Enhancement of sensitivity of the haemagglutination test for echinococcosis by use of *Staphylococcus aureus* protein A

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Summary. A modification of the indirect haemagglutination test is described for the serodiagnosis of echinococcosis. In the modified test the Cowan I strain of *Staphylococcus aureus*, which contains protein A, was used to enhance haemagglutination of sensitised red cells. The test was performed in parallel with the indirect haemagglutination test on 31 sera from surgically-confirmed cases of hydatid disease and on 45 sera from healthy blood donors. Use of *S. aureus* protein A enhanced the sensitivity of the test and greatly increased the titres obtained with most of the sera. None of the sera from healthy blood donors exhibited a titre > 64, whereas all but two of the sera from cases of hydatid disease exhibited titres > 128. The immunoassay is simple, inexpensive and requires little technical skill. It has the potential for wide application in the serodiagnosis of echinococcosis.

Introduction

The need for an immunodiagnostic test for echinococcosis which is simple, rapid and yet sensitive in laboratory and field conditions has resulted in the development of various tests, which include: double diffusion (Ouchterlony, 1958), immunoelectrophoresis (Ardehali *et al.*, 1977), countercurrent immunoelectrophoresis (Kelkar and Kotwal, 1975), cellulose acetate membrane counter-immunoelectrophoresis (Mansueto *et al.*, 1980), complement fixation (Varela-Díaz and Coltori, 1974), latex agglutination (Chemtai *et al.*, 1981), indirect haemagglutination (IHA) (Iacona *et al.*, 1980), enzyme linked immunosorbent assay (ELISA) (Iacona *et al.*, 1980) and dot-ELISA on nitrocellulose paper (Schantz *et al.*, 1984). However no single test provides complete sensitivity and specificity for echinococcosis and a combination of two or three methods may be necessary to obtain reliable results (Chemtai *et al.*, 1981).

Because the IHA test is inexpensive, easy to perform and can be read without errors of subjectivity, it is potentially useful in the diagnosis of echinococcosis (Lewis *et al.*, 1975; Parija *et al.*, 1983). However, the IHA test is not as sensitive as ELISA although use of double-aldehyde stabilised cells enhances its sensitivity (Parija and Ananthakrishnan, 1985).

We describe here the use of *Staphylococcus aureus* protein A to further enhance the sensitivity of the IHA test in the serodiagnosis of echinococcosis.

Materials and methods

Antigen

Hydatid cyst fluid antigen was obtained from patients at surgical operation and processed by the method described by Arabatzis and Papapanagiotou (1963).

Sera

A total of 31 sera from surgically-confirmed cases of hydatid disease and 45 sera from healthy blood donors was collected. All were stored at -20°C.

Bacteria

Two strains of *S. aureus* were used: Cowan I, which possesses protein A and Wood 46, which lacks protein A. The strains were grown on Mueller Hinton Agar at 37°C and stabilised with formalin and heat as described by Camargo *et al.* (1983). After incubation for 24h cultures were harvested at 3000 g for 10 min and washed three times in phosphate-buffered saline (PBS) (pH 7.2) containing sodium azide 0.05% w/v. The pellet was fixed in 10 volumes of formaldehyde 1.5% in PBS at room temperature for 90 min, washed twice in PBS, resuspended in 10 volumes of PBS-azide and heated for 5 min at 80°C. The bacteria were again harvested and washed twice; a 10%

suspension of cells in PBS with sodium azide 0.1% was then prepared and stored in small volumes at 4°C. Before use, stored suspensions were diluted to the desired concentration in pH 7.6 buffer (100 mM NaCl, 50 mM EDTA, 50 mM Tris HCl) containing bovine serum albumin 4 mg/ml of buffer.

Preparation and sensitisation of double-aldehyde stabilised (DAS) cells

Human group O red blood cells were stabilised with pyruvic aldehyde, tannic acid and glutaraldehyde as described by Parija and Ananthakrishnan (1985). The DAS cells were sensitised with the hydatid antigen as described by Farshy and Kagan (1972). DAS red cells were washed twice with PBS. Packed cells were suspended in 10 volumes of various concentrations of antigen in PBS (pH 6.4) and incubated at 50°C for 5 min. The suspension was then kept overnight at 4°C before re-incubation at 50°C for 5 min. The sensitised red cells were washed twice with PBS and then with PBS containing bovine serum albumin (BSA) 0.1%. Finally, a suspension of sensitised cells (1%) was prepared in PBS containing BSA. The optimum sensitising dose of the antigen (the lowest amount of the antigen showing the maximum haemagglutination titre) was determined for each batch of the antigen by chessboard titration against known positive and negative sera.

Indirect haemagglutination (IHA) test

A 25- μ l volume of PBS containing BSA 0.1% was dispensed in each well of a U-bottomed microtitration tray. A 25- μ l volume of serum was added to the first well of the appropriate row. The sera were then serially diluted up to the eleventh well, leaving the last well as a serum-free control. A 25- μ l volume of DAS cells, sensitised with the optimum sensitising dose of antigen, was added to each well. The plate was gently agitated for 2 min and incubated at room temperature for 1h, followed by overnight incubation at 4°C. Controls consisting of known positive and unsensitised DAS cells were included with each test. The IHA pattern was recorded as described by Stavitsky (1954).

