Activity of Spectinomycin Against Anaerobes

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The in vitro inhibitory activity of spectinomycin was tested against various anaerobic bacteria. Different results were obtained with different media and with different initial pH's of the media. The highest minimum inhibitory concentrations for *Bacteroides fragilis* ($\geq 128 \mu\text{g/ml}$) were obtained with the use of Wilkins-Chalgren agar (pH 7.2) and Brucella blood agar (pH 7.0). Brucella blood agar at higher pH's (7.4 and 8.0) and Mueller-Hinton and Diagnostic Sensitivity Test agars produced lower minimum inhibitory concentrations (32 and 64 $\mu\text{g/ml}$). This same relationship between spectinomycin activity and pH of the medium was, in general, observed with these media and other anaerobes, including isolates of *B. melaninogenicus*, *Fusobacterium*, gram-positive cocci, *Clostridium perfringens*, and *C. ramosum*. The variable results observed in this study and in two others make it difficult to predict the clinical usefulness of spectinomycin in the treatment of anaerobic infections. It is probably most appropriate to be guided by results obtained with Wilkins-Chalgren agar and the method proposed as a reference to the National Committee for Clinical Laboratory Standards. These results indicate that spectinomycin is not a potent inhibitor of *B. fragilis* or other clinically significant anaerobes.

Spectinomycin is an aminocyclitol antimicrobial agent that is effective in the treatment of infections due to *Neisseria gonorrhoeae* (1). Although the majority of strains of *Enterobacteriaceae* (excluding *Serratia* and *Proteus*) are susceptible to spectinomycin (9), it has not been used to treat infections caused by these organisms. Phillips and Warren (5) have reported that most of the 100 strains of *Bacteroides fragilis* that they examined were inhibited by 16 μg of spectinomycin per ml. Since this level is well below maximum achievable serum concentrations, they suggested that a therapeutic trial of spectinomycin against *B. fragilis* infections was justified. However, Ferguson and Smith (3) found spectinomycin to be much less active against *B. fragilis*. They concluded that the differing results were caused by the use of different media and methods of incubation. Because of these conflicting studies, we decided to determine the activity of spectinomycin against various anaerobes with the use of several different media and by examining several parameters that might be causes of differing susceptibility results.

MATERIALS AND METHODS

Fifty-five clinical isolates were studied, including *B. fragilis* (25 isolates), *B. melaninogenicus* (6 isolates), *Fusobacterium* (6 isolates), anaerobic gram-positive cocci (13 isolates), *Clostridium perfringens* (3 isolates), and *C. ramosum* (4 isolates). The mini-

mum inhibitory concentration (MIC) was determined by an agar dilution method as described previously (7). Plates were incubated for 48 h in unvented GasPak jars, and the MIC was read as the lowest concentration of spectinomycin that yielded no growth, a barely visible haze, or one colony. Spectinomycin was supplied as Trobicin sterile dry susceptibility powder by the Upjohn Co. and was dissolved and diluted in sterile distilled water. MICs were determined with the following media: Mueller-Hinton agar (MHA), obtained from Baltimore Biological Laboratory, Cockeysville, Md., at an initial pH of 7.4; Diagnostic Sensitivity Test agar (DST, CM 261), obtained from Oxoid Ltd., London, at an initial pH of 7.4; Wilkins-Chalgren agar (WCA) (10), prepared in our laboratory at an initial pH of 7.2; and Brucella agar (BA) obtained from Difco Laboratories, Detroit, Mich., and prepared and tested at three different initial pH's (7.0, 7.4, and 8.0). Each medium, except WCA, was supplemented with 5% lysed sheep blood and 10 μg of vitamin K₁ per ml. pH measurements were read from the surface of agar plates both before and after the completion of anaerobic incubation for each of the media by means of a Markson no. 1207 combination electrode (Markson Science, Inc., Delmar, Calif.).

