PAROMOMYCIN, POLYMYXIN B AND FURAZOLIDONE IN EXPERIMENTAL CHOLERA

By

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Paromomycin, polymyxin B and furazolidone were tested for their ability to inhibit the growth of *V. Cholerae* and their effectiveness in curing rabbit cholera. All the three drugs possess powerful vibriostatic and vibriocidal action. Paromomycin and furazolidone could cure about 50 per cent of the animals suffering from cholera if the treatment was instituted early. Polymyxin B on the other hand possesses only preventive but no curative value. The usefulness of these drugs in clinical cases is discussed.

Cholera may exhibit a range of signs and symptoms from mild diarrhoea to its severe form, the collapse. But the one which attracts the physician' attention follows a characteristic pattern. After an incubation period of twos to five days, the patient generally starts with severe diarrhoea, accompanied or more commonly, followed by vomiting. The stools become watery (rice water) and are excreted in bulk. Acidosis developes unless the water and electrolytes are restored and the case may terminate fatally within a few hrs or days. Such a condition calls for immediate treatment. While quick attention in replacing fluid loss and electrolyte imbalance will benefit a large number of patients, yet there is no drug today which claims to have cured the disease. Experimentally, it has been demonstrated that chloramphenicol neomycin, tetracyclines, etc. may be of value if administered at the early stage of the disease (Dutta and Habbu, 1955 ; Dutta and Colah, 1958, and Dutta, Colah and Vaidya, 1958),but not so when the disease has advanced. The clinical observations also indicate so. A search for a more effective remedy against cholera has, therefore, been continued. Paromomycin, polymyxin B and furazolidone have been chosen for the present study.

Paromomycin is an antibiotic derived from a strain of *Streptomyces rimosus* forma *Paramomycinus*. Chemically it is a glycoside of d-glucosamine. It is water soluble and possesses marked antibacterial activity against a number of gram negative and gram positive bacteria.

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Polymyxin B is obtained from *B. polymyxa*; polymyxin B sulphate is freely soluble in water. It is useful against many gram negative organisms. It is poorly absorbed from gastrointestinal tract and its use has been recognized in the treatment of intestinal disorders such as pseudomonas enteritis, shigellosis and other enteric diseases. It may also be given parenterally,

Furazolidone (3-(5-nitro furfurylideneamino)-2-oxazolidinone ; N-5-nitro-2-furfurylidene)-3-amino-2-oxazolidone) occurs as a yellow crystalline powder, sparingly soluble in water. It displays a wide spectrum of antimicrobial activity. It has inhibiting activity against *E. histolytica* and *Trichomonas foctus*. It is used in Salmonellosis.

METHODS

Bacteriostatic or bactericidal properties.—These tests were carried out by the serial dilution method in which the total volume of the casein hydrolysate medium in each tube was kept constant (10 ml) but the concentrations of the compounds to be tested were varied. All dilutions were made in a protein free casein hydrolysate medium (Sokhey *et al.*, 1950). Rabbit passaged strain of *V. Cholerae*, (Inaba, 569 B), was used as the test organism. The potency of each drug was tested against 10^4 vibrios per ml.

Test on rabbit cholera.—The method of Dutta and Habbu (1955) was followed. The rabbit passaged strain of *V. Cholera* (Inaba, 569 B) was preserved in a lyophilised state. Before each experiment, the vibrios were regenerated, suitably cultured and finally administered to 10-day old rabbits intraintestinally in a dose of 10^4 vibrios per 100g of body weight. The treatment was begun either before or after inoculation of vibrios but the drugs were given every 2 to 6 hrs. A total of 6 or 9 doses were administered orally depending on the condition of the animals. With each set of experiments, control experiments were carried out in which the infected animals were given no treatment. In order to confirm that the death was due to cholera, post mortem examination was done wherever required.

RESULTS

The vibriostatic and vibriocidal properties of paromomycin, polymyxin B and furazolidone are illustrated in the Table I. All were powerful vibriocides against *V. Cholerae*. They were effective in concentrations of 5 to 25 μg/ml.

In Table II it will be seen that when the treatment with paromomycin was begun 1 hr before or 8 hr after infection, it prevented all the signs and

symptoms of the d sease from appearing. All the 4 animals remained normal although the control rabbit died at the end of 30 hr When the treatment was started 16 hr after infection using a dose of 25 mg per 100 g of body weight, about 44 per cent of the animals survived. In 7 animals there were no symptoms except that they appeared to be ill for sometime. When the dose of the drug was raised beyond 25 mg and up to 100 mg and the intervals of administration were reduced from 4 hr to 2 hr, no additional benefit was seen. On the contrary, all the animals so treated, with the exception of one never recovered.

