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Neutralization of the Inhibitory Effect of Sodium Polyanetholesulfonate on *Neisseria meningitidis* in Blood Cultures Processed with the Du Pont Isolator System

RONALD K. SCRIBNER AND DAVID F. WELCH*

Oklahoma Children's Memorial Hospital and Division of Pediatric Infectious Diseases, Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma 73126

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The inhibitory and bactericidal effects of sodium polyanetholesulfonate on *Neisseria meningitidis* were neutralized by blood lysis which occurs within the pediatric Isolator 1.5 Microbial tube (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). Lysed blood was more effective than 2.5% gelatin in preventing the inhibitory effect. All but 1 of 16 *N. meningitidis* strains were killed by 0.06% sodium polyanetholesulfonate in the absence of lysed blood, whereas none were killed by 1.0% sodium polyanetholesulfonate when lysed blood was present. The possible clinical relevance of these results was reflected in the improved detection of meningococcemia in children when the Isolator 1.5 Microbial tube was compared with a conventional broth system.

Sodium polyanetholesulfonate (SPS) is frequently incorporated into commercial blood culture media as an anticoagulant and to counteract the inhibitory factors in fresh human blood (9). Although it has been shown that SPS enhances the rate and speed of recovery of gram-positive cocci and gram-negative rods (3), an adverse effect upon the isolation of *Neisseria* species from blood cultures has been noted (5, 8). Since *Neisseria meningitidis* is an important pathogen in pediatrics, information is needed about the efficacy of the new Isolator 1.5 Microbial tube (E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.), which contains SPS, in the diagnosis of meningococcemia. A preliminary report has indicated that the lysed blood inside the Isolator tube binds SPS and may reverse its antibacterial effects (J. C. Richards, C. Bentsen, J. W. Freytag, and R. R. Hebert, Program Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C76, p. 324). Additionally, it has recently been shown by time-kill studies that *N. meningitidis* is protected from the antimicrobial effects of SPS by the addition of hemoglobin (2). We examined the effect of lysed blood on the susceptibility of *N. meningitidis* to SPS and compared isolation times between the Isolator 1.5 Microbial tube and a conventional broth system in six patients who had meningococcemia. Efficient neutralization of SPS toxicity may be a significant benefit of the Isolator blood culture methodology.

MATERIALS AND METHODS

Bacterial cultures. Organisms used in this study were *N. meningitidis* serogroups A, B, and C, which had been isolated from blood or cerebrospinal fluid of pediatric patients at the Oklahoma Children's Memorial Hospital, Oklahoma City. Identification was performed by carbohydrate utilization tests and agglutination in specific antisera. Isolates were either lyophilized or frozen in brain heart infusion-glycerol medium at −70°C until use.

Isolator 1.5 Microbial tube. The Isolator 1.5 Microbial tube is the pediatric version of the Du Pont Isolator blood culture system. The dimensions of the tube are 10 by 50 mm. It is rubber stoppered under vacuum and contains purified saponin (1 U), SPS (0.96 mg), polypropyleneglycol (0.8 μl), and water in a total volume of 0.1 ml.

Disk diffusion susceptibility. Filter paper disks (Schleicher & Schuell, Inc., Keene, N.H.) were loaded with 20 μl of either undiluted Isolator fluid (SPS, 0.96%; purified saponin, 10 U/ml; polypropyleneglycol, 8 μl/ml), 0.1 ml of Isolator fluid plus 1.5 ml of normal saline (SPS, 0.06%), 0.1 ml of Isolator fluid plus 1.5 ml of 2.5% gelatin (SPS, 0.06%), or 0.1 ml of Isolator fluid plus 1.5 ml of fresh human blood (SPS, 0.06%). The latter specimen simulates the clinical situation in which the Isolator 1.5 Microbial tube is used according to package directions. Disks were air dried for 30 min at room temperature and then applied to the surface of Mueller-Hinton agar plates which had been seeded with *N. meningitidis* by the standard Bauer-Kirby technique (1). Plates were then incubated for 24 h at 35°C in 5% CO_2, and zone sizes were measured to the nearest 0.1 mm.

Inhibitory and bactericidal tests. Determinations of the MIC and the MBC of SPS were performed essentially by standard macrodilution broth methodology (6). Inocula were prepared from overnight cultures in Mueller-broth to give a final density of organisms of 2×10^5 to 6×10^5 CFU/ml. The MIC was defined as the lowest concentration of SPS resulting in no visible growth after 24 h of incubation at 35°C in 5% CO_2. The MBC was defined as the lowest concentration of SPS that prevented growth of more than 20 to 60 colonies, depending on the initial inoculum size, when 0.1 ml from all clear tubes was subcultured to antibiotic-free chocolate agar and incubated as described above. The determination of SPS activity in the presence of lysed blood involved a modification of this technique. Fresh human blood was collected in Isolator tubes from adult volunteers; informed consent was obtained. Three parts of this lysed blood were added to one part Mueller-Hinton broth and dispensed into tubes, and an appropriate amount of 10% SPS was added to achieve twofold dilutions of SPS that ranged from 0.06 to 1% (Table 1).

