Use of Sodium Polyanethol Sulfonate to Selectively Inhibit Aminoglycoside and Polymyxin Antibiotics in a Rapid Blood Level Antibiotic Assay

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Sodium polyanethol sulfonate inhibits aminoglycoside and polymyxin classes of antibiotics in direct proportion to its concentration. Aminoglycoside and polymyxin class antibiotics are selectively inactivated; penicillin, including the semisynthetic penicillins, cephalothin, chloramphenicol, clindamycin, tetracycline, erythromycin, and vancomycin are not inhibited. By incorporating sodium polyanethol sulfonate directly into the test medium it is possible, in a 4-h antibiotic blood level assay, to selectively obviate the activity of the aminoglycosides and polymyxins to determine the concentration of other antibiotics present in the same serum sample.

Sodium polyanethol sulfonate (SPS) is well recognized as an anticoagulant, antiphagocytic, and anticomplementary agent (2). In 1947 May et al. (9) reported that blood levels of streptomycin were consistently low when assays were performed in the presence of SPS. Traub (13) and Traub and Lowrance (15) found that aminoglycoside and polymyxin class antibiotics were inhibited by SPS, whereas other commonly utilized classes of antibiotics were not.

The blood level determination of one antibiotic in a mixture is generally accomplished either by obviating the activity of the unwanted antibiotic(s) with the use of inactivating enzymes (11) or with the use of an organism susceptible only to the antibiotic in question (8). Because aminoglycosides have such a broad spectrum of activity it is difficult to routinely isolate an organism with the proper susceptibility pattern. Specific enzymes are not available for these drugs.

Methods using special conditions that inhibit the action of aminoglycosides have been presented. Sabath and Toftegaard (12) took advantage of the fact that aminoglycoside antibiotics were inactive anaerobically and Ervin and Bullock (7) incorporated calcium into the test medium to determine clindamycin levels in the presence of aminoglycosides.

Based on a modification of an existing rapid blood level method (3), a procedure is presented to assay a broad range of antibiotics in the presence of aminoglycosides and polymyxins. The method requires only 30 min to set up, does not require special equipment or conditions, and is well within the scope of the routine clinical microbiology laboratory. Results are available within 3 to 4 h.

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MATERIALS AND METHODS

Effect of SPS on antibiotics. A standard disk diffusion test was performed according to the method of Ericsson and Sherris (6). Zone sizes of inhibition on media, both free of SPS and containing concentrations of SPS (courtesy of L. J. Sorensen, Hoffmann-LaRoche, Inc., Nutley, N.J.) from 0.01 to 1%, were determined. SPS is available as a 5% sterile liquid (Grobax, Hoffmann-LaRoche, Inc., Nutley, N.J.). Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 (the "Seattle" strain), as well as clinical isolates of S. aureus, S. epidermidis, and representatives of all species of the family Enterobacteriaceae, were tested. Zone sizes were determined using a Nikkon shadowgraph (Nippon Kogaku, Tokyo, Japan).

The effect of different media on the assay was tested by performing all sensitivity tests in parallel on Mueller-Hinton (BBL, Cockeysville, Md.), Trypticase soy (BBL), antibiotic media no. 2 and 5 (Difco Laboratories, Detroit, Mich.), nutrient (BBL), and plate count (BBL) agars. Media were tested both with and without the incorporation of 10% normal pooled serum, which had been screened for antibacterial activity.

The antibiotics tested included streptomycin (10 µg), kanamycin (30 µg), gentamicin (10 µg), polymyxin B (300 IU), penicillin (10 U), carbenicillin (100 µg), ampicillin (10 µg), oxacillin (1 µg), nafcillin (1 µg), cephalothin (3 µg), chloramphenicol (30 µg), tetracycline (30 µg), clindamycin (2 µg), erythromycin (15 µg), and vancomycin (30 µg).

