Effect of pH on the Activity of Erythromycin Against 500 Isolates of Gram-Negative Bacilli

VICTOR LORIAN AND L. D. SABATH

Department of Pathology, Microbiology Division, Bronx-Lebanon Hospital Center, Bronx, New York 10456, and Thordike Memorial Laboratory (Channing Laboratory), Harvard Medical Unit, Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02118

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Erythromycin was found to be a more effective inhibitor of gram-negative bacilli in alkaline medium than in neutral or acid medium. A definite effect was noted with all of 500 recent clinical isolates of Escherichia coli, Klebsiella-Enterobacter, Pseudomonas aeruginosa, and Proteus mirabilis studied, but it was most striking with E. coli. At pH 8.5 all strains, except for 14% of those of P. mirabilis, were inhibited by concentrations of erythromycin readily achieved in urine with common therapeutic doses.

Studies on streptomycin (1, 7) and later on erythromycin (3) indicated that their antibacterial activities vary markedly with the pH of the medium; these two antibiotics have significantly lower minimal inhibitory concentrations (MIC) at high pH than in medium with neutral or low pH. The pH effect on erythromycin activity was first demonstrated against Streptococcus pyogenes and Sarcina lutea (3) and later against staphylococci (2). It was shown that alkaline pH also enhances the activity of erythromycin against gram-negative organisms (6, 9). Zagar (9) and recently Sabath et al. (5) demonstrated that erythromycin at pH 8 was up to 300 times more active against Escherichia coli than at pH 6. Volunteers, taking 3 g of erythromycin estolate together with 12 to 15 g of sodium bicarbonate daily, produced urines sufficiently alkaline with enough erythromycin to inhibit the growth of E. coli in dilutions as high as 1:128 (5).

Because enhancement of the activity of erythromycin against gram-negative bacilli by alkaline pH has been well established and has potential clinical application, a large number of strains of gram-negative bacilli commonly found in urinary infections was tested to determine the proportion that are susceptible to erythromycin at levels of pH readily attainable in the urine.

MATERIALS AND METHODS

Two hundred strains of E. coli and 100 each of Klebsiella-Enterobacter, Pseudomonas aeruginosa, and Proteus mirabilis, all recent isolates from urinary tract infections, were tested at pH 7 and 8.5 by a broth-dilution method as previously described (5). Ten strains of E. coli were also tested at several pH intervals between pH 5.5 and 8.0 to determine more precisely the point at which the antibacterial activity of erythromycin shows the greatest change. Both the MIC and the minimal bactericidal concentration (MBC) were determined by using techniques and criteria previously described (5). The erythromycin used was generously supplied by Eli Lilly & Co., Indianapolis, Ind.

RESULTS

The results (Table 1) indicate that at pH 8.5 all the strains of E. coli were inhibited by 3.12 μg of erythromycin per ml, a concentration easily attainable in urine with therapeutic doses of erythromycin. The strains of Klebsiella-Enterobacter were less sensitive. Only 60% of them were inhibited by 3.12 μg of erythromycin per ml, but 95% were inhibited by 12.5 μg/ml, a concentration also readily achieved in urine (4, 10). Most strains of Pseudomonas were more resistant, but 12 of those tested were sensitive to 6.25 μg/ml and all were inhibited by 50 μg/ml, a concentration achieved in urine by all subjects taking 3.0 g of erythromycin estolate in 24 hr (4). All strains of Proteus were resistant to 12.5 μg/ml, but 90% of them were inhibited by 100 μg/ml or less, concentrations found in the urine of some subjects during therapy (4).