To an aliquot of 1.0 ml of each dilution was added 0.001 ml of an inoculum containing 2×10^5 to 6×10^5 organisms. After incubation, 0.1 ml was subcultured to antibiotic-free

* Corresponding author.

TABLE 1. SPS dilutions used in this study

Amt (ml) of 10% SPS in water	Amt (ml) of 75% lysed blood in Mueller-Hinton broth	Final SPS concn (%)
0.06	9.94	0.06
0.12	9.88	0.12
0.25	9.75	0.25
0.5	9.50	0.5
1.0	9.0	1

medium and incubated as described above. MBC endpoints were read as described above.

Comparison of the Isolator 1.5 Microbial tube and a conventional broth system. Blood was drawn aseptically from patients, inoculated into the Isolator tube and one 50-ml brain-heart infusion blood culture bottle without gelatin (GIBCO Laboratories, Grand Island, N.Y.) containing 0.025% SPS, and transported to the laboratory. The average volume of blood cultured was 0.75 ml in the Isolator tube and 1.8 ml in the broth bottles. The bottles were incubated at 35°C, and the Isolator 1.5 Microbial tubes were processed upon receipt. The contents of the Isolator 1.5 Microbial tube were inoculated onto chocolate agar (ca. 0.3 ml per plate) and incubated in 5% CO_2 at 35°C for 4 days. Agar and broth media were examined routinely at 7:00 a.m., 3:00 p.m., and 8:00 p.m. on each of 4 days. Blind subcultures from bottles were made to chocolate agar plates. An early subculture was performed at 4 to 14 h, depending on the time of receipt. Second and third subcultures were performed at 24 h and 5 days.

RESULTS

Results of disk diffusion susceptibility testing are shown in Table 2. Undiluted Isolator fluid (SPS, 0.96%) had the greatest toxic effect on *N. meningitidis*. Isolator fluid plus saline was more toxic than Isolator fluid plus gelatin or Isolator fluid plus lysed human blood, although the SPS concentration was the same in all cases (0.06%). MICs and MBCs of SPS for *N. meningitidis* are shown in Table 3. The addition of lysed blood to the growth medium had a profound effect on the toxicity of SPS; in all cases, the MBC of SPS exceeded 1%, which represents a concentration considerably higher than that to which bacteria would be exposed when the Isolator 1.5 Microbial tube is used clinically. No differences in susceptibility to SPS among serological groups were detectable in either the disk diffusion or the broth dilution tests. Meningococcemia was diagnosed significantly earlier by the Isolator 1.5 Microbial tube than by the conventional broth system (Table 4). Time to recovery of isolates by the Isolator 1.5 Microbial tube was independent of the bacterial concentration in the blood.

DISCUSSION

Blood culture methodology in which the Isolator 1.5 Microbial tube is used offers more sensitive and earlier detection than broth systems for some organisms (D. F. Welch, R. K. Scribner, and D. Hensel, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 138, 1983). Our results suggest a further advantage which was previously unrecognized. Blood lysis within the Isolator 1.5 Microbial tube reversed the antimeningococcal activity of SPS. Although the primary function of saponin in the system is intended to be lysis of erythrocytes and leukocytes, an important secondary function may be the release of hemoglobin to bind SPS. It has been shown that the addition of gelatin to blood culture media enhances the recovery of *N. meningitidis* (4, 7). Our disk diffusion test results indicate that lysed human blood was more effective than 2.5% gelatin in reversing SPS toxicity. Staneck and Vincent have demonstrated that 2% gelatin or 1% hemoglobin have similar protective effects against SPS toxicity to *N. gonorrhoeae* (8).

The addition of lysed blood to the growth medium raised the MBCs of SPS more than 16-fold for all strains except strain 1780, which showed high resistance to SPS (MBC, 1%). That strains which were highly susceptible to SPS, such as strains 7 and 1981 (MIC, 0.015%), were not killed by 1% SPS when lysed blood was present suggests that SPS toxicity for meningococci should not be a problem with clinical

TABLE 2. Disk diffusion inhibitory zone diameters[a]

Strain	Zone diam (mm) obtained with: Isolator fluid[b]	Isolator + saline[c]	Isolator + gelatin[d]
1	11.2	10.4	7.9
2	9.8	9.2	6
3	10.6	10.0	6
4	12.2	11.0	8.5
7	12.6	12.0	8.0
8	14.1	13.2	8.1
9	13.9	13.0	12.0
12	13.6	13.1	10.1
13	12.0	11.4	10.0
14	13.6	13.2	6
418	12.8	11.0	7.9
1591	13.0	12.0	8.3
1780	8.5	6	6
1981	13.2	12.1	8.3
2020	13.0	11.8	9.0
2075	12.6	11.4	8.1
Mean	12.3	11.3	8.1

[a] No zone of inhibition was observed for any of the strains when 1.5 ml of fresh human blood was added to the Isolator tube and 20 μl was delivered to the disk.
[b] Twenty microliters of undiluted Isolator 1.5 Microbial tube fluid.
[c] A total of 1.5 ml of saline was added to the Isolator 1.5 Microbial tube; then 20 μl was delivered to the disk.
[d] A total of 1.5 ml of 2.5% gelatin was added as described in footnote *c*.

