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Medium-Dependent Inhibition of *Peptostreptococcus anaerobius* by Sodium Polyanetholsulfonate in Blood Culture Media

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Of 13 species of anaerobic cocci, *Peptostreptococcus anaerobius* was the only species tested that was sensitive to 0.1% sodium polyanetholsulfonate (SPS). However, the sensitivity of *P. anaerobius* to SPS varied according to the media in which the cultures were grown. In supplemented peptone (B-D) and brain heart infusion media, most strains of *P. anaerobius* were not inhibited by SPS. Gelatin and proteose peptone were the medium components which were protective. The minimal inhibitory concentration of SPS for *P. anaerobius* was approximately 60-fold higher in media containing gelatin than in regular media. However, the concentration of SPS required to neutralize the bactericidal properties of human serum was only four fold higher in media containing gelatin. In a commercial medium containing SPS (0.03%) and gelatin (1.2%), SPS-sensitive strains of *P. anaerobius* were not inhibited by SPS, and the bactericidal action of human blood on *Escherichia coli* C and *Serratia marcescens* SM 29 was eliminated.

Sodium polyanetholsulfonate (SPS) is used in most blood culture bottles because it prevents coagulation (10) and eliminates the bactericidal action of blood (2, 7, 10). The major disadvantage with the use of SPS is its inhibition of growth of anaerobic cocci (4). Recently, it has been reported that SPS only inhibits one species of anaerobic cocci, *Peptostreptococcus anaerobius* (3, 6). Unfortunately, *P. anaerobius* is the most common anaerobic coccus isolated in clinical laboratories (3); and therefore, the use of SPS may drastically reduce the number of isolations from blood cultures.

Based on his preliminary observation, J. Mehl of Becton-Dickinson & Co. (Rutherford, N. J.) suggested to us that the sensitivity of *P. anaerobius* to SPS might depend on the type of media used. In this paper, we compare the degree of SPS inhibition in several media and identify those components that increased the resistance of *P. anaerobius* to SPS. In addition, we tested the conclusion of Graves et al. that *P. anaerobius* is the only species of anaerobic cocci that is inhibited by SPS (3).

MATERIALS AND METHODS

Bacterial strains. All bacterial strains tested for sensitivity to SPS were clinical isolates from the culture collection of the Anaerobe Laboratory, Virginia Polytechnic Institute and State University (Table 1) as identified by L. V. Holdeman or W. E. C. Moore, according to procedures described in the Virginia Polytechnic Institute Anaerobe Laboratory Manual (5). *Escherichia coli* C and *Serratia marcescens* SM 29, two serum-sensitive strains (7, 11), were gifts from W. H. Traub, Institut für Klinische Mikrobiologie und Infektionshygiene der Universität Erlangen-Nürnberg, West Germany.

Anaerobiosis. An anaerobic chamber, similar to that described by Aranki and Freter (1), manufactured by Coy Manufacturing Co., Ann Arbor, Mich., was used for all plating procedures, incubation, and storage of plates. The atmosphere in the chamber contained 5% CO_2, 10% H_2, and 85% N_2. All other anaerobic procedures were in CO_2-filled tubes as described in the Virginia Polytechnic Institute Anaerobe Laboratory Manual (5).

Sources of chemicals and media. Supplemented peptone broth (Vacutainer culture tube), quadtone, and SPS were supplied by Becton-Dickinson & Co., Rutherford, N. J. The following were purchased from Baltimore Biological Laboratory, Cockeysville, Md.: brucella agar, Schaedler agar, Trypticase soy agar (11043), Columbia broth, Trypticase, and Gelysate. Brain heart infusion (BHI) agar, dextrose, yeast extract, proteose peptone, and gelatin were purchased from Difco Laboratories, Detroit, Mich. Agar (Fisher Scientific Co., Pittsburgh, Pa.), NaCl (Fisher), casein (Fisher), bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.), defibrinated sheep blood (The Brown Laboratory, Topeka, Kan.), and rabbit serum (Pel-Freez Biologicals, Inc., Rogers, Ark.) were also used.