The results (Fig. 1A) indicate that between pH 6 and 7 the inhibitory activity of erythromycin increases sharply. Further increase in activity was also noted up to pH 8. The effect of pH on the MBC did not parallel the effect on MIC; for 4 of the 10 strains studied, the MBC was identical at pH 5.5 and 8 (Fig. 1B).
TABLE 1. Numbers of strains susceptible to erythromycin at pH 7 and pH 8.5

| Organism                     | No. of strains tested | pH of test | No. of strains inhibited by indicated concn² |
|------------------------------|-----------------------|------------|--------------------------------------------|
|                              |                       |            | >200 | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.78 | 0.39 | 0.19 | 0.09 | 0.04 |
| **Escherichia coli**         | 200                   | 7.0        | 9    | 12  | 40  | 107| 32 |       |      |      |      |      |      |      |      |      |
|                              |                       | 8.5        |      |     |     |    |    |       |      |      |      |      |      |      |      |      |
| **Klebsiella-Enterobacter**  | 100                   | 7.0        | 30   | 25  | 35  | 8  | 2  |       |      |      |      |      |      |      |      |      |
|                              |                       | 8.5        | 5    |     |     |    |    |       |      |      |      |      |      |      |      |      |
| **Pseudomonas aeruginosa**   | 100                   | 7.0        | 25   | 44  | 12  | 19 | 39  | 21   | 28   | 12  |      |      |      |      |      |      |
|                              |                       | 8.5        |      |     |     |    |    |       |      |      |      |      |      |      |      |      |
| **Proteus mirabilis**        | 100                   | 7.0        | 100  |     |     |    |    |       |      |      |      |      |      |      |      |      |
|                              |                       | 8.5        | 10   | 4   | 25  | 61 |     |       |      |      |      |      |      |      |      |      |

² Expressed as micrograms per milliliter.

**DISCUSSION**

Williamson and Zinnemann (8) tested 347 strains of *E. coli* and found 50% of the strains were inhibited by 10 μg of erythromycin per ml (pH 6). The strains reported here showed greater resistance; none were inhibited by 10 μg/ml, even at pH 7. However, they were much more sensitive in alkaline medium. In a small clinical study, Zinner et al. (10) found that 71% of 24 patients cleared their urine of bacteria with erythromycin-alkali treatment. The results of the present study suggest that most isolates of *E. coli* and *Klebsiella-Enterobacter* from patients with bacteriuria might be expected to be sensitive to erythromycin in sufficiently alkaline urine. Because strains of *Pseudomonas* and especially *Proteus* had higher MIC, even in alkaline urine, larger doses of erythromycin would appear to be indicated for therapeutic trials of urinary tract infections due to those organisms. Nevertheless, alkalinization of the medium clearly increased the antibacterial activity of erythromycin against virtually all the strains studied.

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**LITERATURE CITED**

1. Abraham, E. P., and E. S. Duthie. 1946. Effect of pH of the medium on activity of streptomycin and penicillin. Lancet 1:455–459.
2. Garrod, L. P., and P. Waterworth. 1956. Behavior in vitro of some new antistaphylococcal antibiotics. Brit. Med. J. 2:61–65.
3. Haight, T. H., and M. Finland. 1952. Observations on mode of action of erythromycin. Proc. Soc. Exp. Biol. Med. 81:118.
4. Sabath, L. D., D. A. Gerstein, P. B. Loder, and M. Finland.
1968. Excretion of erythromycin and its enhanced activity in urine against gram-negative bacilli with alkalization. J. Lab. Clin. Med. 72:916-923.
5. Sabath, L. D., V. Lorian, D. Gerstein, P. B. Loder, and M. Finland. 1968. Enhancing effect on alkalization of the medium on the activity of erythromycin against gram-negative bacteria. Appl. Microbiol. 16:1288–1292.
6. Sylvester, J. C. 1966. Quoted in Erythromycin, a Review of its Properties and Clinical Status, p. 14. Abbott Laboratories, Chicago, Ill.
7. Waksman, S. A., E. Bugie, and S. Schatz. 1947. Isolation of antibiotic substance from soil microorganisms, with special reference to streptothricin and streptomycin. Proc. Staff Meeting Mayo Clinic 19:537–580.
8. Williamson, G. M., and K. Zinner. 1962. The susceptibility of coliform bacilli to erythromycin. Antibiot. Chemother. 12:169–172.
9. Zagar, Z. 1963. Sensitivity of E. coli, Ps. aeruginosa, and B. proteus to erythromycin in various pH culture media. Chemotherapie 6:82–89.
10. Zinner, S. H., L. D. Sabath, J. I. Casey, and M. Finland. 1970. Erythromycin plus alkalization in treatment of urinary infections. Antimicrob. Ag. Chemother.—1969, p. 413–416.