SHORT REPORTS

Rotavirus neutralisation by human milk

There has been considerable publicity recently about the benefits of breast-feeding in giving protection from infection. We have investigated the effect of mother's serum and breast milk, taken daily for five days after birth, on rotavirus infection of cells in vitro.

Materials, methods, and results

Milk and blood samples were taken by staff at Birmingham Maternity Hospital and at Marston Green Maternity Hospital, Birmingham. Milks were clarified by centrifugation, and milks and sera were heated at 56°C for 30 minutes to inactivate complement.

Human rotavirus was obtained from the faeces of children with acute diarrhoea admitted to East Birmingham Hospital. The neutralisation test was carried out as previously described.^{1,2} Human rotavirus infects but fails to replicate in LLC MK₂ cells and the infected cells can be detected by an indirect immunofluorescent technique. The neutralisation titre of a milk or serum was the dilution that gave a 50% or greater reduction of fluorescent foci compared with the control. The specific rotavirus fluorescent antibody titres of sera and milks were determined by their reaction with rabbit rotavirus infected LLC MK₂ cells² in an indirect immunofluorescent test.

The rotavirus neutralising titres of the randomly picked mothers' sera varied from 1/10 to 1/640 (see table). Most of the first milk samples had a neutralising titre close to that of the mother's serum, but this fell rapidly in the puerperium so that, by the fifth day, only the two mothers with the highest serum neutralising titres had milk titres of 1/5 or greater.

All the sera and milks were tested by indirect immunofluorescence for specific reaction with rotavirus infected cells. Specific fluorescence was given by all the sera and the early milks, but the titres were always less than the rotavirus neutralising titres (see table). Four- and five-day milk samples gave no reaction, but it was not possible to test them undiluted because of non-specific fluorescence.

The milk samples that did not neutralise human rotavirus at 1/5 dilution were tested undiluted; the virus was completely inactivated. The same result was obtained with these milks and lamb rotavirus. A high-speed aqueous supernatant of undiluted pasteurised cows' milk and an antibody-free glycoprotein prepared from cows' milk inactivated human rotavirus completely.

Comment

We have shown by neutralisation and immunofluorescent tests that human milk in the early puerperium contains rotavirus antibodies that decline to undetectable levels by five days after birth.

Fifth-day undiluted milks inactivated human rotavirus, but it was not possible to tell whether this was due to specific antibody. As they also inactivated lamb rotavirus, however, and human rotavirus was inactivated by cows' milk and its phenol-extracted glycoprotein, we suggest it may be due to a non-specific antiviral milk factor.^{3,4}

Milk and rotavirus antibodies

Case No	Test	Serum	Milk (days after delivery)							
			1	2	3	4	5	6	7	8
1	NT	640	320	320	80-160	20-40	20			20
	FA	160	320	10	20	5	<5			<5
2	NT	160	5*	80	20	5	5			
	FA	40	<5	20	10	<5	<5	<5		
3	NT	160		> 320	80	20				
	FA	40		20	10	5				
4	NT	80	160	80	20	5				
	FA	<10	<10	<10	<5	<5	<5			
5*	NT	80		40	5	<5	<5	<5		
	FA	10		<5	<5	<5	<5			
6	NT	40	10	5	5	5	<5			<5
	FA	5	<5	<5	<5	<5	<5			<5
7	NT	40		20	5	5	5			
	FA	20		5	<5	<5	<5			
8	NT	20	40	40	10	5				
	FA	10	40	20	10	5				
9	NT	10	5	5	5	<5	<5			
	FA	20	<5	<5	<5	<5	<5			
10	NT	10		10	5	5	<5	<5		
	FA	10		5	<5	<5	<5	<5		

NT = Neutralisation test. The number is the reciprocal of the serum dilution causing 50% reduction in the fluorescent foci compared with the control.

FA = Fluorescent antibody. The number is the reciprocal of the greatest serum dilution showing fluorescence with rotavirus infected cells.

*This sample was very watery.

Rotavirus infection in the first few days of life is frequent and largely asymptomatic, even in breast-fed babies,⁵ at the time when there is a large amount of specific rotavirus antibody in the milk. The fact that undiluted human or cows' milk also inactivates human rotavirus, however, may be an important consideration in the current baby feeding discussions.