General media. Prereduced chopped meat carbo-

TABLE 1. *Species of anaerobic cocci tested for sensitivity to SPS*

Species	No. of strains
Peptostreptococcus anaerobius	26
Acidaminococcus fermentans	5
"Gaffkya" anaerobia	5
Peptococcus magnus	16
Peptococcus prevotii	4
Peptococcus saccharolyticus	3
Peptococcus asaccharolyticus	5
Peptostreptococcus parvulus	2
Streptococcus constellatus	5
Streptococcus intermedius	5
Streptococcus morbillorum	5
Veillonella alcalescens	1
Veillonella parvula	3

hydrate broth, anaerobic dilution fluid, and pyruvate broth with 0.025% Tween 80 were made as described previously (5). The pH of all media used for plates was adjusted to pH 7.2. All media were sterilized by autoclaving (15 min at 15 lb/in^2). Defibrinated sheep blood (5%) was used for blood agar plates. The plated media were dried aerobically in an incubator at 37 C and then stored in the anaerobic chamber overnight.

Composition of supplemented peptone broth. Commercially prepared supplemented peptone broth (B-D Vacutainer culture tube) contained the following ingredients: quadtone (a blend of peptones and yeast extract), 2%; gelatin, 1.2%; NaCl, 0.5%; dextrose, 0.5%; sodium bicarbonate, 0.22%; magnesium sulfate, 0.02%; sodium phosphate, dibasic, 0.014%; adenine, 0.01%; glutamine, 0.01%; *p*-aminobenzoic acid, 0.005%; glutamic acid, 0.005%; proline, 0.005%; SPS, 0.03%; L-cysteine, 0.039%; hemin, 0.005%; nicotinamide adenine dinucleotide, 0.000125%; menadione, 0.0002%; guanine, 0.00015%; cocarboxylase, 0.00015%; and vitamin B_{12}, 0.000005%. For supplemented peptone agar plates, the last seven ingredients were added as a filter-sterilized solution to an autoclaved solution of the other components plus 1.5% agar.

Quadtone basal medium. For most of the experiments, many of the ingredients of supplemented peptone were not necessary; therefore, a simplified medium containing quadtone (2.0%), NaCl (0.5%), dextrose (0.5%), and agar (1.5%) was used. Quadtone was replaced by yeast extract (0.5%) and either proteose peptone (2%), Trypticase (1%), or Gelysate (2%) for certain tests.

Sensitivity to SPS. Thirteen species of anaerobic cocci (Table I) were tested for sensitivity to SPS on the following five agar media: BHI blood, brucella blood, Columbia blood, Schaedler blood, and supplemented peptone. Cultures were tested for sensitivity to SPS on agar plates inoculated with a Steers replicator (9). The inoculum was an overnight culture grown in pyruvate-Tween broth and then anaerobically diluted to an optical density of 0.2 at 650 nm (18-mm light path, B & L Spectronic 20). The replication procedure was performed in the anaerobic chamber, and all plates were incubated in the chamber incubator at 37 C for 48 h. Organisms were considered sensitive when they failed to grow or showed diminished growth in comparison to the control plate without SPS.

Minimal inhibitory concentrations (MIC) of SPS for 16 strains of *P. anaerobius* were determined as described above. The media used were quadtone base plus 5% blood and the quadtone base plus 5% blood and 1.2% gelatin. The MIC was defined as the lowest concentration of SPS that inhibited all growth.

Serum-sensitivity studies. Aerobic quadtone broth (with and without 1.2% gelatin), to which 10% fresh human serum and varying concentrations of SPS had been added, was inoculated with approximately 10^3 cells/ml of log-phase cultures of either *E. coli* C or *S. marcescens* SM 29. Both strains were killed by the human serum used in our tests. Turbidity was recorded after overnight incubation at 37 C.

Growth in simulated blood cultures. Dilutions estimated to contain ten cells, on the basis of a Petroff-Hauser microscopic count of a log-phase culture of two serum-sensitive strains (*E. coli* C and *S. marcescens* SM 29), were injected into B-D Vacutainer culture tubes which contained supplemented peptone broth (45 ml) and freshly drawn human blood (5 ml). The tubes were incubated at 37 C until growth appeared. Several strains of *P. anaerobius* were also tested by the above procedure for ability to grow in the supplemented peptone broth without blood.

