APPLIED MICROBIOLOGY, Sept. 1970, p. 465-468

Vol. 20, No. 3
Printed in U.S.A.

Anticomplementary, Anticoagulatory, and Serum-Protein Precipitating Activity of Sodium Polyanetholsulfonate

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Received for publication 8 May 1970

Sodium polyanetholsulfonate (SPS) at 7.8 μg/ml completely abolished complement-mediated hemolysis of 1:10 diluted fresh guinea pig and human serum; at least twice as much SPS was required to reduce complement activity in 1:2 diluted human serum. The coagulation of 90 and 20% human blood was inhibited by 250 and 125 μg of SPS per ml, respectively. When added to fresh human serum, SPS precipitated beta 1C-globulin (C3), C4, beta lipoproteins, immunoglobulin IgG, IgM, and IgA, though incompletely.

Sodium polyanetholsulfonate (SPS) is an anticomplementary and antiphagocytic synthetic anticoagulant (1, 3, 16). Because of these properties, SPS has found widespread use in blood cultures (12, 15). This substance is also known to inhibit the activity of streptomycin, polymyxin B, kanamycin sulfate, and gentamicin sulfate (4, 8, 13, 14). Likewise, heparin has now been shown to antagonize aminoglycoside and polymyxin antibiotics (5). Wadsworth et al. (16) reported that 0.005% (50 μg/ml) SPS abolished complement activity in 1:10 diluted guinea pig serum. Previous studies in this laboratory indicated that 250 μg (0.025%) SPS per ml neutralized the bactericidal activity of 50% fresh human serum (7); it was of interest to determine the amount of SPS required to antagonize complement-mediated hemolysis of fresh human serum. An attempt was made to quantitate the anticoagulatory activity of SPS in human blood. Finally, immunodiffusion studies served to reveal which components of fresh and heat-inactivated human serum were precipitated by SPS. Previously, Burstein and Samaille (2) had demonstrated that beta lipoproteins were precipitated by SPS from human serum, and Pontieri and co-workers reported that SPS inactivated the third component of porcine complement (10). Years ago, Zunz et al. (17) had shown that SPS precipitated fibrinogen.

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MATERIALS AND METHODS

Sodium polyanetholsulfonate. SPS (batch 099117) was a gift from Hoffman-LaRoche, Inc., Nutley, N.J. The anticoagulant was dissolved in distilled water to yield 10,000 μg/ml (1% aqueous solution). The stock solution was autoclaved and stored at 4 C.

Fresh human serum. Sera from two normal adults (designated as L- and T-serum) were processed as described previously (7).

Antisera. Goat antisera to human immunoglobulin (Ig)G, IgM, IgA, alpha globulins, beta globulins, beta 1C-globulin, C4, transferrin, and gamma globulin were purchased from Hyland, Division of Travenol Laboratories, Los Angeles, Calif.

Human plasma fractions. The following lyophilized fractions of human plasma were obtained from Pentex, Inc., Kankakee, Ill.: albumin, transferrin, fibrinogen, alpha 1-globulin, alpha 4-globulin, alpha globulins, beta globulins, and gamma globulins. The fractions were reconstituted with sterile distilled water and sterile isotonic saline, respectively, to yield physiological concentrations.

Titration of complement. Rabbit anti-sheep hemolysin (Difco), guinea pig complement (Difco), and the complement content of fresh human serum were titrated in accordance with the Laboratory Branch Task Force procedure (6); hemoglobin standards were prepared accordingly. The potency of complement was defined in terms of CH_{50}, the number of which indicated that dilution of complement (serum) which produced 50% hemolysis of the standardized sheep-red blood cell (S-RBC) suspension.

Microimmunodiffusion tests. Tests were carried out with the LKB 6800 A-J immunodiffusion assembly (LKB Produkter AB, Stockholm, Sweden), by the technique of Ouchterlony (9). One per cent Noble

Agar (Difco) with a *p*H of 7.2 served as the stabilizing gel.

Inhibition of blood coagulation. Blood was collected from healthy adult donors and immediately dispensed as 4.5-ml samples into test tubes which contained 0.5 ml of serial twofold dilutions of SPS in Trypticase-Soy Broth (BBL; TSB) to yield final concentrations ranging from 1,000 to 31.25 μg/ml; control tubes received 0.5 ml of isotonic saline. Similarly, 2 ml of blood was added to a series of tubes containing 7.5 ml of TSB and 0.5 ml of twofold diluted SPS; control tubes received saline instead of SPS. The tubes were gently inverted three times and allowed to remain undisturbed at room temperature. The tubes were examined for the presence or absence of blood coagulation at 0.5, 2, 24, and 48 hr. Any grossly visible coagulum was interpreted as positive for blood coagulation.