I thank Dr D A J Tyrrell and Dr K G Nicholson of the Division of Communicable Diseases, the Clinical Research Centre, Northwick Park Hospital, Harrow, for supplying antiviral glycoprotein from cows' milk.

Dr M E Thouless is supported by a grant from the Medical Research Council.

¹ Bryden, A S, *et al*, *Journal of Medical Microbiology*, 1977, **10**, 121.

² Thouless, M E, *et al*, *Archives of Virology*, 1977, **53**, 287.

³ Matthews, T H J, *et al*, *Lancet*, 1976, **2**, 1387.

⁴ Lawton, J W M, and Shortridge, K F, *Lancet*, 1977, **1**, 253.

⁵ Totterdell, B M, Chrystie, I L, and Banatvala, J E, *Archives of Disease in Childhood*, 1976, **51**, 924.

(Accepted 12 July 1977)

Regional Virus Laboratory, East Birmingham Hospital, Birmingham B9 5ST

M E THOULESS, PHD, senior microbiologist

A S BRYDEN, FIMLS, chief technologist

T H FLEWETT, FRCPATH, MRCP, consultant virologist

Response of metastatic breast cancer to combination chemotherapy according to site

Despite the clear efficacy of combination chemotherapy in producing tumour regression in some patients with metastatic breast cancer, its precise role in the overall management of this condition remains controversial. Our experience with two chemotherapy regimens leads us to believe that the major site of symptomatic disease should be the most important factor determining the choice of systemic therapy. We briefly present the evidence for this view below.

Patients, methods, and results

The two regimens were as follows: (1) vincristine 1.5-2.0 mg and doxorubicin (Adriamycin) 40-100 mg intravenously on days 1 and 8; prednisolone 20 mg by mouth daily on days 1 to 14 of a 28-day cycle (VDP); or (2) methotrexate 30-50 mg, 5-fluorouracil 500-1000 mg intravenously on days 1 and 8 with cyclophosphamide 100 mg and prednis-

solone 20 mg by mouth daily on days 1 to 14 of a 42-day cycle (MEFUP). Treatment was continued until disease progressed or until a total dose of 550 mg/m² doxorubicin had been given unless side effects necessitated stopping sooner.

The response of metastatic breast cancer was assessable in 68 patients. Forty-two patients received VDP and 40 received MEFUP. Fourteen patients had both combinations at some time and all patients had at least two courses of the appropriate chemotherapy.

Initial assessment included physical examination, blood count and biochemistry, chest x-ray examination, radiological skeletal survey, bone scan, liver scan, and liver ultrasound. Relevant investigations were repeated at appropriate intervals during and at the end of treatment. Complete remission was defined as no clinical or investigative evidence of tumour in the appropriate site for over two months. Partial remission was defined as decrease by more than half of the estimated volume of all palpable or radiological lesions for over two months, or for bone a decrease in the number of hot spots on the bone scan or radiological sclerosis of lytic metastases, or both.

Disease was assessable in a total of 102 sites (see table). With both combinations soft tissue disease responded better than visceral disease and the response of bony metastases was poor. Median duration of response (CR and PR) was 8 months for MEFUP and 8.5 months for VDP.

Response of metastases to VDP and MEFUP

	VDP				MEFUP			
	No	CR	PR	%	No	CR	PR	%
Lymph nodes ..	21	16	2	86	15	4	5	60
Skin ..	17	5	6	65	13	2	3	38
Breast ..	15	7	4	73	6	3	1	67
Lung ..	6	2	2	67	8	1	3	50
Pleura ..	11	3	4	64	9	2	1	33
Liver ..	9	2	4	67	6			
Bone ..	19		5	26	25			

CR = complete remission
PR = partial remission

Discussion

This wide variation in objective response in different sites is consistent with the experience of others using similar regimens,^{1,2} but has perhaps been inadequately emphasised. While chemotherapy may improve marrow infiltration dramatically³ and sometimes provide transient pain relief, we seldom see sustained objective responses in bone. This cannot be wholly due to the relatively insensitive methods for assessing the response in bone because the same techniques will detect rapid bone healing after successful endocrine treatment.⁴ Possibly such treatment has an anti-osteolytic property which chemotherapy lacks. Moreover, responses to chemotherapy tend to be shorter than those to endocrine treatment so a good endocrine response will be preferable to a good chemotherapy response. Clearly, chemotherapy has a major part to play in the management of metastatic breast cancer and we would agree with Priestman *et al*⁵ that this part may have been underrated. Nevertheless, we think that overall assessment of response to any treatment in a heterogeneous group of patients may be very misleading and that whenever systemic treatment is selected for a particular patient the predominant site of symptomatic disease should be a major consideration.

