with diluted broth cultures.

When it was found that certain strains of pseudomonas would produce fatal infections from a burn involving only the tail, a method of applying local therapy was evolved by placing the tail in a section of rubber tubing and fastening the tube to the loose skin at the base of the tail with Michael clips. For mice under 25 Gm. weight soft latex tubing of $\frac{3}{16}$-inch inside diameter and $\frac{1}{32}$-inch wall was employed. This was cut in sections slightly longer than the tails (8 to 10 cm.), with the end to be stapled cut at a 45 degree angle; the projecting tip was pulled beyond the base of the tail for stapling to the skin. The distal part of the tube was closed with a small cork (size 00).

Most of the experiments were done with a strain of pseudomonas No. 180 (Verder type [13] 1a, 1c, 1d) isolated from the blood of a burned human with septicemia; this strain has been used in our previous studies. Preliminary studies were also done with several other strains of human or animal origin.

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In therapeutic studies the pseudomonas challenge was given within 2 hours after the burn, and therapy was administered 6 hours after the challenge. Local therapy was injected into the tubes in a hydrophylic cream base, and only one application was given. Systemic therapy was administered 6 hours after the challenge and repeated daily for four additional doses, or mixed in the diet of ground pellets. 4-homosulfamilamide acetate (Sulfamylon) was supplied in 8.5% concentration in a hydrophylic cream base, by Sterling Winthrop Chemical Co. Gentamycin sulfate was supplied by the Schering Corp. The remaining drugs were purchased.

Results

Mice were immersed for 3 seconds in water at 70° C. to the upper third of the thigh (approximately 25% of surface area); groups of 10 were challenged within 4 hours by dipping in Pseudomonas 180 cultures in dilutions ranging from 1 to 10,000. All animals succumbed within 7 days. This susceptibility was still present when the challenge was delayed 24, 48 and 72 hours, with a 1 to 50 dilution of the culture. With this burn only 1 of 30 mice without challenge died (8th day). No deaths occurred in unburned mice challenged with a 1 to 1 dilution.

When the tail was immersed to the base in water at 70° C. for 3, 5 and 8 seconds, the maximum susceptibility was produced by 5 seconds, and this was used in subsequent experiments. When challenged within 4 hours after the burn with a 1 to 1 dilution of culture of Strain 180, the mortality in 158 mice was 38% in 3 days, 84% in 7 days and a total mortality of 93% in 21 days. In a single experiment (10 mice each group) the mortality was 100% when the challenge was delayed 24 hours, 40% when challenged after 72 hours, 20% after 96 hours, 10% after 5 days and zero after 7 days. Dilution of the culture greater than 1 to 1 has given variable results in mortality with the tail burn. No deaths occurred in unchallenged mice.

Renal abscesses were invariably present in animals dying on the third day or later, and were frequently observed with a magnifying glass on the second day. The pathology of pseudomonas septicemia has been described in mice,[5] rats[2] and humans.[3]

Local Treatment

Sulfamylon (homosulfanilamide acetate) cream (8.5% concentration) injected locally into the tube produced 92% survival in 25 mice (Table 1). Chloramphenicol applied in the same vehicle produced 80% survivors in 0.14% concentration, and 100% in 0.25% strength. Gentamycin at 0.5%, neomycin at 0.4%, streptomycin at 1% concentrations likewise gave complete protection. Sulfadiazine at 0.5% concentration was also highly effective.

Colistin (Colimycin) 0.1%, polymyxin 0.05 and 0.2%, penicillin G 0.25%, silver nitrate 0.5%, and tetracycline 0.25% were without effect. Silver nitrate may be rapidly neutralized by the local exudate from the burn or by the vehicle employed, and further work must be done to evaluate its effectiveness. The hydrophylic cream alone did not influence survival.

It is thus observed that six of the drugs employed were highly effective locally, and a survey of others would undoubtedly demonstrate many more. The required concentration of gentamycin (0.5%) might involve hazards from systemic toxicity when applied to large areas of burned surface.

The tubes frequently came off after the first week but this did not affect survival; this is in accord with the absence of mortality when the challenge was delayed 7 days.

Delayed Local Therapy. Lindberg *et al.*[2] reported a high degree of protection with Sulfamylon when therapy was delayed for

24 to 72 hours. We have done one series of experiments with sulfamylon and chloramphenicol in which therapy was delayed for 24 hours. Survival in both cases was reduced to 60%.

