LABORATORY AND CLINICAL STUDIES OF POLYMYXIN B AND E[1, 2]

By EDWIN J. PULASKI,[3] HINTON J. BAKER,[3] MILTON L. ROSENBERG,[4] and JAMES F. CONNELL, JR.[4]

(From the Surgical Research Unit, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas)

The antibiotics, polymyxin A B C D and E, have been described in the recent literature. Their antibacterial spectra are similar. They differ from one another in amino acid content. It is the purpose of this presentation to describe our studies of the antibacterial activity, pharmacology, untoward reactions, and clinical experience with polymyxin B and polymyxin E.[5]

IN VITRO ACTION

We found that the susceptibility to polymyxin of 78 strains of ten different genera by the tube dilution test (Table I) is marked against Salmonella, Shigella, Klebsiella, microorganisms of the *coli aerogenes* groups, and most importantly, Pseudomonas. Brucella and many staphylococci are moderately sensitive, while Proteus and hemolytic streptococci are refractory. It is of interest that a strain of Pseudomonas, which developed high resistance to streptomycin in three daily transfers, did not become resistant to polymyxin after 27 transfers. Size of inoculum and human serum reduced but slightly polymyxin activity. The concentration of polymyxin required to inhibit Klebsiella and Pseudomonas is not reduced significantly by the addition of subinhibitory amounts of streptomycin, aureomycin, sulfadiazine or penicillin, either singly or in several combinations. Polymyxin antagonizes the cumulative streptomycin-penicillin action on Proteus.

The administration of polymyxin to patients in divided doses by the intramuscular route in a daily dosage of 2 to 4 mgm. per kilogram of body weight results in blood serum concentrations of 1 to 8 μg. per milliliter. The peak occurs 30 minutes to two hours following injection; one-half the peak level is noted at six hours, and the drug can be detected in blood serum up to 12 hours. When the injections are repeated at six-hour intervals, blood serum levels in excess of 30 μg. per milliliter may be obtained after four doses. No cumulative increase in blood levels has been noted when the drug is administered every 12 hours in a dosage of 1 mgm. per kilogram. Polymyxin is excreted in the urine less rapidly than penicillin and streptomycin. The amount detected in the first 12 hours after injection is less than 0.1% of the injected dose. After 12 hours, excretion increases progressively (Figure 1). On a dosage of 3 mgm. per kilogram of body weight per day levels in the urine range between 40 and 400 μg. per milliliter after 24 hours of therapy.

TABLE I

Sensitivity to "aerosporin" polymyxin B

Organism	No. strains tested	Sensitivity range in μg./ml.	Mean sensitivity μg./ml.
Salmonella	5	0.08– 0.3	0.18
Shigella	7	0.08– 0.6	0.3
Klebsiella	5	0.16– 1.2	0.48
Coli-aerogenes	17	0.06– 1.6	0.8
Pseudomonas	19	0.3 – 3.1	1.2
Paracolobacter	2	1.2 – 1.6	1.4
Staphylococcus	6	1.2 – 5.0	2.0
Brucella	8	0.6 –12.0	4.1
Beta hemolytic streptococcus	6	3(1).–>12.0	>12.0
Proteus	5	100.–>100.	>100.
Total	78		

We have noted that less than 1% polymyxin passes through Zeitz or sintered glass ultra fine filters. It is practical to sterilize urine for assay by heating the sample to 60° C. for one hour.

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[3] Major, M.C., United States Army.

[4] Captain, M.C., United States Army.

[5] Kindly supplied by Burroughs Wellcome and Company.

