Sensitised chick cells in the indirect haemagglutination test for echinococcosis

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Summary. An indirect haemagglutination (IHA) test was performed with chick red blood cells (RBC) on sera from 26 confirmed cases of echinococcosis and 45 control sera. The results were compared with those obtained in an IHA test with sheep RBC on the same batches of sera; both tests were equally sensitive. The chick cells settled quickly and the results could be determined within 30–45 min. Heterophilic antigen was not a problem. This study also showed that chick cells stabilised by the double-aldehyde method, could be sensitised with the antigen and then stored at 4°C for up to 31 days before use in the IHA test without loss of sensitivity. The use of sensitised double-aldehyde stabilised (DAS) chick cells in IHA tests provides a rapid diagnostic test in echinococcosis.

Introduction

The indirect haemagglutination (IHA) test for echinococcosis is technically simple, specific and sensitive (Iacona *et al.*, 1980; Chemtai *et al.*, 1981; Parija and Ananthakrishnan, 1985). A method for the preparation of double-aldehyde stabilised (DAS) sheep red blood cells (RBC) that are more sensitive than cells treated with single aldehyde in the IHA test for echinococcosis has been described by Parija and Ananthakrishnan (1985).

The IHA test has the disadvantage of being slow and time consuming. We report an evaluation of the use of chick RBCs, which are readily available nucleated cells, in this test. Sensitised DAS chick cells were stored at 4°C for up to 45 days to investigate the effect of storage on subsequent use in IHA.

Materials and methods

Antigen

Hydatid-cyst fluid was obtained from patients during surgical operation and processed by the method of Aratzis and Papapanagiotu (1963).

Sera

Serum samples were separated from the blood of 26 confirmed cases of echinococcosis in JIPMER Hospital during 1981–84. Negative control serum samples were obtained from 45 normal healthy persons. All sera were stored at –20°C until required.

Chick RBC

Blood was collected from chicks into Alsever's solution. The cells were washed thrice in phosphate-buffered saline (PBS; pH 7.2, 0.15 M) and treated with the stabilising agent within 48 h.

Preparation of sensitised double-aldehyde stabilised (DAS) chick RBC

DAS chick cells were prepared by treating chick cells sequentially with pyruvic aldehyde, tannic acid and glutaraldehyde as previously described (Parija and Ananthakrishnan, 1985). The DAS cells were sensitised with the hydatid antigen as described by Farshy and Kagan (1972). Briefly, one volume of packed DAS cells was suspended in 10 volumes of an optimum sensitising dose of the antigen. The optimum sensitising dose determined for the particular batch of antigen was a dilution of 1 in 16 (1 volume of undiluted antigen in 15 volumes of PBS, pH 6.4). The cell plus antigen mixture was incubated at 50°C in a water bath for 5 min, followed by overnight storage at 4°C then incubation at 50°C again for 5 min. The sensitised cells were washed thrice with PBS (pH 7.2) and stored in small volumes as a 10% suspension in PBS (pH 7.2) at 4°C until used.

IHA test

Stored sensitised cells were used to prepare a 1% suspension in PBS with bovine serum albumin (BSA). This suspension was used in the IHA test on the same day, and after storage at 4°C for 7, 14, 31 and 45 days. The test was performed as described by Herbert (1978). The results were read by observing the settling patterns of the cells in the plate after incubation for 15, 30, 45, 60 and

Table I. Results of IHA tests on sera from patients with echinococcosis

Serum no.	Titre of IHA antibody with	
	DAS sheep RBC*	DAS chick RBC*
1	256	256
2	128	128
3	1024	512
4	512	512
5	8	8
6	256	512
7	256	128
8	512	512
9	256	256
10	256	256
11	1024	512
12	256	128
13	32	64
14	128	128
15	256	256
16	128	128
17	128	128
18	512	512
19	512	512
20	1024	1024
21	256	256
22	Not done	128
23	Not done	256
24	Not done	128
25	Not done	4
26	Not done	128

* RBC were used on the day of preparation.

120 min at room temperature and after holding the plates overnight at 4°C.

Results

The chick cells settled quickly and showed a definite agglutination pattern with positive sera after incubation at room temperature of 30–45 min. After overnight incubation at 4°C, the agglutination pattern of the cells was observed to be distorted. A titre of ≥ 128 was considered to be diagnostic of echinococcosis (Parija and Ananthakrishnan, 1985). Table I shows the results of IHA tests on 26 sera from patients with echinococcosis with the sensitised DAS chick cells prepared fresh and used on the same day. These results are compared with those for the test with DAS sheep cells obtained on 21 sera from the same batch previously (Parija and Ananthakrishnan, 1985). Table II shows the results of IHA tests on 45 normal control sera with DAS chick cells only. Table III shows the results of the IHA test with sensitised DAS chick cells on 11 sera after storage at 4°C for 7, 15, 31 and 45 days.

