APPLIED MICROBIOLOGY, Nov. 1971, p. 805-808

Vol. 22, No. 5
Printed in U.S.A.

Detection of Bacteremia in Children with Sodium Polyanethol Sulfonate: a Prospective Clinical Study

ROBERT MINKUS AND HUGH L. MOFFET
Children's Memorial Hospital, Chicago, Illinois 60614

Received for publication 12 July 1971

A prospective study was made of 1,000 consecutive duplicate blood cultures obtained from sick children to evaluate the usefulness of sodium polyanethol sulfonate (SPS). With the small volumes of blood (1 to 5 ml) usually obtained for blood cultures in children, SPS did not increase the frequency of recovery of organisms judged to be associated with clinical infections, with the possible exception of *Diplococcus pneumoniae*. However, the use of SPS was associated with an increased frequency of recovery of organisms judged to be contaminants, such as *Staphylococcus epidermidis* and propionibacteria, possibly because SPS enhanced the recovery of a very small inoculum of skin bacteria.

Sodium polyanethol sulfonate (SPS) is a heat-stable anticoagulant which can be added to blood culture bottles to increase the frequency of recovery of bacteria from blood. The major mechanism of action is probably the anticoagulant effect, which prevents the formation of clots which can trap bacteria (11). In addition, SPS decreases the phagocytic properties of leukocytes, inactivates complement (5, 7), and inhibits the bactericidal effects of human serum (5, 10). All of these effects may conceivably aid in the recovery of bacteria from blood.

Although there have been numerous in vitro studies which demonstrate the ability of SPS to enhance the survival of bacteria in blood, it has received few prospective clinical trials (4, 9). In one study, SPS added to thioglycolate resulted in 35% more positive cultures than thioglycolate alone and 22% more positive cultures than thioglycolate medium containing another anticoagulant, sodium citrate (9). However, the frequency of contaminants, the clinical criteria used to define contaminants, and the effect of SPS on the recovery of contaminants were not reported. In addition, that study required three separate blood cultures 2 hr apart with 5 ml of blood in each. These requirements can rarely be met for blood cultures obtained from children and are often impractical in clinical situations involving adult patients. Because of the importance of improving current blood culture methods and the encouraging results and unanswered questions cited above, it was decided to do a prospective clinical trial in a children's hospital.

MATERIALS AND METHODS

The study was designed to fit into the established blood culture routine at this hospital. Physicians obtained the blood cultures, and the specimens were processed by the hospital's clinical bacteriology laboratory. All patients having blood cultures obtained for any reason were included in the study until 1,000 consecutive pairs of cultures were obtained.

Preparation of culture materials. Parallel cultures were done, one by the laboratory's standard method and the other by the identical method with SPS added. The culture medium used was thioglycolate broth with CO_2 (B-D Vacutainer) without additives such as dextrose or indicator. Prefilled screw-top, rubber-stoppered vacuum bottles were used, each containing 50 ml of broth. SPS was added to some blood culture bottles aseptically with a sterile needle and syringe in the laboratory to achieve a final concentration of 0.05%, as recommended by the manufacturer. Two bottles were taped together, one containing only the thioglycolate broth, the other containing thioglycolate broth with added 0.5 ml of 5% SPS solution (Grobax, Hoffman-LaRoche Co.) The two bottles, one with SPS and one without SPS, were marked with a code letter scratched on the glass. After preparation, the bottles were incubated for 24 hr at 37 C and then examined for possible growth of contaminants before use. Several sets of bottles in each batch were held for 14 days at 37 C to exclude the possibility of contamination with slow growing or minimal numbers of bacteria.

Inoculation of blood into media. Paired bottles were distributed to all areas of the hospital and kept at room temperature until used. The nursing and medical staff were informed of the study and were asked not to separate the two bottles. The physicians were instructed to divide the blood obtained from the patient

into two equal samples, each between 1 and 5 ml, and to inject an equal quantity into each of the two bottles. In this children's hospital, the amount of blood obtained for culture was usually less than 5 ml per bottle because many patients were infants or small children and because a portion of the blood obtained was often used for other tests.

Blood for culture was obtained from an antecubital or external jugular vein. Before blood was drawn for culture, the venepuncture site was swabbed with tincture of iodine, allowed to dry, and then was swabbed with sterile alcohol. A sterile disposable needle and syringe were used to collect the blood which was then inoculated immediately into each culture bottle through the rubber stopper after wiping with alcohol. Paired bottles were then incubated at 37 C until they were picked up and delivered to the bacteriology laboratory 2 to 15 hr later.

