Trial of human diploid cell rabies vaccine in human volunteers

Preliminary studies of a recently developed human diploid cell vaccine (HDCV) against rabies showed that it gave good protection to animals.¹ Since the vaccine produced a high level of antibodies in the pre-exposure treatment of human volunteers,²⁻⁵ trials were initiated in Aurangabad and Bombay, both areas where rabies is endemic, as a first step to determine whether it might be an alternative to the Semple vaccine for the postexposure treatment of rabies.

Patients, methods, and results

HDCV was prepared in WI-38 human fibroblasts and supplied in lyophilised form by the Institut Merieux (Lyon, France). The vaccine was reconstituted with distilled water and had an antigenic value of about 3. HDVC (4 ml) was injected into the deep subcutaneous tissue of the upper arm of volunteers in one of two dosage schedules. Group 1 (18 volunteers) received 1 ml vaccine each on days 0, 3, 7, and 14; group 2 (23) received 2 ml vaccine each on days 0 and 10. Semple type vaccine, a heat- and phenol-inactivated emulsion of brains from rabies-infected sheep, supplied by the Haffkine Institute, Bombay, was given to 30 patients attending the antirabic treatment centre at the Medical College Hospital, Aurangabad, after being bitten by dogs in 14 daily injections of 5 or 10 ml into the deep subcutaneous tissue of the abdominal wall. In group 3 (23) rabies infection was not proved; in group 4 (7) the dog was proved rabid.

There were few reactions to HDCV. Seven volunteers complained of local pain and swelling, usually after the fourth injection of 1 ml. No reactions were reported among those who received two inoculations of 2 ml of vaccine (group 2). One volunteer in group 1 developed local pain and swelling with high fever lasting 48 hours after each of the two doses of HDCV. Reactions among patients receiving Semple vaccine were not recorded. The level of virus neutralising antibody at different times after HDCV vaccination was similar in both schedule groups (see table). Ten days after the first inoculation all volunteers in group 1, and all but one in group 2 had detectable antibodies. When sera were tested three months after the beginning of vaccination the antibody level had decreased, but the residual levels were still high. Only seven of 21 patients sampled from groups 3 and 4 who were exposed to rabies and treated with Semple type brain vaccine had detectable antibodies on the day they received the 10th daily inoculation. Patients from group 4 showed higher antibody levels than those of group 3, even though they were treated with the same vaccine and by an identical immunisation schedule. Possibly the rabies virus-infected saliva entering the body of the exposed person acted as an additional antigen and was responsible for increased antibody production.

Comparison of rabies virus neutralising antibody titres in persons immunised with human diploid cell vaccine (HDCV) and persons treated after exposure to rabies with Semple brain tissue vaccine. Results are geometric mean titres*

Day after inoculation	HDCV		Semple	
	Group 1 (n = 18)	Group 2 (n = 23)	Group 3 (n = 23)	Group 4 (n = 7)
0	<0.1	<0.1	<0.1	<0.1
10	1.0 (0.1-25.0)	0.6 (0.1-18.0)	0.1 (<0.1-3.1)	0.1 (0.1-3.1)
14	3.9 (0.3-30.0)	3.5 (0.9-25.0)	0.4 (0.2-6.2)	
30	4.6 (0.9-30.0)	3.0 (0.3-60.0)	0.5 (0.2-2.5)	4.6 (3.2-18.7)
90	1.8 (0.3-12.0)	2.0 (0.3-44.0)		1.5 (0.1-12.5)

*Virus neutralising antibody levels were determined by plaque reduction technique, and are expressed in International Units (IU). Standard NIH rabies antibody preparation containing 1 IU/ml caused 50% rabies virus plaque reduction at dilution of 1/160.

Discussion

These trials showed that a two- or four-dose schedule of HDCV could give an antibody response equal to that of 14 doses of Semple vaccine and, in addition, the antibody response to HDCV in most volunteers seemed to develop faster. On the basis of early antibody development at 10 days the four-dose schedule must be considered better than the two-dose schedule. Judged by the criterion of antibody development by the 10th day the Semple vaccine performed poorly.

Although no definite correlation has yet been found between neutralising antibody levels in the blood after post-exposure treatment and protection against the disease, preliminary tests in animals⁵ indicate that one to three inoculations of HDCV can protect animals if treatment is started several hours after contact with street rabies virus. If further study confirms these results, it should be possible to offer the tissue culture vaccine to people requiring protection against rabies as an alternative to Semple vaccine with fewer injections.

This investigation was supported in part by USPHS research grant AI-09706 from the National Institute of Allergy and Infectious Diseases, Contract No NIH-71-2292 with the National Institutes of Health, funds from the World Health Organisation, and funds from the Merieux Institute, Lyon, France. We are indebted to Mrs Doris Grella and Ms Meryl Harrison for excellent technical help.

Requests for reprints should be addressed to Dr Stanley A Plotkin, The Wistar Institute, 36th Street at Spruce, Philadelphia, Pa 19104, USA.