¹ Brambilla, C, *et al*, *British Medical Journal*, 1976, **1**, 801.

² Canellos, G, *et al*, *British Medical Journal*, 1974, **1**, 218.

³ Dady, P, *et al*, *British Medical Journal*, 1977, **1**, 554.

⁴ Stoll, B A, in *Endocrine Therapy in Malignant Disease*, ed B A Stoll, p 111. London, Saunders, 1972.

⁵ Priestman, T J, *et al*, *British Medical Journal*, 1977, **1**, 1248.

(Accepted 29 July 1977)

Royal Marsden Hospital, Sutton, Surrey

J A RUSSELL, MB, MRCP, senior medical registrar

J W BAKER, FRCP, consultant radiotherapist

P J DADY, MB, MRCP, medical registrar

H T FORD, MB, FRCP, consultant radiotherapist (also at the Combined Breast Clinic, St George's Hospital, London SW17)

J C GAZET, MS, FRCS, consultant surgeon and honorary senior lecturer (also at the Combined Breast Clinic)

J C McKINNA, FRCS, consultant surgeon

A G NASH, FRCS, consultant surgeon (also at St Helier Hospital, Carshalton, Surrey)

T J POWLES, PhD, MRCP, senior lecturer and honorary consultant physician (also at the Combined Breast Clinic and the Institute of Cancer Research, London)

Meningeal reaction to starch powder in the cerebrospinal fluid

There have been many reports of surgical glove powder causing granuloma formation in various tissues of the body,¹ most notably peritoneum, but not of reaction to starch after neurosurgical operation. We report what is apparently a meningeal reaction to surgical glove powder in the cerebrospinal fluid.

Case report

A previously fit 53-year-old bricklayer developed ataxia and vertigo over eight months. EMI scan showed a large 4th ventricle lesion that was removed at craniectomy in 1977. Histology disclosed a subependymoma. Post-operatively he was treated with betamethasone, initially 6 mg three times a day and then in decreasing doses over the next two weeks. His symptoms improved, and at 13 days he was transferred back to his original hospital for further convalescence before going home. After transfer he developed a pyrexia of 38°C associated with general malaise and neck stiffness. A lumbar puncture 14 days after operation disclosed a large number of cells, many of which could not be identified as polymorphs or lymphocytes. He was returned to our care 15 days after operation.

Starch granules cuffed by inflammatory cells under polarised light showing birefringence. (Stained with haematoxylin and eosin and shown at a magnification of $\times 656$.)

On return he was pyrexial (38.5°C), with neck stiffness. The white cell count was $11.1 \times 10^9/l$ ($11 \times 10^9/mm^3$). A further lumbar puncture was performed, which produced bright yellow fluid at a pressure of 180 mm cerebrospinal fluid (CSF) with free rise and fall and the following composition—white cell count $150 \times 10^6/l$ ($150/mm^3$) (80% polymorphs, 20% mononuclears), red cell count $30 \times 10^6/l$ ($30/mm^3$), protein 1.7 g, and sugar 1.0 mmol/l. A spun deposit of CSF showed polymorphonuclear leucocytes and many large phagocytic cells. Throughout the films were rounded bodies that stained positively with iodine and periodic acid Schiff and showed maltese-cross birefringence under polarised light. These reactions are typical of starch granules. Almost all these bodies were surrounded by cells. No bacteria were seen, and culture was negative. A presumed diagnosis of starch meningitis was made, and he was again treated with betamethasone. A further lumbar puncture was performed five days later, removing 30 ml of CSF in an attempt to dilute the starch concentration. This showed a similar picture, but with less starch and a higher percentage of phagocytes. The pyrexia and neck stiffness settled over five days, and he was discharged after a further five days.

Comment

We think that this is a genuine meningeal reaction to starch. In both CSF specimens examined at this hospital almost every starch granule was surrounded by a collar of inflammatory cells, and occasional macrophages with large cytoplasmic vacuoles, suggestive of absorption of starch granules, were present. This unusual appearance may explain the initial difficulty in identifying some mononuclear cells. Although no starch granulomata were seen, these observations make it unlikely that the appearances were due to simple contamination.