RESULTS

Table 1 shows the MICs of the various anaerobes for each medium used. The initial pH of the medium is shown, and studies were carried out with BA at three different initial pH's. Mode values are given, with the range of values given in parentheses. MICs of spectinomycin

TABLE 1. Susceptibility of anaerobes to spectinomycin with different agar media^a

Organism	No. of strains	MIC ($\mu\text{g/ml}$) ^b					
		BA			MHA (7.4)	Oxoid DST (7.4)	WCA (7.2)
		7.0 ^c	7.4	8.0			
<i>B. fragilis</i>	25	128 (64–128)	32 (32–64)	32 (16–32)	32 (16–64)	64 (2–128)	>128 (128– >128)
<i>B. melaninogenicus</i>	6	32 (16–64)	16 (8–16)	8 (4–16)	8–32	16–32	64 (32–64)
<i>Fusobacterium</i>	6	64 (32–64)	32 (16–32)	16 (8–16)	32 (4–32)	32 (8–32)	64 (32–128)
Gram-positive cocci	13	16 (4–128)	16 (4–128)	8 (2–32)	32 (4–64)	16 (8–128)	64 (8–128)
<i>C. perfringens</i>	3	>128	128	64	128 (32–128)	128 (64–128)	>128
<i>C. ramosum</i>	4	64 (32–64)	32	16	32 (16–32)	32 (32–64)	64 (64–128)

^a Each agar medium (except WCA) was supplemented with 5% lysed sheep blood and vitamin K₁ (10 $\mu\text{g/ml}$).

^b Mode value of MIC. The range of MICs is given in parentheses. The range of values outside parentheses indicates an equal number of results at each of the MIC values.

^c pH of medium before initiation of anaerobic incubation.

for *B. fragilis* varied among different media, and with BA they varied with the different initial pH's of the agar. The mode MIC with WCA was greater than 128 $\mu\text{g/ml}$, and the value for BA at pH 7.0 was 128 $\mu\text{g/ml}$. Mode MICs for BA at pH 7.4 and 8.0 were both 32 $\mu\text{g/ml}$ and were similar to values for MHA and DST agar, which were 32 and 64 $\mu\text{g/ml}$, respectively. These data suggest that relatively high MICs are associated with media having low initial pH's and that, as the pH is raised, MIC values become lower.

For BA the same correlation between MIC values and pH of the medium was present with the other anaerobes studied. In particular, *B. melaninogenicus* showed a progressive lowering in mode MIC from 32 to 16 to 8 $\mu\text{g/ml}$ with the initial pH of the agar changing from 7 to 7.4 to 8.0. Likewise, the *Fusobacterium* and *C. ramosum* isolates showed spectinomycin MICs going from 64 to 32 to 16 $\mu\text{g/ml}$ with pH changes from 7 to 7.4 to 8.0, respectively. For this group of anaerobes the highest MICs were found with WCA; mode values were 64 $\mu\text{g/ml}$, except for *C. perfringens*, for which the value was greater than 128 $\mu\text{g/ml}$. Each of these MICs was one to two dilutions greater than the values obtained with MHA and DST agar, which had pH's of 7.4, which was 0.2 pH unit greater than that for WCA. When BA is prepared according to the directions of the manufacturer, the initial pH will be 7.0. Therefore, the mode MIC values at this pH should be the ones used when comparing results with those obtained with the other media. These BA values are very close to

(within one dilution) those obtained with WCA, which has a similar low pH of 7.2; an exception was the gram-positive cocci, in which there was an unexplained fourfold difference in mode MIC values.

These data suggest that for most anaerobes, and in particular for *B. fragilis*, the initial pH of the medium is an important factor in influencing the ultimate MIC value of spectinomycin. Those media with low pH's (7.0 to 7.2) will give higher MIC values than those with high initial pH's (7.4 to 8.0) when each susceptibility study is performed with incubation under the same anaerobic conditions.

pH measurements of each agar medium were obtained just before and after 48 h of incubation at 37°C in GasPak jars. Each of the single medium plates contained 128 μg of spectinomycin per ml. The average change during incubation was 0.08 pH unit, and in each case except one this represented an increase in pH. WCA showed a decrease of 0.05 pH unit during incubation. These results do not show a significant change in pH of the medium during GasPak incubation.