Table II. Results of IHA tests with DAS chick RBC on 45 normal control sera

IHA titre	Number of sera (%)
<8	24 (52.3)
16	8 (17.8)
32	8 (17.8)
64	5 (11.1)

Table III. Results of IHA tests with sensitised DAS chick RBC after storage at 4°C

Serum no.	IHA titre with RBC stored for (days)			
	7	15	31	45
1	256	256	128	32
2	128	128	128	64
3	512	512	512	512
4	512	512	512	512
5	8	8	8	8
6	512	512	256	128
7	128	128	128	64
8	512	512	512	128
15	256	256	256	64
18	512	512	512	256
21	256	256	256	128

Discussion

Non-nucleated RBC from sheep (Chemtai *et al.*, 1981; Parija and Ananthakrishnan, 1985) and man (group O; Herbert, 1978) and nucleated RBC from turkey (Singh *et al.*, 1980) and goose (Mannen *et al.*, 1984) have been used successfully in IHA tests in various infections. Turkey and goose cells are usually recommended because, being nucleated, they are heavier, they settle quickly and the results can be obtained rapidly. Because goose and turkey RBC are scarce in this part of India, it was decided to evaluate chick RBC as alternative nucleated RBC for the IHA test for echinococcosis. Although chick cells have been used extensively in virus serology (Skaug *et al.*, 1975), they do not appear to have been used previously in serological tests for echinococcosis.

The haemagglutination of chick cells in this study was observed after a minimum of 30 min and all tests were readable by a maximum of 45 min with different dilutions of sera and with the sensitised DAS chick cells incubated at room temperature. Because the results could be read within 45 min,

there is an advantage over the use of non-nucleated cells that need > 6 h to settle and agglutinate.

Heterophilic antigens were not a problem with chick cells and no prior absorption of the test sera was required, as is needed with sheep cells. Moreover, use of this common domesticated bird as the source of cells ensures that chick RBC are constantly available for laboratory use.

The need to prepare sensitised cells (tanning the stabilised cells for 15 min at 37°C followed by washing and sensitisation with antigen at 37°C for 30 min and washing) is a tedious and time-consuming procedure if it were necessary before each test. The effects of storage of the sensitised DAS cells at 4°C on the sensitivity of the test was evaluated. We found (table III) that sensitised chick DAS cells retain their sensitivity for up to 31 days after preparation when stored at 4°C. After storage for 45 days

there was a gradual fall in sensitivity. This stability leads to an economy in time and makes IHA a rapid diagnostic test. Moreover use of DAS cells has been shown to increase the sensitivity of the test in comparison with single-aldehyde stabilised cells (Parija and Ananthakrishnan, 1985).

The results of tests with sensitised DAS chick cells on 21 sera, in comparison with IHA tests with sensitised DAS sheep cells freshly prepared and used immediately (Parija and Ananthakrishnan, 1985) (table I) show similar sensitivity.

In conclusion, sensitised DAS chick cells, stored at 4°C for up to 31 days can be used directly in the IHA test to make it a rapid test in echinococcosis. The test is equally sensitive to IHA with sheep RBCs, use of chick RBC does not require an absorption of the sera to be tested, and the results can be obtained within 45 min.

REFERENCES

- Arabatzis G, Papaganagiotou J 1963 Laboratory tests in hydatid disease: a comparison of the indirect haemagglutination, complement-fixation and intradermal tests. *Bulletin of the World Health Organization* **28**:266–268.
- Chemtai A K, Bowry T R, Ahmad Z 1981 Evaluation of five immunodiagnostic techniques in echinococcosis patients. *Bulletin of the World Health Organization* **59**:767–772.
- Farshy D C, Kagan I G 1972 Use of stable sensitized cells in indirect microhemagglutination test for malaria. *American Journal of Tropical Medicine and Hygiene* **21**:868–872.
- Herbert W J 1978 Passive haemagglutination with special reference to the tanned cell technique. In: Weir D M (ed) *Handbook of experimental immunology*, 3rd edn, Chapter 20. Blackwell, Oxford, p 1.
- Iacona A, Pini C, Vicari G 1980 Enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of hydatid disease. *American Journal of Tropical Medicine and Hygiene* **29**:95–102.
- Mannen K, Ishikawa K, Tachibana J, Mifune K 1984 Methods of increasing the sensitivity of the haemagglutination inhibition test for rabies virus antibody. *Bulletin of the World Health Organization* **62**:883–892.
- Parija S C, Ananthakrishnan N 1985 Evaluation of stabilised cells in the indirect haemagglutination test for echinococcosis. *Journal of Medical Microbiology* **19**:95–98.
- Singh M *et al.* 1980 Studies on human filariasis in Malaysia. The application of an indirect haemagglutination technique for immuno diagnosis. *American Journal of Tropical Medicine and Hygiene* **29**:548–552.
- Skaug K, Orstavik I, Ulstrup J C 1975 Application of the passive haemolysis test for the determination of rubella virus antibodies. *Acta Pathologica et Microbiologica Scandinavica B* **83**:367–372.