¹ Wiktor, T J, *et al*, *Journal of Infectious Diseases*, 1972, **126**, 408.

² Wiktor, T J, Plotkin, S A, and Grella, D W, *Journal of the American Medical Association*, 1973, **224**, 1170.

³ Bahmanyar, M. (Teheran). *Symposia Series in Immunological Standardization*, 1974, **21**, 226.

⁴ Aoki, F Y, Tyrrell, D A J, and Hill, L E, *Lancet*, 1975, **1**, 660.

⁵ Wiktor, T J, *et al*, *Journal of Infectious Diseases*, in press.

Aurangabad Medical College, Aurangabad, India

USHA SHAH, MD, professor (present address: B J Medical College, Poona, India)

G S JASWAL, MD, lecturer

The Haffkine Institute, Bombay, India

H J MANSHARAMANI, MD, scientific officer

Wistar Institute of Anatomy and Biology, World Health Organisation Collaborative Centre for Reference and Research in Rabies, Philadelphia, Pennsylvania 19104

S A PLOTKIN, MD, member

T J WIKTOR, DVM, member

Neonatal appendicitis

Acute appendicitis in a neonate is rare. I describe a case in a 2-week-old baby.

Case report

A girl, born at full term on 6 February 1975, was admitted to the British Military Hospital, Munster, on 21 February 1975. She was bottle-fed, and had no feeding problems up to the day before admission. She refused her evening feed on 20 February, and during the night vomited yellow material and passed loose green faeces. She continued to refuse feeds. On admission she was apyrexial, not dehydrated, with a moderately distended soft abdomen and active bowel sounds. Bile-stained vomiting continued over the next 24 hours, but then vomiting became faeculent. She was still apyrexial, but the abdomen was more distended and the rectum empty. Erect and supine abdominal x-ray films showed small-bowel distension with fluid levels.

Laparotomy was performed 52 hours after the initial symptoms. The peritoneal cavity contained turbid fluid, and the dilated small bowel was coated with purulent exudate. A kinked loop of ileum, adherent to the ileocaecal region, was causing the obstruction. Further dissection showed an acutely inflamed gangrenous appendix. Routine appendicectomy with stump burial was performed. Peritoneal fluid culture grew predominantly *Klebsiella* spp. Pathological examination confirmed acute inflammatory changes, but no perforation. Postoperatively she was treated with gentamicin and benzylpenicillin. Recovery was uneventful, and she was discharged on the eighth day.

Discussion

Appendicitis in the neonate is rare. In 1960 Schaupp *et al*¹ reviewed reports published in the English-speaking world and found only 19 cases, to which they added five of their own. Only four of these babies survived—a mortality rate of 83%. Since then 19 cases have been reported (making a total of 43 cases). Eight of these survived, giving a total mortality rate of 70%.

The authors all emphasise the difficulty of diagnosis. The presenting symptoms are usually non-specific—anorexia, vomiting, and diarrhoea. Signs likewise are all too often unhelpful—dehydration and minor degrees of abdominal distension. Fever is often absent—Hardman and Bowerman² found this in 11 out of 16 cases. They also found a raised white cell count present in only five out of nine

cases. Firor and Myers³ pointed out one of the main reasons for acute appendicitis being so rare in the infant. Assuming luminal obstruction to be one of the major causes, they quoted the original observations by Treves that the appendix is characteristically funnel-shaped for the first 9 to 12 months of life. Thus blockage of the lumen by faecalith, oedema, or lymphoid hyperplasia is less likely to occur.

The presence of intestinal obstruction in this case led to surgical referral and intervention before perforation had occurred. Perforation increases the mortality rate to 85%: only four patients are recorded as surviving. Knowledge of fluid and electrolyte balance and techniques of neonatal anaesthesia have greatly improved recently and it is now less risky to operate on neonates. In cases of neonatal abdominal distension for which no definite diagnosis has been made, laparotomy should be considered at an early stage.

I thank Brigadier N G Kirby, Command Consultant in Surgery, BAOR, for his help in managing this patient and for his encouragement and advice during the preparation of this paper.

¹ Schaupp, W, Clausen, E G, and Ferrier, P K, *Surgery*, 1960, **48**, 805.

² Hardman, R P, and Bowerman, D, *American Journal of Diseases of Children*, 1963, **105**, 99.

³ Firor, H V, and Myers, H A P, *Surgery*, 1964, **56**, 581.

British Military Hospital, Iserlohn, Germany

G L FOWKES, MB, FRCS, major, surgical specialist (present address: Inverell 2360, NSW, Australia)

Reduction in incidence of rash using polymer-free ampicillin

Rash is a common complication of ampicillin treatment with a particularly high incidence in certain diseases, including lymphatic leukaemia.¹ It has been suggested that two impurities of high molecular weight contribute to the rash. One is a penicilloylated protein and the care now taken to ensure that this impurity is removed during the manufacture of ampicillin has resulted in a significant reduction in rash incidence in man.² The second impurity is a polymer, whose role in the production of rash in man has not been investigated. In this study polymer-free ampicillin was given to patients suffering from lymphoproliferative disease with infective complications. The incidence of exanthemata was recorded and supplementary information obtained by skin testing.

