ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1983, p. 325-327 Vol. 23, No. 2
0066-4804/83/020325-03$02.00/0

Effect of Carbon Dioxide on Erythromycin

ELLIE J. C. GOLDSTEIN[1,2]†* AND VERA L. SUTTER[3]

Medical[1] and Research[3] Services, Wadsworth Veterans Administration Medical Center, Los Angeles, California 90073, and Department of Medicine, University of California at Los Angeles School of Medicine, Los Angeles, California 90024[2]

Received 14 June 1982/Accepted 1 December 1982

Exposure of erythromycin solutions to CO_2 or air for 18 h resulted in minimal deterioration under both conditions. Saturated disks exposed to the two atmospheres resulted in greater erythromycin deterioration, with slightly more deterioration occurring in the presence of CO_2. Reduction of activity was greatest when assays were conducted in a CO_2 environment.

Erythromycin is a macrolide antibiotic elaborated by *Streptomyces erythraeus*. Early studies showed acid pH to be detrimental to the activity of erythromycin, whereas its activity was increased with alkalinity, approximately 10-fold for each pH unit (3, 12). Clinically, the efficacy of erythromycin in treating urinary tract infections has been augmented by alkalinization of the urine (11, 17). Significant differences in the antibacterial activity of erythromycin have been reported and attributed to variables in methodology such as pH of the medium and presence or absence of CO_2 in the atmosphere. The effect of CO_2 and pH on the in vitro activity of erythromycin has been noted most markedly with anaerobic bacteria, especially *Bacteroides fragilis* (1, 2, 4–7, 9, 13–16).

The synthesis of erythromycin by *S. erythraeus* is inhibited 40% when submerged fermentations are sparged with 11% CO_2. This effect was unrelated to the growth of *S. erythraeus* or pH of the medium (8). To determine whether CO_2 might inactivate erythromycin as well as inhibit its synthesis, we investigated the effect of CO_2 on the activity of erythromycin.

Micrococcus luteus ATCC 9341 was used as the test organism. The isolate was removed from −70°C storage, transferred twice to ensure purity, plated onto nutrient agar (Difco Laboratories, Detroit, Mich.), and incubated at 35°C for 24 h. Growth was suspended in tryptose phosphate broth (Difco) to obtain an optical density of 0.47 at 550 nm (Coleman Junior spectrophotometer). Five-milliliter portions were refrigerated at 2 to 8°C and stored for 4 to 6 weeks. When ready for use, 0.04 ml of the micrococcus suspension was added to 20 ml of nutrient agar, and plates (150 by 15) were poured.

† Present address: R. M. Alden Research Laboratory, 11980 San Vicente Boulevard, Suite 103, Los Angeles, Calif. 90049.

Antibiotic solutions were prepared by dissolving laboratory standard powder in 95% ethanol and subsequently diluting it in sterile distilled water. Serial dilutions from 100 to 1.56 μg/ml were made, and four concentrations (25, 12.5, 3.12, and 1.56 μg/ml) were chosen for testing. Twenty microliters of antibiotic standard solution were used to saturate sterile 0.25-in. (0.625-cm) filter paper disks having a surface area of 28.3 mm^2. Portions of each standard solution (5 ml in plastic petri dishes [15 by 100 mm] having an inside surface area of 6,362 mm^2) and sets of each antibiotic standard saturated disk were placed in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) containing either 10% CO_2 and air (obtained by evacuating and replacing 10% of the atmosphere with CO_2) or room air. The jars were placed on a shaker and left at room temperature for 18 h. Sets of sterile filter paper disks were then saturated with 20 μl of the previously prepared erythromycin solutions. A total of four experimental sets were prepared. Fresh erythromycin standards were prepared and used to saturate another set of paper disks.

Sets of saturated disks from each of the four experimental groups were assayed along with sets of freshly prepared and saturated disks. Four experimental disks, one for each erythromycin concentration in each set, were assayed along with the four corresponding freshly prepared standard disks per plate. All plates were incubated at 35°C for 18 h and then examined. The experiments were run in triplicate. In addition, sets of fresh standard disks were assayed alone against the test organism and incubated in either room air or 10% CO_2 environments at 35°C for 18 h. The growth of isolated colonies of *M. luteus* in 10% CO_2 and room air without antibiotic disks present was also observed and measured.

Standard curves were constructed from the mean values of the triplicate determinations, and

TABLE 1. Effect of various conditions on erythromycin activity

Condition	Erythromycin concn (μg/ml)			
Assayed in air				
Fresh standard solution	25.0	12.5	3.12	1.56
Standard solution exposed to CO_2	23.5	11.5	2.8	1.5
Standard solution exposed to air	22.0	11.0	2.8	1.1
Standard solution, applied to disks exposed to CO_2	12.0	7.6	2.6	1.1
Standard solution, applied to disks exposed to air	17.5	8.5	3.1	1.2
Assayed in CO_2				
Fresh standard solution	10.1	5.2	1.3	0

estimates of erythromycin concentrations were made with the results of the fresh standard solution assays as the absolute values.

In addition, measurement of the pH of the erythromycin test solution and the saturated disks were made in triplicate before and after incubation in both atmospheres with paper pH strips (Colorplast, E. M. Reagent; E. Merck AG, Darmstadt, Germany). Measurements of the pH of the agar surface of both inoculated and uninoculated plates before and after incubation in both atmospheres were made with a Beckman Expandomatic SS-2 meter with a flat bulb combination electrode. The pH of the control plates containing no erythromycin was also determined.