RESULTS

In the first series of experiments, serial twofold dilutions of fresh guinea pig serum (1:10 through 1:320) were exposed to various concentrations of SPS (range, 500 to 7.8 μg/ml). As little as 7.8 μg of SPS per ml significantly reduced the activity of complement in 1:10 diluted guinea pig serum (Table 1), whereas SPS at the same concentration completely abolished complement activity in 1:10 diluted fresh human serum (Table 2). However, at least twice as much SPS was required to neutralize complement in 1:2 diluted fresh human serum (Table 3). No sharp end points were obtained in these latter titrations, in that SPS over a range of concentrations markedly but incompletely reduced the complement activity.

Coagulation of 90 and 20% human blood was inhibited by 250 and 125 μg of SPS per ml, respectively, over a period of 48 hr (Table 4).

SPS at 500 μg/ml was found to precipitate the following human plasma components: beta globulins, fibrinogen, and pooled gamma globulins.

TABLE 1. *Anticomplementary activity of sodium polyanetholsulfonate (SPS) in guinea pig serum*

Final concn of added SPS (μg/ml)	Dilutions of fresh guinea pig serum					
	1:10	1:20	1:40	1:80	1:160	1:320
500	0[a]	0	0	0	0	0
250	0	0	0	0	0	0
125	0	0	0	0	0	0
62.5	5	0	0	0	0	0
31.25	20	0	0	0	0	0
15.6	20	0	0	0	0	0
7.8	30	20	0	0	0	0
None (control)	100	100	90	85	55	20

[a] Numbers denote per cent of hemolysis as compared with hemoglobin standards.

TABLE 2. *Anticomplementary activity of sodium polyanetholsulfonate (SPS) in fresh human serum*

Final concn of added SPS (μg/ml)	Dilutions of L-serum					
	1:10	1:20	1:40	1:80	1:160	1:320
500	0[a]	0	0	0	0	0
250	0	0	0	0	0	0
125	0	0	0	0	0	0
62.5	0	0	0	0	0	0
31.25	0	0	0	0	0	0
15.6	0	0	0	0	0	0
7.8	0	0	0	0	0	0
None (control)	100	95	60	35	5	0

[a] See footnote to Table 1.

TABLE 3. *Anticomplementary activity of sodium polyanetholsulfonate (SPS) in fresh human serum*

Final concn of added SPS (μg/ml)	L-serum		T-serum	
	Dilutions		Dilutions	
	1:2	1:5	1:2	1:5
500	0[a]	0	10	0
250	10	0	10	5
125	10	0	20	5
62.5	20	15	15	10
31.25	20	20	10	10
15.6	30	20	20	0
7.8	65	0	50	0
None (control)	100	100	100	100

[a] See footnote to Table 1.

TABLE 4. *Anticoagulatory activity of sodium polyanetholsulfonate (SPS) in 90 and 20% fresh human blood*

Final concn of added SPS (μg/ml)	90% Blood				20% Blood			
	0.5 hr	2 hr	24 hr	48 hr	0.5 hr	2 hr	24 hr	48 hr
1,000	−[a]	−	−	−	−	−	−	−
500	−	−	−	−	−	−	−	−
250	−	−	−	−	−	−	−	−
125	−	+	+	+	−	−	−	−
62.5	−	+	+	+	−	−	−	+
31.25	−	+	+	+	−	−	+	+
None (control)	+	+	+	+	+	+	+	+

[a] Symbols denote absence or presence of macroscopically visible blood coagulation.

Albumin, glycoprotein, transferrin, and alpha globulins remained in solution after the addition of SPS.