In the case of polymyxin B (Table III), all animals survived when the administration of the drug began 8 hr after infection or earlier with a dose of 100 mg/kg. With a higher dose of 250 mg/kg, none of the anima remained alive when the treatment began 16 hr after vibrio inoculation.

Furazolidone (Table IV) in a dose of 100 mg/kg protected all the animals when the drug was given 1 hr before or 8 hr after infection. This dose, however, was ineffective when the administration of the drug first began at a later period, i.e., 16 hr after infection. When the dose was raised still further to 500 mg/kg, furazolidone afforded protection to 50 per cent of the animals. Further increase of the dose to 1 mg/kg had no benefit.

The animals which had received no treatment but were infected with *V. cholerae* died without exception with typical signs and symtoms of cholera.

TABLE I

Paromomycin, polymyxin B and furazolidone against 10^4 V. cholerae/ml

Drugs	Vibriostatic conc.	Vibriocidal conc.
Paromomycin	5 μg/ml	10 μg/ml
Polymyxin B	10 μg/ml	25 μg/ml
Furazolidone	10 μg/ml	25 μg/ml

TABLE II

Action of paromomycin (oral) on infant rabbits infected with V. cholerae (Inaba 569 B) 10^4/100 g body weight

Treatment started (hr before or after inf.)	Treated infected rabbits: Dose/100 g	Mean time of onset of diarrhoea (hr)	Mean survival time hr)	Mortality	Untreated infected rabbits (control): Mean time of onset of diarrhoea (hr)	Mean survival time (hr)	Mortality
1 hr before	10 mg. 6 hrly, 8 doses	——	——	0/2	22	30	1/1
8 hr after	—do—	——	——	0/2			
16 hr after	25 mg, 4 hrly, 8 doses	18 (9)	23	9/16	18	20	1/1
16 hr after	30 mg, 4 hrly, 7 doses	25 (2)	32	2/2	19	23	1/1
16 hr after	50 mg, 4 hrly, 7 doses	25 (4)	36	4/4	20	25	1/1
16 hr after	100 mg, 4 hrly, 6 doses	26 (3)	30	3/4	18	28	1/1
16 hr after	100 mg, 2 hrly, 9 doses	20 (4)	31	4/4	20	28	1/1

Figures in the parenthesis indicate the number of animals suffered from diarrhoea

TABLE III

Action of polymyxin B (oral) on infant rabbits infected with V. cholerae (Inaba, 569 B) 10^4/100 g body weight

Treatment started (hr before or after inf.)	Treated infected rabbits. Dose/100 g	Mean time of onset of diarrhoea (hr)	Mean survival time (hr)	Mortality	Untreated infected rabbits (control) Mean time of onset of diarrhoea (hr)	Mean survival time (hr)	Mortality
1 hr before	10 mg, 6 hrly, 8 doses	——	——	0/4	22	32	1/1
8 hr after	10 mg, 6 hrly, 8 doses	——	——	0/4	20	30	1/1
16 hr after	25 mg, 4 hrly, 5 doses	20	31 (6)	6/6	18 (2)	28	2/2

Figures in parenthesis indicate the number of animals suffered from diarrhoea

TABLE IV

Action of furazolidone (oral) on infant rabbits infected with V. Cholerae (Inaba 569 B) 10^4/100 g body weight

Treated infected rabbits					Untreated infected rabbits (control)		
Treatment started (hr before or after inf.)	Dose/100 g	Mean time of onset of diarrhoea (hr)	Mean survival time (hr)	Mortalit	Mean time of onset of diarrhoea (hr)	Mean survival time (hr)	Mortality
1 hr before	10 mg, 6 hrly, 8 doses	——	——	0/2	22	30	1/1
8 hr after	10 mg, 6 hrly, 8 doses	1	——	0/4	18	20	1/1
16 hr after	10 mg, 6 hrly, 8 doses	23 (4)	33	4/4	19	24	2/2
16 hr after	50 mg, 6 hrly, 7 doses	24 (2)	34	2/4	22	32	1/1
16 hr after	100 mg, 6 hrly, 5 doses	28 (4)	32	4/4	18	34	1/1