Results on the effect of various conditions on the activity of erythromycin are shown in Table 1. Exposure of erythromycin solutions to CO_2 or air resulted in minimal deterioration of activity. Exposure of disks saturated with the 25-μg/ml standard solution resulted in approximately 50 and 30% loss of activity in CO_2 and air, respectively. Disks saturated with the lower concentration standard solutions were less dramatically affected. When fresh standard solutions were assayed in a CO_2 environment, there was a marked decrease in the activity of erythromycin. The sizes of the colonies of the assay organism were slightly larger when they were incubated in room air as compared with their sizes in the CO_2 environment.

When the effects of exposure to air and CO_2 on pH measurements under various conditions were studied, it was shown that the change in pH after exposure to room air was always less than that after exposure to the 10% CO_2 environment. The standard solution exposed to air had either no pH change or an increase of 0.2, whereas exposure to 10% CO_2 resulted in a pH decrease of 0.2 to 0.6. Disks saturated with the various concentrations of erythromycin showed a similar trend, with a maximum pH decrease of 0.2 after exposure to air but a pH decrease of 0.5 to 0.8 after exposure to a 10% CO_2 atmosphere.

Though the pHs of both the standard solutions and saturated disks were between 5.5 to 5.9 on initial measurement, the pH of uninoculated nutrient agar plates without erythromycin disks was between 6.50 and 6.58. These plates showed a pH decrease of 0.54 in the CO_2 environment and of 0.03 in the room air environment. After exposure to room air, inoculated and uninoculated nutrient agar plates with erythromycin disks showed a decrease in pH of 0.11 to 0.20 and 0.11 to 0.04, respectively. After exposure to 10% CO_2, there was a decrease of 0.48 to 0.62 and 0.48 to 0.65 in the agar surface pH of inoculated and uninoculated plates, respectively.

From these results, it appears that CO_2 does not inactivate erythromycin, since solutions exposed to CO_2 or air showed little difference in activity. With the exception of the highest concentration of erythromycin, results were similar when solutions were applied to disks. Although the growth of the assay organism was slightly diminished in the CO_2 environment, the apparent activity of erythromycin was less and the pH was reduced. Our results lend support to the observations of Hansen et al. (4) that uncontrolled pH in a CO_2 atmosphere results in apparent reduction of erythromycin activity.

We thank Phyllis Stewart for valuable technical assistance.
This study was supported by Medical Research Service, Veterans Administration, Los Angeles, Calif.

LITERATURE CITED

1. **Appelbaum, P. C, and S. A. Chatterton.** 1978. Susceptibility of anaerobic bacteria to ten antimicrobial agents. Antimicrob. Agents Chemother. **14**:371–376.
2. **Goldstein, E. J. C., V. L. Sutter, Y. Y. Kwok, R. P. Lewis, and S. M. Finegold.** 1981. Effect of carbon dioxide on in vitro susceptibility of anaerobic bacteria to erythromycin. Antimicrob. Agents Chemother. **20**:705–708.
3. **Haight, T. H., and M. Finland.** 1952. The antibacterial action of erythromycin. Proc. Soc. Exp. Biol. Med. **81**:175–183.
4. **Hansen, S. L., P. Swomley, and G. Drusano.** 1981. Effect of carbon dioxide and pH on susceptibility of *Bacteroides fragilis* group to erythromycin. Antimicrob. Agents Chemother. **19**:335–336.
5. **Ingham, H. R., J. B. Selkon, A. A. Codd, and J. H. Hale.** 1970. The effect of carbon dioxide on the sensitivity of *Bacteroides fragilis* to certain antibiotics in vitro. J. Clin. Pathol. **23**:254–258.
6. **Kislak, J. W.** 1972. The susceptibility of *Bacteroides fragilis* to 24 antibiotics. J. Infect. Dis. **125**:295–299.

7. **Lorian, V., and L. D. Sabath.** 1970. Effect of pH on the activity of erythromycin against 500 isolates of gram-negative bacilli. Appl. Microbiol. **20**:754–756.
8. **Nash, C. H., III.** 1974. Effect of carbon dioxide on synthesis of erythromycin. Antimicrob. Ag. Chemother. **5**:544–545.
9. **Rosenblatt, J. E., and F. Schoenknecht.** 1972. Effect of several components of anaerobic incubation on antibiotic susceptibility test results. Antimicrob. Ag. Chemother. **1**:433–440.
10. **Russell, A. D.** 1974. Factors influencing the activity of antimicrobial agents: an appraisal. Microbios **10**:151–174.
11. **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 alkalinization. J. Lab. Clin. Med. **72**:916–923.
12. **Shoemaker, E. H., and E. M. Yow.** 1954. Clinical evaluation of erythromycin. Arch. Intern. Med. **93**:397–406.
13. **Stalons, D. R., C. Thornsberry, and V. R. Dowell, Jr.** 1974. Effect of culture medium and carbon dioxide concentration on growth of anaerobic bacteria commonly encountered in clinical specimens. Appl. Microbiol. **27**:1098–1104.
14. **Sutter, V. L., and S. M. Finegold.** 1976. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. Antimicrob. Agents Chemother. **10**:736–752.
15. **Sutter, V. L., Y. Y. Kwok, and S. M. Finegold.** 1973. Susceptibility of *Bacteroides fragilis* to six antibiotics determined by standardized antimicrobial disk susceptibility testing. Antimicrob. Ag. Chemother. **3**:188–193.
16. **Toala, P., C. Wilcox, and M. Finland.** 1970. Effect of pH of medium and size of inoculum on activity of antibiotics against group D *Streptococcus* (*Enterococcus*). Appl. Microbiol. **19**:629–637.
17. **Zinner, S. H., L. D. Sabath, J. I. Casey, and M. Finland.** 1970. Erythromycin plus alkalinization in treatment of urinary infections, p. 413–416. Antimicrob. Agents Chemother. 1969.