Fresh and heat-inactivated L- and T-serum were exposed to 500 μg of SPS per ml. The result-

TABLE 5. *Precipitation of proteins from fresh human serum by sodium polyanetholsulfonate*

Source	Goat anti-human					
	Beta 1C glob-ulin	Beta glob-ulin	C4	IgA	IgG	IgM
Fresh human serum (control)	+[a]	+	+	+	+	+
Precipitate (unwashed)	+	+	+	+	+	+
Supernatant fluid	+	+	+	+	+	+
Precipitate (first wash)	+	+	+	+	+	+
Precipitate (second wash)	−	−	−	−	+	−
Precipitate (third wash)	−	−	−	−	+	−

[a] Symbols denote presence or absence of distinct precipitation bands (Ouchterlony microimmunodiffusion).

ing precipitates were washed three times in aqueous SPS (500 μg/ml). The sediment and supernatant fluid from each of the washes were tested for the presence of serum components with the Ouchterlony microimmunodiffusion technique. It was found that SPS at this concentration precipitated, though incompletely, beta 1C-globulin, C4, beta lipoproteins, IgG, and to some extent IgA and IgM from fresh serum (Table 5). However, only IgG regularly was precipitated from heat-inactivated serum by SPS; IgA and IgM were insolubilized irregularly from heat-inactivated serum. No attempt was made to quantitate the amounts of precipitated individual serum components.

DISCUSSION

It was found that less SPS (15.6 μg/ml) was required to inhibit complement-mediated S-RBC hemolysis in contrast to the roughly 16-fold higher concentration of SPS (250 μg/ml) necessary to neutralize completely the bactericidal activity of 1:2 diluted fresh human serum against serum-sensitive strains of *Escherichia coli* (7), at least under our experimental conditions. This raises the question of whether one may equate complement-mediated hemolysis with complement-antibody-mediated bacteriolysis (11).

With respect to the anticoagulatory activity of SPS, it was shown that 250 and 125 μg of SPS per ml were sufficient to prevent the coagulation of 90 and 20% human blood, respectively, for at least 48 hr. Thus, SPS at 250 μg/ml (0.025%) would appear to be adequate to inhibit the coagulation of 90% or less blood and to abolish the bactericidal activity of 1:2 diluted human serum. SPS at this concentration certainly would inhibit the coagulation and bactericidal activity of 20% blood, i.e., that amount of blood commonly employed for the culture of human blood specimens. The inhibition of aminoglycoside antibiotics by 500 μg of SPS per ml was shown to be a media-dependent phenomenon (14); thus, one should employ nutrient broth with added SPS for the cultivation of blood specimens from those patients who received, for example, gentamicin sulfate at the time the specimens were drawn. Preliminary experiments indicated that SPS at 250 and 500 μg/ml markedly antagonized the activity of kanamycin and gentamicin sulfate in nutrient broth.

The finding that SPS precipitated beta lipoproteins and fibrinogen confirmed previous observations (2, 17). Two-dimensional immunodiffusion studies disclosed that SPS also precipitated beta 1C-globulin (C3) and C4 from fresh human serum; previously Pontieri et al. (10) demonstrated that SPS inactivated C3 in porcine serum. Thus the anticomplementary activity of SPS appears to be due to the precipitation of at least two components of human complement, namely C3 and C4.

Unexpected was the finding that SPS precipitated IgG from fresh human serum. However, the supernatant fluid of SPS-treated fresh serum still contained IgG as well as the other proteins that were detected in the precipitates, indicating that precipitation of these proteins was incomplete. Stuart (12) noted that SPS-treated human sera still could be employed for Widal agglutination tests; apparently SPS precipitated only an insignificant amount of IgM, thus causing no reduction of the titer of anti-*Salmonella* agglutinins. Antistreptolysin O antibodies are known to belong to the IgG class of immunoglobulins. Preliminary ASO titrations in our laboratory employing control and SPS-treated (500 μg/ml) sera failed to detect a marked difference in titers, an observation also indicating that precipitation of IgG was incomplete. Other preliminary experiments showed that the addition of SPS to hyperimmune influenza sera (rabbit) did not reduce the titers of hemagglutination-inhibition (HAI) antibodies.

It would be desirable, of course, to demonstrate the suitability of SPS with regard to the rapid "chemical" inactivation of complement in the sera of patients for diagnostic serological procedures, such as ASO tests and presumptive heterophile agglutination tests, a procedure that would save the clinical laboratory considerable time. Similarly, one might wish to employ SPS to inactivate complement in sera prior to the performance of viral neutralization tests and to remove nonspecific inhibitors (lipoproteins) from sera to be titrated for myxovirus, paramyxovirus, or rubella virus HAI antibodies.

ACKNOWLEDGMENT

This study was supported by a grant from the United Medical Research Foundation of North Carolina.

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