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RAPID DIAGNOSIS OD ADULT DIARRHEA ROTAVIRUS (ADRV): DETECTION OF VIRAL ANTIGENS IN FAECAL SAMPLES USING STAPHYLOCOCCAL CO-AGGLUTINATION TEST

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Summary. — *Staphylococcus aureus* Cowan I rich in protein A when sensitized with guinea pig antiserum to adult diarrhea rotavirus (ADRV) at 1 : 16 gave a strong co-agglutination with ADRV-positive faecal samples as previously confirmed by electron microscopy (EM) and enzyme-linked immunosorbent assay (ELISA). The bacteria sensitized with normal guinea pig serum did not give any co-agglutination. Blocking tests using rabbit ADRV-specific antiserum for the treatment of twelve ADRV-positive samples abolished the reaction. All the fifty ELISA-confirmed ADRV-positive faecal samples gave positive co-agglutination, whereas all the forty-eight ELISA-negative faecal samples from healthy subjects gave negative results. The test has been proved to be rapid, simple, specific, and economic, useful for rapid diagnosis even in remote areas, so that the ADRV infection can definitely be differentiated from some of acute bacterial diarrheas.

Key words: *bacterial co-agglutination test*; *rapid diagnosis*; *rotavirus*

Introduction

Major epidemics caused by ADRV occurred in various parts of China since 1983; the virus has been isolated by Hung *et al.* (1983 and 1984*a*). A simple method for rapid diagnosis of ADRV has been in need to monitor the huge epidemic areas of the disease. Therefore, in addition to ELISA (Wang *et al.*, 1986), we developed a bacterial co-agglutination test for the detection of faecal ADRV antigen.

Staphylococcal co-agglutination test of turkey and calf rotaviruses (Skaug *et al.*, 1983; Kang *et al.*, 1985) has been reported previously in the faeces. The procedures described were modified and inproved by us, and the specificity of the test using a faecal ADRV was further confirmed in the present study.

Materials and Methods

Preparation of specific antisera. The technique has been described previously (Wang *et al.*, 1985*a*). Briefly, ADRV antigen was extracted from the diarrheal faeces of confirmed ADRV cases and purified by ultracentrifugation on sucrose cushion. Guinea pigs and rabbits were immunized to obtain the first generation of hyperimmune sera. These antisera were applied in crossed immunoelectrophoresis for further purification of the virus antigen. The purified antigen was employed for immunization to obtain the second generation of hyperimmune sera used in the present study. From these the guinea pigs serum was used for coating of bacteria; its titre as determined by counter immunoelectrophoresis was 1 : 128.

Sensitization of bacteria. Staphylococcus aureus strain Cowan I rich in protein A (Lyophilized product of the Shanghai Institute of Biological Products, Shanghai) reconstituted to 1 ml per vial and containing 10 % (w/v) bacterial suspension was washed once and made up to 50 % bacterial suspension with normal saline. The suspension was mixed with an equal volume of guinea pig hyperimmune serum diluted 1 : 16 (equivalent to 8 counter immunoelectrophoresis units) in normal saline and incubated at 37 °C in a water bath for 20 min at occasional mixing. The mixture was then washed three times with phosphate buffered saline (PBS), pH 7.4, containing 0.5 % Tween 20 (T-PBS) and centrifuged at 3,500 rev/min for 5 min. The pellet of sensitized bacteria was resuspended to make up a 2—2.5 % suspension in T-PBS as a working suspension for the co-agglutination test. This suspension was still active when stored at 4 °C for more than one month.

Collection of faecal samples. 1. From diarrheal adult cases, faecal samples were collected during the epidemics which occurred in Lanzhou City of Gansu Province, Jinzhou City of Liaoning Province, Quindao City of Shandong Province and Chengde City of Hebei Province during 1983—1984. All samples had been proved ADRV-positive by ELISA (Wang *et al.*, 1986), by the analysis of viral ribonucleic acid (RNA), which confirmed the pattern of ADRV (Wang *et al.*, 1985*b*). A part of the samples was analysed by EM (Hung *et al.*, 1984*b*). 2. Healthy subjects. Faecal samples from healthy subjects which were collected in Beijing City contained no ADRV antigen detectable by ELISA (Wang *et al.*, 1986).