RESULTS

Medium dependence of SPS inhibition. Of the 13 species of anaerobic cocci tested (Table 1), *P. anaerobius* was the only species that was inhibited by 0.1% SPS. The toxicity of SPS for *P. anaerobius* was dependent on which medium was used for the test (Table 2). SPS was much less toxic in supplemented peptone (Vacutainer culture tube medium) and BHI media than in the other media we tested (Table 2).

Media components that prevented SPS inhibition. The major components of supplemented peptone medium were tested to determine what was responsible for the lack of SPS inhibition in that medium. Quadtone, a blend of peptones

TABLE 2. *Sensitivity of 24 strains of P. anaerobius to SPS on several agar media*

Media	Strains inhibited (no.)	
	0.05% SPS	0.1% SPS
Supplemented peptone agar	1	3
BHI agar	7	15
BHI blood agar	7	15
Schaedler blood agar	21	24
Trypticase soy agar	23	23
Brucella blood agar	24	24
Columbia blood agar	24	24

and yeast extract, was not protective. Gelatin, the other major ingredient, was protective. Addition of 1.2% gelatin to the quadtone basal medium allowed 23 of 24 strains of *P. anaerobius* to grow in the presence of 0.1% SPS, and 0.6% gelatin protected 10 of 24 strains. The effect of gelatin was not dependent on the media used. Addition of 1.2% gelatin also protected *P. anaerobius* from SPS in Schaedler, brucella, and Columbia blood agar (Table 3). Gelatin was effective regardless of whether it was autoclaved with the media or added afterwards as a filter-sterilized solution.

Proteose peptone appeared to be responsible for the lower toxicity of SPS in BHI (Difco Laboratories, Detroit, Mich.) medium. In a medium containing 2% proteose peptone, 13 of 26 strains of *P. anaerobius* grew in the presence of 0.1% SPS. We did not test brain and heart infusions, which are the other major ingredients of BHI, because they were not available commercially. The activity of proteose peptone was sufficient, however, to account for the effect of BHI.

TABLE 3. *Reversal of SPS toxicity by gelatin for 24 strains of P. anaerobius in various media*

Base media	No. of strains inhibited			
	0.05% SPS	0.05% SPS + 1.2% gelatin	0.1% SPS	0.1% SPS + 1.2% gelatin
Schaedler	24	1	24	4
Brucella	24	1	24	10
Columbia	24	0	24	6

TABLE 4. *Effect of proteins other than gelatin on reversing the toxicity of SPS for P. anaerobius*

Additives to quadtone basal media	No. of strains tested	No. of sensitive strains
Casein, 1.2%	25	9
Casein, 2.4%	25	0
Bovine serum albumin, 1.2%	21	21
Rabbit serum, 20%[a]	25	24
Rabbit serum, heat inactivated, 20%[a]	25	22
Defibrinated sheep blood, 20%[a]	25	24

[a] This concentration of blood was equal to a protein concentration of 1.2% in the medium (8).

Other compounds tested. Addition of blood, serum, and bovine serum albumin to media did not affect SPS toxicity. However, casein did protect *P. anaerobius* from SPS (Table 4). Trypticase, a pancreatic digest of casein, was not protective, nor was Gelysate, a pancreatic digest of gelatin (Table 4).

Extent of protection by gelatin. To measure the extent of protection provided by gelatin, we determined the MIC of SPS in a simple quadtone agar medium with and without 1.2% gelatin. The 16 strains tested had MIC values of less than 0.006% SPS in the absence of gelatin, and gelatin raised the MIC values by an average of 64-fold (Table 5). The three strains that were most sensitive to SPS had MIC values of 0.125% SPS in the presence of gelatin. However, in supplemented peptone broth, which contains 0.03% SPS and 1.2% gelatin, all three strains grew to maximum turbidity within 48 h from inocula of approximately 10 cells per 50 ml of broth. This may have been due to the better growth conditions of the complete medium.