Processing of cultures in the laboratory. Blood culture bottles were incubated at 37 C as soon as they were received. Equal samples of 0.5 ml were withdrawn from the bottles and subcultured at 24 hr, 72 hr, and 10 days after receipt. Gram stains and subcultures of both bottles were done immediately if visual inspection of the bottles, done twice a day, revealed any suspicion of bacterial growth. Each subculture was inoculated into thioglycolate broth and onto chocolate agar for incubation in 5% carbon dioxide. Penicillinase was not added to any of the cultures.

Definition of contaminants. Before the study began, prospective criteria were defined for classifying any organisms recovered as either pathogens or contaminants. Since any organism can be pathogenic, clinical factors were included in these criteria. The prospective criteria for *Staphylococcus epidermidis* to be considered pathogenic were as follows. (i) The patient must have had a plastic intravenous catheter or other foreign body in a vein for at least 48 hr prior to blood culture; or (ii) the organism had to have been recovered from more than one blood culture or from both blood and cerebrospinal fluid. The criteria for accepting alpha-hemolytic *Streptococcus* as a pathogen were: (i) patient had rheumatic or congenital heart disease and had two or more consecutive isolations of the organism from a blood culture, or (ii) the patient had an alpha-hemolytic *Streptococcus* urinary tract infection, as well as the same organism in a blood culture. The criteria for accepting *Candida albicans* as a pathogen were: (i) any isolation from a patient undergoing hyperalimentation with a plastic intravenous catheter; or (ii) two or more consecutive isolations of *C. albicans* from the blood of patients with intravenous catheters in place for at least 48 hr prior to culture. Propionibacteria and *Bacillus* species were always considered as contaminants because they are rarely associated with human disease.

RESULTS

One thousand consecutive double-bottle cultures were obtained from October 1969 through August 1970. Of these 1,000 cultures, 92 (9.2%) were positive for organisms defined as pathogenic by the prospective criteria listed above. Of these 92 cultures, 66 were positive in both bottles, 13 were positive only in the bottle to which SPS had been added, and 13 were positive only in the bottle without SPS (Table 1). The most common pathogenic bacteria recovered (Table 2) were *S. aureus* (29), *Haemophilus influenzae* (17), *Klebsiella pneumoniae* (12), *Diplococcus pneumoniae* (8), and group A *Streptococcus* (7). One culture, from a patient with terminal leukemia, grew two organisms, *Hafnia* species and *K. pneumoniae*.

TABLE 1. *Effect of sodium polyanethol sulfonate (SPS) on recovery of pathogens and contaminants*

Organism	No. recovered			
	SPS only	Non-SPS only	Both bottles	Total
Pathogen.....	13	13	66	92
Contaminant.....	26	12	28	66

The positive cultures were also divided into gram-positive and gram-negative organisms (Table 3). SPS did not appear to aid in the preferential recovery of either group. The addition of SPS did not result in earlier detection of positive cultures, with the timing of subcultures used in this laboratory.

Of the 92 positive blood cultures judged to be positive for pathogenic bacteria, 23 were recovered while the patient was receiving an antibiotic, 5 from a bottle with SPS, 4 from a bottle without SPS, and 14 from both bottles. Of the organisms which were susceptible to an antibiotic which the patient was receiving, 10 were recovered in bottles with SPS and 7 were recovered in bottles without SPS.

Of the 1,000 pairs of cultures, 66 (6.6%) had an organism recovered which was considered to be a contaminant by the prospective definitions above (Table 4). Of these contaminants, 28 were recovered from both bottles, 26 were recovered only in the bottle with the SPS additive, and 12 were recovered only in the bottle without the SPS. The most common contaminants were *S. epidermidis* (46) and propionibacteria (15), which are normal flora of the skin.