Systemic Therapy

Experiments were carried out with the oral or parenteral injection of drugs to evaluate systemic treatment of pseudomonas infection. Most of the studies were done

TABLE 1. *Mice with Tail Burn Challenged with P. aeruginosa, Strain 180, 1 to 1 Dilution. Local Therapy Inserted into Tube Enclosing Tail, 6 Hours After Challenge*

Drug	No. Mice	Deaths Days After Burn 1–3	4–7	8–11	12–15	16–21	Mortality (%)
Sulfamylon							
8.5%	25	1			1		8
Chloramphenicol							
0.14%	20		2	1	1		20
0.25%	20						0
Gentamycin							
0.03%	10	2	6	1			90
0.06%	20		6	4			50
0.15%	10		2				20
0.25%	10		2	2	1		50
0.5%	10						0
Neomycin							
0.17%	10		3	3			60
0.4%	10						0
Streptomycin							
0.25%	10		4	1	1		60
0.5%	10			1			10
1.0%	10						0
Sulfadiazine							
0.5%	10				1		10
Polymyxin							
0.05%	10	2	6	1			90
0.2%	10	1	5	1			70
Colistin							
0.1%	10	2	4	2		1	90
Silver nitrate							
0.5%	20	8	8	4			100
Tetracycline							
0.25%	10	2	7	1			100
Penicillin G							
0.25%	10	4	3	1			80
Hydrophylic cream	10	5	3	1			90
Controls	158	61	71	12	2	1	93

with Pseudomonas Strain 180 challenge and the tail burn. Systemic therapy was begun 6 hours after the challenge and no tubes on the tails or local therapy were used.

It was found that streptomycin subcutaneously and sulfadiazine orally in 5 daily doses (or in the diet) were highly effective in preventing death (Table 2). Chloramphenicol, although effective locally, was without benefit on oral or intraperitoneal administration. Colistin and gentamycin had little effect in doses within the clinical range, while polymyxin at 10 mg./Kg. brought about 50% survival. Human gamma globulin produced 60 to 80% sur-

TABLE 2. *Systemic Therapy Administered 6 Hours After Challenge with Strain 180, and Repeated Daily for Four Additional Doses*

Drug mg./kg.	No. Mice	Deaths Days After Burn 1-3	4-7	8-11	12-15	16-21	Total Mortality (%)
Sulfadiazine							
100 (oral)	10						0
150	10						0
500	20						0
0.1% in Diet	10						0
Streptomycin							
100 (s.c.)	20			1	1		10
Gentamycin							
2.5 (s.c.)	10	2	8				100
5.8	10	2	5				70
10	10	1	4	2			70
15	10	2	1	1			40
Neomycin							
15	10	2	6				80
Chloramphenicol							
50 (oral)	10	2	7				90
150	10	5	2	1			80
50 (i.p.)*	10	6	2	1			90
Colistin							
2.5 (s.c.)	10	4	5				90
10	10	5	3	1			90
Polymyxin							
2.5 (s.c.)*	10	10					100
10	10		2	2			40
Tetracycline							
150 (oral)	10	8	2				100
0.1% in Diet	30**	13	16	1			100
Gamma globulin human							
100 (i.p.)	10		1	1			20
400	10		2	2			40
Controls	158	61	71	12	2	1	93

* 25% burn.

** 10 each with tetracycline, oxy-, and chlortetracycline.

TABLE 3. *Relation of in vitro Sensitivity to in vivo Action of Drugs With Pseudomonas Strain 180*

Drug	Minimal Inhibitory Concentration (mcg./ml.)	% Survival			
		Local Therapy		Systemic Therapy	
		Dosage (%)	Survival (%)	Dosage (mg./Kg.)	Survival (%)
Colistin	1.0	0.1	0	10	0
Polymyxin	1.0	0.2	10	10	60
Gentamycin	1.0	0.25	50	10	30
Tetracycline	5	0.25	0	150	0
Chlortetracycline	50			150	0
Oxytetracycline	5			150	0
Neomycin	5	0.4	100	15	0
Chloramphenicol	25	0.25	100	50–150	0
Sulfadiazine	25	0.5	90	100–500	100
Streptomycin	25	0.5	90	100	90
Diaminodiphenylsulfone	100			100	0
Homosulfanilamide	625	8.5	92	1000	0
Penicillin G	625	0.25	0	200	0

vival, a result similar to that obtained in previous experiments with other technics.[7, 10] Penicillin G, 200 mg./Kg. orally, diaminodiphenylsulfone 100 mg./Kg. orally or 0.04% in the diet, homosulfanilamide HCl 1 Gm./Kg. orally, and the tetracyclines, were without benefit. The animals receiving tetracyclines and diaminodiphenylsulfone died appreciably faster than their corresponding controls, suggesting the possible demonstration of a broad spectrum antibiotic bringing about an increased susceptibility to pseudomonas invasion; this possibility has been frequently stressed in the clinical literature.

Systemic Therapy with Other Strains of Pseudomonas. In order to confirm the curative action of streptomycin and sulfadiazine, two other strains were selected on a basis of being sufficiently virulent to kill mice with a 25% surface area burn by local challenge, and sensitive to these drugs *in vitro* at 25 to 50 mcg./ml. Strain Mills (Verder Type 4,4,5) was from a human source, while 13 RA was isolated by Dr. R. C. Millican from a blood culture of an irradiated mouse. Therapeutic tests with streptomycin and sulfadiazine systemically on these strains (Table 4) demonstrated an activity similar to that with Strain 180.

One strain of pseudomonas (No. 181) was sufficiently virulent to kill mice with the tail burn, but it was insensitive to streptomycin and sulfadiazine (> 625 mcg./ml.). Certain of the drugs tested against Strain 180 were used against this strain. Streptomycin, sulfadiazine, chloramphenicol, chlortetracycline, and colistin were inactive systematically. Gentamycin (10 mg./Kg. s.c.) produced 60% survival, and polymyxin in the same dosage 100% survival.