Figures in parenthesis indicate the number of animals suffered from diarrhoea

DISCUSSION

Before attempting to interpret the results it would be necessary to examine the infant rabbit model in relation to other methods commonly used in the laboratory for evaluation of drugs against cholera. In this respect mice have been used extensively. Rao and Ganapathi (1941), Griffitts (1942), Bhatnagar (1948) and others (reported by Pollitzer, 1960) have demonstrated the usefulness of sulphonamides in protecting mice against cholera. Felsenfeld and Soman (1952) observed that poorly absorbed antibiotics such as streptomycin, neomycin and bacitracin were of no use in mice infected with *V. cholerae*, but chloramphenicol and chlortetracycline gave better results and oxytetracycline, the best, with 98 per cent survival. In mouse cholera where vibrios are injected intraperitoneally with mucin, one deals with a condition of bacteraemia. Any drug which possesses vibriostatic or vibriocidal action should be useful provided it is absorbed in the system. It is natural that substances like streptomycin, neomycin and bacitracin, which are not easily absorbed, would but be ineffective in the mouse infection.

Felsenfeld and Soman (1952) have used monkeys to demonstrate the effect of drugs in cholera. They showed that *Macaca rhesus* heavily infected with *V. cholerae* intragastrically could be saved when treated with oxytetracycline or neomycin either given orally or parenterally.

Collier, Hall and Waterhouse (1949) had developed the "mouse faecal suspension test" for evaluation of remedies for cholera, but the method had no relation to clinical cholera.

Burrows (1953) used guineapigs to demonstrate the efficacy of sulphonamides, giving each drug to groups of cholera infected animals. He found that sulfadiazine and sulphaguanidine reduced the total number of bacteria and percentage of cholera vibrios in the stools.

In clinical cases, the vibrios remain localized within the gut and the individual suffers from massive loss of fluid and extreme dehydration which are the primary reasons for death. Neither the mice or the guineapigs nor the monkeys exhibit such symptoms. A parallel condition, however, is seen in infant rabbits infected intraintestinally with *V. cholerae* (Dutta and Habbu, 1955). In the rabbit, one observes the fatal diarrhoea with characteristic rice water stools (Jenkin and Rowley, 1959) and other morphologic changes in the gut (Norris, Dutta, Finkelstein, Formol and Sprinz, 1963) akin to human biopsies. The vibrios too remain within the intestines (Dutta and Habbu, 1955).

Now turning towards the therapeutic tests as a criterion in judging the experimental model for its closeness to the human disease, one would observe that both the sulphonamides and the antibiotics were effective in mice, monkeys and guineapigs, but no such actions of the drugs have been seen in well developed clinical cases. In rabbit cholera too, these drugs had little value once the diarrhoea had set in. In short, both from the point of symptomatology and the results of drug treatment in clinical cases and in the rabbit cholera, one would like to conclude that there is no behavioural differences between the two models.

In the rabbit infected with *V. cholerae*, diarrhoea makes its appearance, on an average, at the end of 24 hr and the animal lives for another 10 or 12 hr. In this study, as well as in earlier investigations, the schedule of treatment has been arbitrarily divided into four groups. To the first group, the treatment began 1 hr before infection, to the second 8 hr after infection, to the third 16 hr after and in the last, the therapy begun only after the onset of diarrhoea By drawing an analogy from human cases where the incubation period is greater and the physician gets more time to treat, it may be presumed that "one hr before infection" treatment schedule would correspond to the prophylactic value of a drug. If there be any benefit from treatment which begins 8 or 16 hr after infection then one may perhaps presume, it has been due to treating an early case of cholera. Drug therapy in a rabbit where diarrhoea has set in would indicate, as if, treating a well established case of cholera. None of the drugs used here, i e., paromomycin, polymyxin B and furazolidone have proved their usefulness once the diarrhoea had begun. Nevertheless, they could be used in the prevention of disease in contact cases Some benefit was noted when the treatment had started 8 or 16 hr after infection, with paromomycin or furazolidone. Or in other words they would be of some value if given at the early stage of clinical cholera.

Generally speaking by the time the patient offers himself for treatment as a cholera case, it is probably too late for any vibriostatic or vibriocidal drug to have any real benefit. By that time there has been a massive multip-li cation of vibrios followed by lysis with the release of cholerogenic material (s). Wheather the latter is secreted or leached out of the cells is yet to be decided. The manifestatioan of the disease is due to the local and systemic actions of the released material(s). Under the circumstances, bacteriostatic and bactericidal actions of the drug will have a limited value in arresting the further growth of the bacteria and freeing the stools of vibrios earlier, provided the subject survives. Work of Dutta and Panse (1963) that berberine which has no vibriostatic or vibriocidal actions could as well be effective as

sulphonamides or antibiotics in the treatment of experimental cholera confirms such presumption. Therefore, the real treatment of cholera still lies in fluid and electrolytes replacement. Paromomycin and polymyxin B may at least be used as supportive treatment.

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