TABLE 3. MICs and MBCs of SPS[a]

Strain	MIC (%)	MBC (%)
1	0.06	0.06
2	0.03	0.06
3	0.06	0.06
4	0.06	0.06
7	0.015	0.03
8	0.03	0.03
9	0.03	0.03
12	0.06	0.06
13	0.06	0.06
14	0.06	0.06
418	0.06	0.06
1591	0.06	0.06
1780	0.5	1.0
1981	0.015	0.3
2020	0.03	0.06
2075	0.06	0.06

[a] MBCs for all strains were >1.0 when lysed blood was added to the Isolator tube.

TABLE 4. Diagnosis of meningococcemia by broth and Isolator systems

Patient isolate	CFU/ml of blood	Time (h) to recovery by:	
		Isolator tube	Broth system
418	>1,000	22	46
1780	>1,000	24	50
1981	20	18	42
2020	50	15	39
2075	180	24	49
6666	3	43	NR[a]

[a] NR, Not recovered.

use of the Isolator 1.5 Microbial tube. Strains such as these may not be recovered by broth culture techniques since most broth media contain at least 0.025% SPS. However, one must recognize that the effect of SPS on a given strain would be unpredictable in clinical situations because of variables such as spontaneous hemolysis and influence of inoculum size. The quantity of free hemoglobin is directly related to binding and neutralization of the antibacterial properties of SPS: an SPS/hemoglobin ratio of 1:6 provides optimum SPS inactivation (2).

N. meningitidis was recovered from the blood of five patients by both the Isolator and broth systems. In the sixth case, *N. meningitidis* was recovered from the Isolator tube only. No difference in time to recovery was seen in either system between a strain that was resistant to SPS (strain 1780) and one that was highly susceptible (strain 1981). Although strain 1780 required 0.5% SPS for inhibition of 10^5 CFU/ml in these experiments, it is possible that the dilution effect of the broth, which reduced the initial bacterial inoculum to ca. 20 CFU/ml, caused delayed detection in the clinical situation. The mean time to recovery of the five isolates recovered from both systems was 20.6 h by the Isolator 1.5 Microbial tube and 45.2 h by the broth system ($P < 0.001$, paired t test). Along with the results of our in vitro studies, this finding provides a basis to speculate that the Isolator system may facilitate the recovery of *N. meningitidis* because the blood lysis frees hemoglobin and SPS toxicity is thereby avoided. However, larger studies in which the recovery of SPS-susceptible organisms is compared with that of SPS-resistant organisms in the Isolator and broth systems may be required to fully support these observations.

LITERATURE CITED

1. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**:493–496.
2. **Edberg, S. C., and M. K. Edberg.** 1983. Inactivation of the polyanionic detergent sodium polyanetholsulfonate by hemoglobin. J. Clin. Microbiol. **18**:1047–1050.
3. **Eng, J.** 1975. Effect of sodium polyanethol sulfonate in blood cultures. J. Clin. Microbiol. **1**:119–123.
4. **Eng, J., and E. Holten.** 1977. Gelatin neutralization of the inhibitory effect of sodium polyanethol sulfonate on *Neisseria meningitidis* in blood culture media. J. Clin. Microbiol. **6**:1–3.
5. **Eng. J., and H. Iveland.** 1975. Inhibitory effect in vitro of sodium polyanethol sulfonate on the growth of *Neisseria meningitidis*. J. Clin. Microbiol. **1**:444–447.
6. **National Committee for Clinical Laboratory Standards.** 1983. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Tentative standard M7-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Pai, C. H., and S. Sorger.** 1981. Enhancement of recovery of *Neisseria meningitidis* by gelatin in blood culture media. J. Clin. Microbiol. **14**:20–23.
8. **Staneck, J. L., and S. Vincent.** 1981. Inhibition of *Neisseria gonorrhoeae* by sodium polyanetholesulfonate. J. Clin. Microbiol. **13**:463–467.
9. **Traub, W. H., and B. L. Lowrance.** 1970. Anticomplementary, anticoagulatory, and serum-protein precipitating activity of sodium polyanetholsulfonate. Appl. Microbiol. **20**:465–468.