Treatment of faecal samples. Suspensions of diarrheal or normal faecal samples diluted with PBS were centrifuged in micro-centrifuge tubes at 3,500 rev/min for 15 min, and 20 μl of the supernatants were added to equal amounts of 50 % *Staphylococcus* bacterial suspension in plastic tubes to remove IgG. The mixture was incubated in a water bath at 37 °C for 20 min, with occasional mixing, and then centrifuged at 7,000 rev/min for 5 min. The final supernatant was used for the co-agglutination test.

Co-agglutination test. One drop or 20 μl of the treated faecal sample was mixed with equal amount of 2—2.5 % sensitized bacterial suspension in a circle marked with a marker pen on a slide and the results were read in 2—3 min.

Blocking test of co-agglutination. To prove the specificity of the co-agglutination test, 10 μl ADRV-positive faecal sample were mixed with 10 μl of 1 : 2 diluted ADRV-specific rabbit antiserum and incubated at 37 °C in a water bath for 1 hr. Then 20 μl of 50 % bacterial suspension were added to the mixture and treated in the same manner as mentioned above. The supernatant of antibody-blocked and the PBS treated control samples were examined by the co-agglutination test.

Results

Co-agglutination activity of bacteria sensitized with different dilutions of specific antiserum

To determine the optimal dilution of the hyperimmune serum for sensitization of bacteria, serial dilutions of ADRV-specific guinea pig antiserum were made for sensitization of small aliquots of 50 % bacterial suspension. After sensitizing and washing of the bacteria, a 2.5 % working suspension was made up for the co-agglutination test with an ADRV-positive faecal sample. As shown in Table 1, bacterial suspensions sensitized with the anti-

Table 1. Bacterial co-agglutination with an ADRV-positive faecal sample using bacteria sensitized with various dilutions of ADRV-specific guinea pig antiserum

Antiserum dilution	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	Normal guinea pig serum
Co-agglutination	++++	++++	++++	++++	+++	–

serum at dilutions 1 : 2 through 1 : 16 gave strong (++++) co-agglutination, whereas the further dilution at 1 : 32 gave a weaker reaction (+++). Thus the dilution at 1 : 16 for this lot of antiserum was accepted for sensitization of a large batch of bacterial suspension.

Bacterial co-agglutination with various dilutions of an ADRV-positive faecal sample

A faecal sample which has been proved to be ADRV-positive by EM, ELISA, and by the RNA electrophoretic pattern was serially diluted with T-PBS for co-agglutination with the sensitized bacteria. As shown in Table 2, this faecal sample gave strong reaction at dilutions from 1 : 1 through 1 : 10, whereas the reactions were decreasing at 1 : 20 and higher, and became completely negative at 1 : 80.

Detection of clinical faecal samples from diarrheal patients

Using bacterial co-agglutination for the detection of 50 faecal samples which had been proved by ELISA as ADRV-positive, all gave positive results scored as follows: 22 were '++++', 20 were '+++', 7 were '++', and 1 was '+'. 38 faecal samples from healthy subjects gave negative results. Other 10 normal faecal samples which were treated by sonification and chloroform for extraction of ADRV antigens (Wang *et al.*, 1986) also gave negative results.

Table 2. Bacterial co-agglutination with various dilutions of an ADRV-positive faecal sample using bacteria sensitized with 1 : 16 ADRV-specific guinea pig antiserum

Dilutions of faecal sample	1 : 1	1 : 5	1 : 10	1 : 20	1 : 30	1 : 60	1 : 80	Normal faecal sample (1 : 1)
Co-agglutination	++++	++++	++++	+++	++	+	–	–

Table 3. Further blocking test for bacterial co-agglutination of ADRV-positive faecal samples

ADRV-positive faecal sample No.	Faecal samples[a] treated with	Guinea pig sera for sensitization	Co-agglutination
1[b]	ADRV-specific rabbit antiserum	ADRV-immune	–
	ADRV-specific rabbit antiserum	Normal	–
	PBS control	ADRV-immune	++++
	PBS control	Normal	–
2	ADRV-specific rabbit antiserum	ADRV-immune	–
	ADRV-specific rabbit antiserum	Normal	–
	PBS control	ADRV-immune	++++
	PBS control	Normal	–