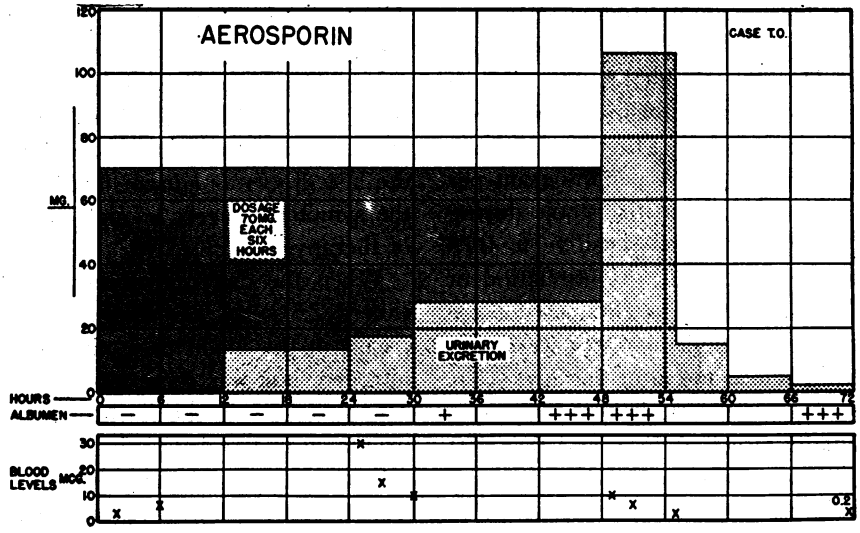

FIG. 1

Solutions of the drug are not inactivated by this treatment, but loss of potency is noted after heating to 100° C. for ten minutes. Cysteine does not appear to antagonize polymyxin activity.

CLINICAL EXPERIENCES

The therapeutic effectiveness of polymyxin has been tested at Brooke General Hospital in a series of patients with infections of the urinary tract and infections in granulating wounds. Also, the drug orally administered has been evaluated as an intestinal antiseptic in preparation of the bowel for surgery.

Twenty patients with severe urinary tract infections were treated with polymyxin intramuscularly administered over periods of one to six days in dosages of 2 to 5.6 mgm. per kilogram of body weight per day, divided in two, four, or six doses. The average, and we now believe the optimum dose, was 2.5 mgm. per kilogram per day divided in four doses for three days. Pain at the site of injection was common, but obviated by solution of the drug in 1% aqueous procaine. Nineteen of the 20 patients had extended previous trials of other chemotherapy. Ten of the 20 patients were regarded unequivocally as *benefited*. Improvement in most instances was observed after 24 hours, as manifested by marked reduction or elimination of bacteria from the urine, regression of symptoms, decrease in fever and gross and microscopic improvement in the urine. In all patients listed as benefitted, there was no bacteriological relapse up to three weeks after cessation of treatment. The most dramatic responses are obtained in polymyxin-sensitive, acute pseudomonas pyelonephritis. Usually, 12 hours after polymyxin therapy is begun the infection appears under control. The results of therapy in seven of the 20 patients are considered doubtful, either because there was little symptomatic improvement, or the urine was not rendered completely or permanently bacteria-free. In three of the 20 patients treatment was regarded as an outright *failure*. Naturally drug-resistant organisms were present in one and multiple cortical abscesses in scarred kidneys in the other two.

It is of importance that drug resistance did not develop in any of the cultures of these patients. There can be little doubt of the therapeutic effectiveness of polymyxin for the treatment of urinary tract infections produced by susceptible gram-negative bacilli, particularly acute pseudomonas pyelonephritis.

Polymyxin orally administered does not produce detectable blood levels. It exerts a rapid bacteriostatic action on most coliform bacteria, as well as on some types of cocci. Solutions of the drug were administered by mouth to 14 subjects in doses of either 200 or 400 mgm. per kilogram per day. All coliform organisms except Proteus were suppressed in these 14 subjects within 24 to 72 hours (Figure 2). Cocci were inconstantly reduced in

number, while Clostridia and Monilia were unaffected. Suppression of coliforms was maintained for 11 to 24 days, at which times the drug was withdrawn. Coliform organisms returned one to six days following cessation of therapy. In only one subject ingesting 2.5 mgm. per kilogram per day did the *E. coli* reappear in the stools during treatment. This organism was sensitive to 0.78 μg. per milliliter of polymyxin. Continuation of therapy for five additional days eliminated these bacteria. All coliform organisms tested were sensitive to polymyxin before and after therapy. It is apparent at this stage of the study that 200 mgm. a day is a minimally effective dose, and that 400 mgm. is a more consistently suppressive dose.