DISCUSSION

The use of SPS has been the subject of many nonclinical studies, most of which have shown it to be of potential advantage in increasing the likelihood of recovering bacteria in clinical bacteremias. Roome and Tozer added various amounts of blood to broth and then added a small inoculum of a serum-sensitive strain of *Escherichia coli*, to determine the relationship between the concentration of serum and the sur-

TABLE 2. *Pathogens recovered*

Organism	No. of pathogens recovered from			
	SPS only[a]	Non-SPS only	Both bottles	Total
Staphylococcus aureus	3	4	22	29
Haemophilus influenzae	0	0	17	17
Klebsiella pneumoniae	1	1	10	12
Diplococcus pneumoniae	3	1	4	8
Group A *Streptococcus*	0	1	6	7
Pseudomonas aeruginosa	2	2	1	5
S. epidermidis	2	1	2	5
Escherichia coli	0	1	2	3
Candida albicans	1	1	0	2
Neisseria meningitidis	0	0	1	1
Citrobacter	1	0	0	1
Enterobacter cloacae	0	0	1	1
Hafnia and *Klebsiella pneumoniae*	0	1	0	1
Totals	13	13	66	92

[a] SPS, sodium polyanethol sulfonate.

vival of serum-sensitive bacteria (8). They found that the blood must be diluted 1:30 or more to obtain bacterial growth. However, when SPS was added to the broth before the addition of blood, the bactericidal effect of serum was abolished, and dilution of the blood was no longer necessary to obtain a positive culture.

Lawrence and Traub tested the time required for fresh serum to kill a large inoculum of serum-sensitive *E. coli* (5). They also found that SPS abolished the serum bactericidal effect, with lesser concentrations of SPS required for lesser concentrations of serum. This beneficial effect of SPS disappeared after heating the serum to 45 C, implying that the serum bactericidal effect is complement-dependent. The turbidity of the serum noted when SPS was added was presumably secondary to precipitated complement.

Morello and Ellner cited unpublished experiments in their own laboratory which "failed to demonstrate any stimulatory effect of (SPS) on the growth of bacteria in the absence of blood," but, believed it to be beneficial in the presence of blood (6).

Ellner and Stoessel studied the effects of both temperature and anticoagulants on blood cultures (2). They found that both citrate and SPS increased the recovery of many strains of bacteria, but that SPS was clearly superior to citrate. SPS was especially beneficial for the growth of *D. pneumoniae* and *Pseudomonas* species.

Evans et al. studied the growth of bacteria in simulated blood cultures and found an anticoagulant to be beneficial and, specfiically found SPS to be superior to heparin, ethylenediaminetetraacetic acid, and citrate (3).

Rosner compared three different media in a clinical trial: thioglycolate alone, thioglycolate with 0.03% SPS, and thioglycolate with citrate (9). Positive cultures were detected earlier when incubated with added SPS. The specimens incubated with added SPS had a significantly higher recovery rate than with thioglycolate alone or with thioglycolate with added citrate. SPS did not increase the recovery of gram-negative bacteria, in contrast to what might be expected if SPS inhibited the bactericidal effect of serum on serum-

TABLE 3. *Effect of sodium polyanethol sulfonate (SPS) on recovery of gram-negative or gram-positive pathogens*

Organism	No. recovered from			
	SPS only	Non-SPS only	Both bottles	Total
Gram-negative	4	5	32	41
Gram-positive	9	8	34	49
Totals	13	13	66	90

TABLE 4. *Contaminants recovered*

Organism	No. recovered from			
	SPS only	Non-SPS only	Both bottles	Total
Staphylococcus epidermidis	18	8	20	46
Propionibacteria	5	3	7	15
Alpha *Streptococcus*	2	0	1	3
Bacillus subtillis	1	1	0	2
Totals	26	12	28	66

sensitive gram-negative bacteria. However, *D. pneumoniae* and alpha, beta, and anaerobic streptococci all were recovered more often when SPS was present. Positive cultures were analyzed only if the organism was recovered in all three cultures. Any organism isolated from one or two of the cultures was excluded as if it were a contaminant, regardless of the organism or the clinical factors involved. Thus all of the data on the effect of SPS on the recovery of presumed contaminants were excluded.

These exclusions also may have eliminated some clinically significant bacteremias. If three cultures are drawn, the isolation of an organism from one or two of them might be sufficient grounds for treatment, if the clinical situation is appropriate. A bacteremia that was intermittent, transient, or associated with a low concentration of organisms might be missed in one of three cultures, thus eliminating some legitimate positive cultures from the study.

Finegold et al., in a study of 100 positive and negative blood cultures, found SPS of some benefit in recovering bacteria from the blood. They noted an increased number of contaminants when SPS was added but suggested no explanation for this phenomenon (4).