Notes — a) All the faecal samples were pretreated with sonification and chloroform extraction
b) No. 1 faecal sample not treated with sonification and chloroform also gave the same results in a parallel experiment

Specificity of bacterial co-agglutination

Out of the upper-mentioned ADRV-positive faecal samples 10 were treated with equal amounts of 1 : 2 ADRV-specific rabbit antiserum while the control samples were treated with PBS. The results showed that the co-agglutination of all the ADRV-positive samples were blocked by the ADRV-specific antiserum.

In a further experiment shown in Table 3, the co-agglutination of two ADRV-positive faecal samples (No. 1 and No. 2) was not only blocked by ADRV-specific antiserum, but also these samples did not react with the bacteria coated with normal guinea pig serum, whereas they strongly reacted with ADRV-specific antiserum sensitized bacteria.

The specificity of the co-agglutination could also be observed by the patterns of agglutination, as shown in parallel in Figure 1 (observation by naked eye) and in Figure 2 (observed under the light microscope). A strong specific co-agglutination was found only when ADRV-positive faecal sample No. 1 reacted with ADRV-specific antiserum-sensitized bacteria (I). The same ADRV-positive sample when blocked with specific rabbit antiserum (II) gave negative results similarly as the faecal sample from normal subject (III) and the PBS control (IV).

Discussion

Bacterial co-agglutination using *Staphylococcus aureus* for the diagnosis of ADRV infection by direct detection of the viral antigens from faecal samples has been proved to be rapid, simple, specific, and economic. The

test can be performed anywhere without sophisticated equipment, even in remote areas, in case diarrheal epidemic occurs.

In the present study, the procedures have been improved by the use of optimally diluted hyperimmune antiserum for sensitization of bacteria to economize the antiserum consumption, and the use of 0.5 % Tween 20 in PBS to prevent from non-specific agglutination by the faecal samples. No non-specific reaction was observed in testing of forty-eight faecal samples from normal subjects. The blocking test to confirm the specificity of the technique appears very important, yet this test had not been done in the previous studies on calf and avian rotaviruses (Skaug *et al.*, 1983; Kang *et al.*, 1985). The specificity of the test is summarized as follows:

— Bacteria sensitized with normal guinea pig serum did not co-agglutinate with the ADRV-positive faecal samples, whereas only ADRV-specific antiserum sensitized bacteria was capable of co-agglutinating these samples (Tables 1 and 3).

— All faecal samples, which had been confirmed as ADRV-positive by ELISA, by EM and according to RNA electrophoretic pattern gave positive co-agglutination, whereas faecal samples from normal subjects free from ADRV antigen as confirmed by ELISA gave negative results in the co-agglutination test.

— ADRV-specific rabbit antiserum was capable of blocking the specific co-agglutination (Table 3 and Fig. 1-II and 2-II.

The presented bacterial co-agglutination is useful not only for the diagnosis of patients in hospitals, but also for epidemiological investigations. It is extremely important to differentiate ADRV diarrhea from bacterial diarrheas promptly due to their different prophylactic measures. The present co-agglutination test could possibly be used as a promising tool for this purpose.

Furthermore, we succeeded to sensitize *Staphylococcus* bacteria with monoclonal antibodies to Japanese encephalitis virus (JEV) and to perform co-agglutination test for detection of JEV antigens. The work is in progress and the data will be published in a separate paper (Zhang *et al.*, 1989).

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Legends to Figures (Plate IX):

Fig. 1. Staphyloccocal coagglutination test with naked eye

Fig. 2. Staphyloccocal coagglutination test observed under the light microscope

I. ADRV-positive faecal sample vs. quinea pig ADRV-specific antiserum-sensitized bacteria.

II. ADRV-positive faecal sample blocked by rabbit ADRV-specific antiserum vs. quinea pig ADRV-specific antiserum-sensitized bacteria.

III. Faecal sample from healthy subjects vs. guinea pig ADRV-specific antiserum-sensitized bacteria.

IV. PBS vs. guinea pig ADRV-specific antiserum-sensitized bacteria.