Several granulating wounds infected with *Ps. aeruginosa* were treated by the topical application of 1% polymyxin in salt solution or carbowax base, with eradication of the organisms in all instances, except where devitalized tissue was present.

TOXICITY

No patients in this series developed any of the usual drug sensitivity reactions. No evidence of toxicity was noted following topical or oral administration of the drug. In the case of intramuscular injection, in nearly every instance neurologic disturbances were present, as manifested by paresthesias and hypesthesias mainly about the face and scalp, mild dizziness and weakness. These symptoms usually appeared with the first dose, and persisted throughout the course of treatment. The only objective neurologic abnormality was circumoral flush. Neurotoxic symptoms disappeared in every instance within 24 hours after the last dose was administered. In no case were the symptoms severe enough to warrant cessation of therapy.

When the daily intramuscular dosage did not exceed 2.5 mgm. per kilogram, there was no increase in the blood urea nitrogen or non-protein nitrogen, nor was there any clear-cut evidence of nephrotoxicity in the form of tubular damage attributable to the drug. It is reiterated that most subjects given the drug initially had abnormal urines. Patients with normal urines did not present nephrotoxicity after three to five days on a dosage of 2 mgm. per kilogram of body weight. When the dosage was 4 mgm. per kilogram or more, tubular damage was usually noted, as evidenced by increased albuminuria, red cells, white cells, renal cells and inconstantly, granular casts in the urine. Oliguria and fixation of the specific gravity at a low level were not demonstrated in any of our cases. Urinary abnormalities produced as a result of drug therapy disappeared within four days after the drug was stopped. Both polymyxin B and polymyxin E are essentially similar in their toxic effects. We are encouraged by the relative absence of neurotoxicity in a recently tested batch of polymyxin E (EA 113).

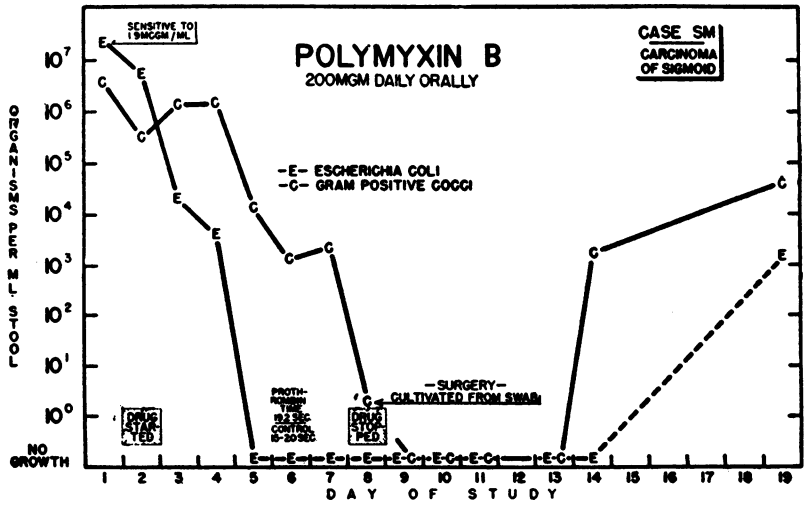

FIG. 2

SUMMARY

Polymyxin B and polymyxin E are potent chemotherapeutic agents against susceptible gram-negative urinary tract infections, including those produced by *Ps. aeruginosa*. They are useful topically applied in eradicating these organisms in granulating wounds. Polymyxin is a potent intestinal antiseptic. Drug resistance of bacteria incident to therapy does not occur. Certain reversible neurotoxic phenomena occur following intramuscular injection of the drug, both with polymyxin B and polymyxin E, even with a single 50 mgm. dose. When the daily dosage exceeds 2.5 mgm. per kilogram, intramuscularly administered every six hours, nephrotoxicity is encountered. Further toxicity studies on patients receiving 2 mgm. per kilogram per day intramuscularly are in progress.

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