In the present study, SPS did not increase the number of clinically significant positive blood cultures, with the possible exception of *D. pneumoniae*. When the isolates were divided into gram-positive and -negative organisms, there was no preferential recovery in cultures containing SPS. However, the addition of SPS markedly increased the isolation rate of organisms considered to be clinically insignificant contaminants, particularly *S. epidermidis* and propionibacteria.

In the preparation of the double bottles, precautions were taken to avoid contamination, and no evidence of contamination was found in incubated bottles. Although it is impossible to rule out the laboratory as a source of contamination after the specimens were received, the two bottles were handled in an identical fashion by the laboratory, and any laboratory contamination would be randomized; that is, each bottle would have had an equal risk of contamination.

The most likely explanation for the increased frequency of contaminants in bottles with SPS is that small numbers of skin organisms were inoculated randomly in some of the bottles, but grew out most frequently from the bottle with SPS. SPS may lead to a greater frequency of positive cultures when small numbers of organisms are present in the inoculum. The increased number of *D. pneumoniae* isolated with SPS may have occurred because pneumococcemias are often transient, with small numbers of bacteria. In this series, none of the eight isolates of *D. pneumoniae* was associated with serious clinical illness. In four, the positive blood culture was an unexpected finding when correlated with the clinical findings.

Much of the evidence from other studies cited above indicates that SPS may increase the likelihood of obtaining an isolate of an organism when relatively large volumes of blood are used, as is the case for most blood cultures in adults, where at least 5 ml of blood is usually drawn for blood cultures. For pediatric patients, only small amounts of blood are usually available for culture so that SPS does not appear to be of any significant advantage for routine use. Use of SPS may result in an increased detection of contaminant organisms in some acutely ill infants or small children who only have one blood culture obtained. In this situation, the use of SPS may be a disadvantage since reports of isolates which are probably skin contaminants often complicate the problem faced by the clinician caring for the patient.

ACKNOWLEDGMENTS

We acknowledge the technical assistance and advice of Ernest R. Burkholder and staff of the Clinical Microbiology Laboratories, Children's Memorial Hospital, Chicago.

This study was supported by a general research support grant 5SO1FRO5475 to R.M. from the National Institutes of Health.

LITERATURE CITED

1. Allgoewer, M. 1947. Ueber die Wirkung von Heparin, polyanetholsaurem natrium (Liquoid Roche) und tribasischem natriumcitrat auf menschliche Leukocyten *in vitro*. Schweiz. Med. Wochenschr. 77:40–43.
2. Ellner, P. D., and C. J. Stoessel. 1966. The role of temperature and anticoagulant on the in vitro survival of bacteria in blood. J. Infec. Dis. 116:238–242.
3. Evans, G. L., T. Cekoric, R. L. Searcy, and L. R. Hines. 1966. Effects of anticoagulants on antibacterial action of blood. Clin. Res. 14:484.
4. Finegold, S. M., I. Ziment, M. L. White, W. R. Winn, and W. T. Carter. 1968. Evaluation of polyanethol sulfonate (Liquoid) in blood cultures. Antimicrob. Ag. Chemother. 1967, 692–696.
5. Lowrance, B. L., and W. H. Traub. 1969. Inactivation of the bactericidal activity of human serum by Liquoid (sodium polyanetholsulfonate). Appl. Microbiol. 17:839–842.
6. Morello, J. A., and P. D. Ellner. 1969. New medium for blood cultures. Appl. Microbiol. 17:68–70.
7. Pontieri, G. M., and O. J. Plescia. 1965. A comparison of methods for the inactivation of third component of guinea-pig complement. Experentia 21:81–82.
8. Roome, A. P. C. H., and R. A. Tozer. 1968. Effect of dilution on the growth of bacteria from blood cultures. J. Clin. Pathol. 21:719–721.
9. Rosner, R. 1968. Effects of various anticoagulants and no anticoagulant on ability to isolate bacteria directly from parallel clinical blood specimens. Amer. J. Clin. Pathol. 49:216–219.
10. Von Haebler, T., and A. A. Miles. 1938. The action of sodium polyanethol sulfonate (Liquoid) on blood cultures. J. Pathol. Bacteriol. 46:245–252.
11. Wright, H. D. 1925. The bacteriology of subacute infective endocarditis. J. Pathol. Bacteriol. 28:541–578.