DISCUSSION

The results of this study indicate that spectinomycin activity against anaerobes will vary depending on the medium used and that an important reason for this variation is a differing initial pH of the medium. MICs of spectinomycin were highest with the use of BA at pH 7.0 and WCA at pH 7.2, presumably because spectinomycin is less active at lower pH values.

This phenomenon has been demonstrated previously for aminoglycosides (6) and erythromycin and lincomycin (4). Our results are similar to those obtained by Ferguson and Smith (3), who found that most of their *B. fragilis* isolates had MICs of spectinomycin ≥ 70 $\mu\text{g/ml}$. This resistance pattern was not found by Phillips and Warren (5), who reported that all 38 of the *B. fragilis* isolates that they studied were inhibited by 16 μg of spectinomycin per ml. They did acknowledge that different media pH's and different inoculum sizes could affect the results, but only at extreme ranges. Using the same medium (DST) as Phillips and Warren used, we obtained higher spectinomycin MICs (mode of 64 versus 16 $\mu\text{g/ml}$). Both studies used the GasPak system, but our inoculum was higher (10^5 versus 10^3 organisms). Ferguson et al. (2) attributed the lower spectinomycin MICs of Phillips and Warren to low CO_2 concentrations in the GasPak system (0.5 to 2.7%), whereas they had used an evacuation-replacement anaerobic jar method, which produced 7.5% CO_2 . This excess CO_2 would tend to lower the pH of the medium. However, this explanation is not entirely satisfactory, since in the study of Ferguson et al. the pH of the medium (starting at 7.4) did not decrease below 7.0 during incubation in 7.5% CO_2 ; 7.0 was the starting pH of the medium of Phillips and Warren. Ferguson et al. did use a different medium (Oxoid Sensitest, CM 409) and a larger inoculum (10^4 organisms). The above discussion illustrates that it is difficult to pinpoint the exact reasons for the different results in these three studies. However, we would conclude that different media and their differing initial pH's as well as different inoculum sizes all contributed in an additive fashion.

It is difficult to choose which set of conflicting results is the most accurate and reliable, especially in the absence of in vivo documentation of therapeutic activity (or inactivity) of spectinomycin in anaerobic infections. However, the agar dilution method employing WCA has been proposed to the National Committee for Clinical Laboratory Standards (NCCLS) as a reference method for susceptibility testing of anaerobes (V. L. Sutter, A. L. Barry, W. J. Martin, J. E. Rosenblatt, V. R. Dowell, Jr., C. Thornsberry, T. D. Wilkins, and R. J. Zabransky, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 16th, Chicago, Ill., Abstr. no. 453, 1976). It would, therefore, seem most appropriate to accept data obtained with this method. Our results with WCA indicate that *B. fragilis*

clinical isolates are resistant to greater than 128 μg of spectinomycin per ml and that various other anaerobes will most often be inhibited by ≥ 64 $\mu\text{g/ml}$. Since reported peak levels of spectinomycin in blood after a 2.0-g intramuscular dose are approximately 100 $\mu\text{g/ml}$ (8), it does not appear likely that this agent would be effective in the treatment of anaerobic infections. However, having said this, we must recognize that prior studies with the proposed NCCLS method have not included aminocyclitols and that the pH of WCA (7.2) does not favor the activity of these agents. It is possible that results with this method are not good predictors of in vivo spectinomycin activity against anaerobes. This possibility must, in turn, be tempered with our observations that the pH's of infected body fluids (other than blood) are generally low (5.0 to 6.0). Aminocyclitols may not be optimally active in this environment. The controversy will be settled only when appropriate in vivo studies are carried out.

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