Relation of *in vitro* Sensitivity to *in vivo* Effect

While some correlation existed between *in vitro* sensitivity of Pseudomonas 180 to antibacterial agents and *in vivo* effect, several important exceptions were present. The *in vivo* effect should be considered along with the dose range employed, as shown in Tables 1 and 2; because of limitations due to toxicity these vary widely, and it must be pointed out that insufficient data have been obtained for an exact analysis.

Of the three antibitotics with the highest *in vitro* activity against pseudomonas 180 (1 mcg./ml.), only gentamycin exhibited some local action; systemically, polymyxin and gentamycin were moderately effective in maximum tolerated doses (Table 3).

TABLE 4. *Systemic Therapy with Streptomycin and Sulfadiazine Administered in 5 Daily Doses, Beginning 6 Hours After Challenge; 25% Body Surface Burn and 1 to 1 Dilution of Organisms*

Pseudomonas	Minimum Inhibition Concentration (mcg./ml.)	No. Mice	Deaths in Days					Mortality
			1–3	4–7	8–11	12–15	16–21	
Strain Mills								
Streptomycin	25							
100 mg./Kg. (s.c.)		10		1	1			20
Sulfadiazine	25–50							
0.5 Gm./Kg. (oral)		9			1	1		22
0.05% in diet		10						0
0.1% in diet		10						0
Controls		20	14	3		1		90
Strain 13-RA								
Streptomycin	25							
100 mg./Kg. (s.c.)		9			1	1		22
Sulfadiazine	25–50							
0.5 Gm./Kg. (oral)		9			1		1	22
0.05% in diet		10		1				10
0.1% in diet		10						0
Controls		20	10	5	1		3	95

Next in order of sensitivity were tetracycline and neomycin; the former was inactive and the latter showed only local activity. Chloramphenicol (25 mcg./ml.) was quite effective locally, but without systemic action. Sulfadiazine and streptomycin exhibited the same sensitivity as chloramphenicol, but with similar dosage both were effective locally as well as systemically. Homosulfanilamide had a low *in vitro* activity (625 mcg./ml.) but was quite effective locally in the high concentration used; Lindberg *et al.*[2] have reported the *in vitro* sensitivity to range from 78 to 625 mcg./ml. Penicillin and diaminodiphenylsulfone were without *in vitro* or *in vivo* activity.

The clinical use of drugs for pseudomonas septicemia has been largely guided by *in vitro* tests of sensitivity, since few studies have been carried out in experimental infections in animals. Our results indicate that *in vitro* tests are not a reliable index of therapeutic activity; this is borne out by the fact that clinical results with the antibiotics now in use have been uniformly unsuccessful.

Discussion

These experiments were designed to afford a simple method for comparing the relative effectiveness of various drugs against pseudomonas infections. The duration of therapy and the dosage employed were not necessarily optimum for survival, but afford an index of their relative effectiveness.

This preliminary survey of therapy reveals that a considerable number of drugs are effective locally in the prevention of death from pseudomonas septicemia. The choice must depend upon considerations of toxicity and the frequency of development of resistant strains.

For systemic treatment streptompcin and sulfadiazine were the only agents that were highly effective in doses that are not hazardous. Since they are prone to the development of resistant strains, it would

seem advisable to restrict their use in a hospital ward to treatment of septicemia. It is suggested that when blood cultures are taken aliquots be placed in two tubes of broth containing 50 mcg./ml. of streptomycin and 50 mcg./ml. of sulfadiazine or other sulfonamide. Should the strain be resistant to both drugs, our studies would indicate that polymixin in maximum tolerated doses (10 mg./Kg.) would be the present alternative.

Pulaski and Sprinz [9] surveyed the sensitivity to streptomycin of 98 strains of pseudomonas and found that 84% fell within the range of 2 to 32 mcg./ml.

Since renal lesions are a feature of pseudomonas septicemia, a sulfonamide more soluble than sulfadiazine might be preferable.

We have developed a technic for measuring healing of the burn wound by determining the length of viable tail.[11] A good correlation has been found between the effectiveness of local or systemic therapy and viable tail length. This indicates that streptomycin and sulfadiazine in systemic treatment not only prevent septicemia but also suppress the local infection.

Summary

A method for the production of systemic pseudomonas infections in burned mice is described and also a technic for the supplication of local therapy to the burned tail.

A preliminary survey of therapeutic agents applied locally revealed that homosulfanilamide, chloramphenicol, streptomycin, sulfadiazine, neomycin and gentamycin were highly effective in preventing death from infection. Colistin and polymyxin locally were inactive in the concentrations used.

Systemic treatment with streptomycin and sulfadiazine resulted in survival of nearly all animals when the infecting organisms were sensitive to these drugs. Polymyxin was moderately active but the dosage required approached the limit of tolerance.

In vitro activity is not a reliable index of therapeutic effect.

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