Protein A-antibody mediated haemagglutination

The method was similar to IHA except that a suspension of *S. aureus* Cowan I strain, prepared as described above, was added to the diluted sera before addition of sensitised cells. The optimal concentration of *S. aureus* cells was determined by chessboard titration with various suspensions of bacteria (0.1, 0.2, 0.3, 0.4 and 0.5%) and known positive and negative sera. The lowest concentration of bacterial cells which showed the maximum haemagglutination was considered to be the optimum concentration of *S. aureus* valid for that batch of the cells.

A 25- μ l volume of PBS containing BSA was dispensed in all the rows of a U-bottomed microtitration plate. A

25- μ l volume of serum was added to the first well of each row. The sera were then serially diluted in twofold steps up to the tenth wells. A 25- μ l volume of *S. aureus* suspension at the optimal concentration (0.2%) was dispensed into each well. The plates were then agitated at 70 oscillations/min for 30 min at room temperature. A 25- μ l volume of sensitised DAS cells was then added to each well. The plates were gently agitated for 2 min and incubated at room temperature for 1h, followed by overnight incubation at 4°C. The co-haemagglutination patterns obtained were scored as for the IHA test by the method of Stavitsky (1954).

Appropriate controls were included in each test. As a control of the effect of *S. aureus* protein A, tests were repeated with the Wood 46 strain of *S. aureus* which lacks protein A.

Results

The results of the IHA test were compared with those of the protein A-modified assay for sera from 31 patients with surgically-confirmed echinococcosis. Titres ≥ 128 were obtained with 25 of 31 (80.6%) sera tested by the IHA method and 29 of 31 (93.5%) sera tested with the protein A-modified assay (table). All titres obtained in the presence of *S. aureus* protein A were at least double those observed by IHA; in most cases, much greater increases in titre were obtained and in one case a titre of 128 by IHA was 262 144 in the protein A-modified assay.

Serum-free controls revealed no haemagglutination by the *S. aureus* suspensions alone. In tests with the Wood 46 strain of *S. aureus* instead of the Cowan I strain, titres were identical to those of the unmodified IHA test. None of 45 sera from healthy blood donors exhibited a titre > 64 when tested by the protein A method.

Discussion

The Cowan I strain of *S. aureus* bearing protein A specifically binds IgG without affecting its immunological activity (Langone, 1982). This property of protein A is employed in several diagnostic tests for the demonstration of antibodies (Bergquist and Waller, 1983; Camargo *et al.*, 1983) and antigens (Kronvall, 1973; Sivadasan *et al.*, 1984).

The IHA test used for the serodiagnosis of echinococcosis is simple, specific and moderately sensitive (Iacona *et al.*, 1980; Chemtai *et al.*, 1981; Parija and Ananthakrishnan, 1985). However it is not as sensitive as ELISA or radio-immunoassay because some of the IgG bound to red cells fails to cross link the cells so that haemagglutination does not occur. Cross-linking could be achieved by use of

Table. Comparison of antibody titres obtained by two tests on 31 sera from patients with echinococcosis.

Serum no.	Antibody titres	
	IHA test	SAPA-IHA test
H19	512	16 384
H20	1024	65 536
H21	256	512
H22	128	2048
H23	256	2048
H24	128	16 384
H25	4	32
H26	128	512
H27	1024	32 768
H28	256	4096
H29	4	512
H30	256	1024
H31	512	1024
H32	256	131 072
H33	128	2048
H34	256	512
H35	2	64
H36	512	16 384
H37	4	256
H38	256	512
H39	512	32 768
H40	128	8192
H41	1024	131 072
H42	16	256
H43	128	262 144
H44	128	256
H45	256	8192
H46	128	4096
H47	128	2048
H48	128	512
H49	16	256

IHA = Indirect haemagglutination;
SAPA = *S. aureus* protein A

anti-immunoglobulin (Coombs *et al.*, 1953). Alternatively *S. aureus* bearing protein A can be used because this protein will bind IgG and subsequently agglutinate antigen-sensitized RBCs (Jagannath *et al.*, 1984).

The results of this study show that use of *S. aureus* cells bearing protein A greatly enhances the sensitivity of the IHA test. Since the Wood 46 strain of *S. aureus*, which lacks protein A did not alter the sensitivity of the IHA and since *S. aureus* cells bearing protein A did not agglutinate sensitized DAS cells in the absence of antibody, co-haemagglutination appears to be mediated through protein A and occurs only in the presence of specific antibody. The modified test showed greater sensitivity (93.5%) than the unmodified IHA test (80.6%) and much increased antibody titres in positive sera.

If the threshold of positivity is set at a titre of 128, no false positives were observed among 45 normal sera; however, it is possible that such excellent discrimination may not extend to patients with liver disease other than that due to hydatid disease.

We did not compare the results of our modified test with those of ELISA in echinococcosis. However, earlier studies have shown that the sensitivity of protein A-mediated haemagglutination tests compares well with that of ELISA in the serological diagnosis of tuberculosis (Jagannath *et al.*, 1984) and filariasis (Parija and Rao, unpublished data).

Reagents for the modified test are inexpensive, easily available and stable. The sensitized cells can be stored at 4°C for a long period with no loss of sensitivity (Jagannath and Sengupta, 1981 and Parija *et al.*, 1986). Other studies have shown that prepared suspensions of *S. aureus* can be stored at 4°C or at room temperature for 8–12 weeks without loss of activity (Camargo *et al.*, 1983). In contrast, the reagents for the ELISA test are expensive, difficult to obtain and perishable in tropical conditions.

The protein A-mediated haemagglutination test is simple, sensitive, inexpensive and does not require technical skill. The test deserves further evaluation in the serodiagnosis of echinococcosis.

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