Effect of gelatin on beneficial effects of SPS. SPS is used in blood culture media to eliminate the bactericidal effect of human blood for serum-sensitive organisms. Because gelatin essentially eliminated the inhibitory effect of SPS on *P. anaerobius*, we considered that gelatin might also eliminate the beneficial effect of SPS. However, this did not appear to be so since two serum-sensitive organisms, *E. coli* C and *S. marcescens* SM 29, grew from inocula of approximately 10 cells/50 ml in the Vacutainer culture tube (0.03% SPS, 1.2% gelatin) in the presence of 10% fresh human blood.

Gelatin did have some effect on the ability of SPS to eliminate serum killing. When the two serum-sensitive strains were grown in a medium containing 10% human serum, the amount of SPS required to allow growth was increased fourfold by addition of 1.2% gelatin (Table 6). However, in the presence of gelatin

TABLE 5. *Minimal inhibitory concentration (MIC) of SPS for 16 strains of P. anaerobius on quadtone plate medium*

Media	MIC (final % of SPS in media)									
	<0.00075	0.0015	0.003	0.006	0.0125	0.025	0.05	0.1	0.2	>0.2
Base medium with 5% blood	6[a]	3	5	2	0	0	0	0	0	0
Base medium with 5% blood plus 1.2% gelatin	0	0	0	0	3[b]	0	2	6	3	2

[a] Number of strains having the MIC.

[b] These strains were not inhibited by 0.03% SPS in B-D supplemented peptone broth which contained gelatin.

TABLE 6. *Protection of two serum-sensitive strains (E. coli C and S. marcescens SM 29) in medium containing SPS and gelatin*

Strain[a]	Additives to base medium + 10% serum	Minimal effective concentration of SPS (final % of SPS in media)
E. coli C	None	0.003
	1.2% gelatin	0.0125
S. marcescens SM 29	None	0.0015
	1.2% gelatin	0.006

[a] Inoculum was approximately 10^3 cells/ml.

the amount of SPS required for protection from serum killing (0.0125 to 0.006%) was still much less than the amount of SPS currently used in blood culture media (0.03 to 0.05%).

DISCUSSION

Our results are in agreement with the conclusion of Graves et al. (3) that SPS only inhibits *P. anaerobius* and does not inhibit other common clinical isolates of anaerobic cocci. However, we have not tested a large enough number of strains of each species to detect possible strain variation. The SPS disk test for identification of *P. anaerobius* (3) should prove useful for clinical laboratories, but larger scale tests will be necessary to document the accuracy of the test. Our study demonstrates that the test should be standardized with a single medium such as the Schaedler agar used by Graves et al. (3). Media for use with the SPS disk test should not contain proteose peptone, gelatin, or casein. Schaedler medium does not contain these ingredients.

It is not yet known how SPS inhibits growth of *P. anaerobius*. This makes it difficult to explain how gelatin and casein overcome the inhibition. The activity of these proteins seems to be related to their secondary or tertiary structure, since their pancreatic digests, consisting of single amino acids and short peptides, were not active. The activity of proteose peptone may result from its content of large peptides ("proteoses") which are probably derived from gelatin; both proteose peptone and gelatin have an unusually high glycine content of 23 to 25%. SPS may be inactivated by binding to portions of gelatin and casein molecules just as it is known to bind to the serum proteins responsible for serum killing (10). However, gelatin only increased by fourfold the concentration of SPS needed to neutralize the bactericidal effect of human serum, whereas it increased 64-fold the concentration needed to inhibit *P. anaerobius*.

Whatever its mechanism of action, gelatin appears to be a useful addition to blood culture media since it allows growth of *P. anaerobius* while at the same time it does not prevent SPS from neutralizing the bactericidal effect of blood. Additional simulated and clinical blood culture studies should be performed to confirm these results because SPS and serum sensitivity might vary among strains, fresh clinical isolates might behave somewhat differently than our stock cultures, and the bactericidal activity of blood from different sources might vary as well.

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