Patients, methods, and results

Of the 10 patients studied, eight had chronic lymphatic leukaemia and two had lymphosarcoma affecting the spleen and marrow but with apparently normal peripheral blood. The indication for treatment was respiratory infection in seven patients and urinary tract infection in three. A 10-day course of polymer-free ampicillin was given, five patients receiving 2 g/day

and the remainder 1 g/day. Four weeks after starting ampicillin skin tests were performed and repeated after 12 weeks in patients who developed a positive reaction or rash. Skin testing was carried out in three stages, starting with a preliminary prick test with minor determinants. Negative reactors were then given 0.01 ml intradermally of polymer-free ampicillin (10^{-2} mol/l) and ampicillin polymer (10^{-6} mol/l). Patients who reacted negatively were given 0.1 ml intradermally of polymer-free ampicillin (10^{-1} mol/l) and ampicillin polymer (10^{-4} µg/l). Reactions were read at 20 minutes, 48 hours, and 72 hours. Biopsy specimens from those with positive skin test reactions and rashes were obtained when possible.

The results are shown in the table. One patient developed a maculopapular rash which was first noticed three days after stopping ampicillin. Microscopic examination of her skin showed non-specific inflammatory change, and she did not have a positive skin test reaction. Three patients were positive on skin testing to high-dose ampicillin polymer at four weeks. Biopsy in one case showed no evidence of hypersensitivity. In all three patients this phenomenon had disappeared by 12 weeks.

Comment

Commercial ampicillin is now virtually free of penicilloylated protein but still contains polymer. Our study was therefore an attempt to elucidate the role of polymer in the production of ampicillin rash. In a previously reported series 10 out of 12 patients with lymphatic leukaemia receiving ampicillin developed a rash.¹ In the present study only one patient out of 10 with similar disease given polymer-free ampicillin developed a rash. Although half our patients received a smaller ampicillin dose than those of Cameron and Richmond,¹ there is no evidence that the amount of ampicillin and the degree of rash are related.³ It is unlikely that concurrent administration of prednisolone suppressed the reaction as corticosteroids have been found to be ineffective in preventing ampicillin exanthemata.⁴

The positive reactions on skin tests observed in three patients after four weeks were transient and had disappeared by three months. In their time relations and evanescence these positive reactions resembled the Jones-Mote reaction⁵ but were histologically different. It is interesting that all three positive skin reactions were due to ampicillin polymer, but this does not clarify the underlying mechanism of their production. Clearly there were too few patients for firm conclusions to be drawn. The results do give clinical support for the efforts made by manufacturers to ensure the lowest possible level of polymeric materials in antibiotics.

We are grateful to Beecham Research Laboratories for the supply of polymer-free ampicillin.

¹ Cameron, S J, and Richmond, J, *Scottish Medical Journal*, 1971, **16**, 425.

² Knudsen, E T, Dewdney, J M, and Trafford, J A P, *British Medical Journal*, 1970, **1**, 469.

³ Weuta, H Von, *Arzneimittel-Forschung*, 1972, **22**, 1300.

⁴ Pullen, H, Wright, N, and Murdoch, J McC, *Lancet*, 1967, **2**, 1176.

⁵ Jones, T D, and Mote, J R, *New England Journal of Medicine*, 1934, **210**, 120.

Department of Haematology, Royal Infirmary, Edinburgh

A C PARKER, BSC, MRCP, lecturer

University of Sheffield, Royal Hospital, Sheffield S1 3SR

J RICHMOND, MD, FRCP, professor, Academic Division of Medicine

Details of patients receiving polymer-free ampicillin

Case No	Age (years)	Sex	White blood count ($\times 10^9/l$)	Absolute lymphocyte count ($\times 10^9/l$)	Ampicillin (g/day)	Concomitant therapy	Skin test result	Rash
1	62	M	31.6	29	1	Chlorambucil 4 mg, allopurinol 150 mg		—
2	61	F	15.8	10.2	1			—
3	80	F	170	160	1		Positive at 4 weeks to high-dose ampicillin polymer	—
4	88	M	40.9	37.5	2	Prednisolone 10 mg		—
5	66	M	72	67	2			—
6	72	M	91	83	2	Chlorambucil 2 mg	Positive at 4 weeks to high-dose ampicillin polymer	—
7	49	M	223	210	1	Cyclophosphamide 100 mg, prednisolone 10 mg		—
8	61	F	83	80	1	Chlorambucil 8 mg, prednisolone 15 mg	Positive at 4 weeks to high-dose ampicillin polymer	—
9	66	F	5.1	2	2			—
10	66	F	5.1	2.2	2	Prednisolone 10 mg		+

Conversion: SI to traditional units—White cell and lymphocyte counts: $1 \times 10^9/l = 1000/mm^3$.