Determination of antibiotic blood levels. Four-
hour antibiotic blood level measurements were performed by a modification of a previously described method (5). Plate count agar was chosen, due to its relative lack of inhibitory activity against SPS and its good growth support properties, as the test medium. Aliquots (6-ml) were dispensed into test tubes and stored at 4°C for up to 4 weeks. For each specimen two tubes of agar were melted and brought to 50°C. Depending on the antibiotic to be assayed, an organism susceptible to it was selected from the day's routine Kirby-Bauer sensitivity tests (4). A 0.5 MacFarland turbidity standard was made from this strain in saline, and 1.2 ml of this suspension was added to each tube of plate count agar. While still at 50°C, using a 24-gauge needle and a 2-ml syringe, 0.8 ml of sterile 5% SPS solution was added to each tube, to make a final SPS concentration of 0.6%. Pour plates were made and allowed to solidify. Wells (4 mm in diameter) were cut with a cork borer, and dilutions of the antibiotic standard, dependent on the assayed antibiotic, and the patient's serum were added (3). As a control, each plate included one well containing a high concentration of the obviated aminoglycoside or polymyxin; 20 μg of gentamicin, 40 μg of kanamycin or streptomycin, and 20 μg of polymyxin B per ml were employed. There should be no zone of inhibition around these controls. Incubation was at 35°C. The antibiotic concentration in the patient's serum was calculated after 3 to 4 h by semilogarithmic graph in the usual manner.

RESULTS

Effect of SPS on antibiotics. In a concentration range of 0.01 to 1.0%, SPS did not inhibit penicillin, carbenicillin, ampicillin, oxacillin, nafcillin, cephalothin, chloramphenicol, tetracycline, clindamycin, erythromycin, and vancomycin.

Streptomycin, kanamycin, gentamicin, and polymyxin B were inhibited in direct proportion to the amount of SPS added. On plate count and nutrient agars, they were totally inhibited at a concentration of 0.6%. In Mueller-Hinton, antibiotic media no. 2 and 5, and Trypticase soy agars, concentrations of 0.8 to 1.0% were required to completely inhibit these antibiotics.

Effect of SPS on bacterial strains. Neither the Seattle strains of E. coli and S. aureus nor any of the representative species of the genus Staphylococcus or family Enterobacteriaceae were inhibited by concentrations of SPS up to 1.0%. There were no growth differences, in terms of colony-forming units, between organisms grown on SPS-free media and media containing 0.6% SPS.

Effect of media on the activity of SPS. Nutrient and plate count agars were the least inhibitory of the agars tested. SPS was two to eight times more active in these media than the others.

Antibiotic assay. Patient specimens obtained from the Special Antibiotic Section of the Division of Microbiology and Immunology of this hospital were assayed both by this method and by that of Sabath and Toftegaard (12). A Clostridium septicum and a Bacteroides fragilis subsp. fragilis were used as test organisms. Both methods agree within 10% (Table 1).

Figure 1 demonstrates a serum clindamycin assay from a patient receiving clindamycin and gentamicin. Only one plate of a duplicate set is shown. Gentamicin activity is completely inhibited, allowing the rapid determination of the clindamycin level.

DISCUSSION

A rapid antibiotic assay has been developed to determine the concentration of circulating antibiotics in the presence of aminoglycosides or polymyxins. The method was based upon the addition of a commercially available anionic detergent, SPS, to the culture medium. This

### Table 1. Comparison of the SPS and anaerobic rapid assay methods

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of specimens</th>
<th>Discrepancy between the two methods (%)</th>
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<tr>
<td></td>
<td></td>
<td>0-5%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>56</td>
<td>46</td>
</tr>
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<td>Carbenicillin</td>
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<td>44</td>
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<td>Cephalothin</td>
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<td>Chloramphenicol</td>
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<td>Clindamycin</td>
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</tr>
<tr>
<td>Vancomycin</td>
<td>6</td>
<td>67</td>
</tr>
</tbody>
</table>

* Patients concomitantly receiving either streptomycin, kanamycin, gentamicin, or polymyxin B parenterally. Results are rounded to the nearest whole percent.
of SPS added and the amount of antibiotic inhibited. Unlike inhibition occurring under anaerobic conditions or following the addition of calcium, SPS appeared to exert its effect directly on the antibiotic by combining with it to form a precipitate (S. C. Edberg and C. J. Bottenbley, unpublished data).

SPS has been used for years in blood culture media (1). Incorporated at low concentrations, in the range of 0.05%, the percentage of positive cultures markedly increases (10). The action is primarily due to its anticomplemental and antiphagocytic activity. Enterobacteriaceae and Staphylococcus are quite unsusceptible to concentrations up to 1.25% (10). Only anaerobic peptostreptococci and occasional strains of Neisseria meningitidis were reported to be inhibited by SPS (5).

The direct incorporation of 0.6% SPS to determine the concentration of circulating antibiotics mixed with aminoglycosides or polymyxin agreed ±10% with published methods. The SPS procedure, however, offers the advantages of simplicity and speed in performing mixed blood level antibiotic assays, in which it is necessary to selectively inhibit aminoglycoside and polymyxin antibiotics.

ACKNOWLEDGMENTS

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LITERATURE CITED


