Cryptococcal meningitis complicating systemic lupus erythematosus: two patients treated with flucytosine and amphotericin B

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SUMMARY Two fatal cases of cryptococcal meningitis complicating adrenocorticosteroid-treated systemic lupus erythematosus are reported. In one patient who was treated with flucytosine alone, after an initial period of improvement cryptococci resistant to flucytosine were isolated, and subsequent amphotericin B treatment did not alter the progress of the disease. In the second patient, who received both drugs concurrently, resistant cryptococci did not appear and the patient recovered sufficiently to return home. Flucytosine-resistant mutants could be demonstrated *in vitro* in the original cryptococcal isolates from both patients. The use of flucytosine and amphotericin B in combination is discussed.

Disseminated cryptococcal infection is a rare disease in Britain and almost invariably associated with severe impairment of host defences, particularly the immunosuppression of transplant surgery. Khan and Sbar (1975) noted the association of cryptococcal meningitis with systemic lupus erythematosus, especially after treatment with adrenocorticosteroids, and pointed out that diagnosis of this complication is rarely made during life, although successful treatment is possible.

Amphotericin B and, more recently, flucytosine (5-fluorocytosine) have been successful in the treatment of cryptococcal meningitis, though neither agent is ideal. The greatest discrimination must be shown in the administration of these chemotherapeutic agents if the infection is to be eradicated from the patient, whose own defences can barely assist this process, and the patient is to be unharmed by the treatment. We here present two patients seen within a year at this hospital, both of whom died, but in whom the clinical course and laboratory observations give indications for successful treatment of the condition.

Laboratory methods

Cryptococcus neoformans was isolated on Sabouraud's dextrose-peptone agar and identified in

secretions and in culture by morphology in India ink preparations. Its identity was confirmed by morphology in cornmeal agar, and assimilation and fermentation tests (after Lodder, 1970), by pigmentation on a medium containing an extract of *Guizotia abyssinica*—'Niger seed' (Staib, 1962), and by pathogenicity on intracerebral injection in suckling mice. Blood was cultured by adding 5 ml to nutrient broth with 0.05% sodium polyanethol sulphonate, and a pour plate also was prepared by adding 1 ml of blood to 19 ml of molten Sabouraud's agar.

All experiments on the antifungal activity of flucytosine were performed using yeast nitrogen base (Difco) with dextrose and asparagine (Shadomy *et al.*, 1969), or yeast morphology agar (Difco). In experiments with more than 40 hours' incubation flucytosine was assayed in the culture medium at the end of the experiment to ensure that no significant decline in antifungal activity had occurred. Viable counts were estimated by the method of Miles and Misra (1938). Minimum inhibitory concentrations (MIC) for flucytosine and amphotericin were estimated by the methods of Shadomy *et al.* (1969). Serum and CSF concentrations of flucytosine were estimated by a plate diffusion method, using surface inoculation with a sensitive *Candida albicans* isolate. Amphotericin B does not affect flucytosine assay in this system (Kaspar and Drutz, 1975). Amphotericin in the presence of flucytosine was assayed by a

Received for publication 12 July 1976

similar method, with the addition of 0.01% cytosine to the medium to inactivate flucytosine (Holt, 1975).

Evidence of synergy between flucytosine and amphotericin B was sought using the methods of Medoff *et al.* (1971). Mutational resistance was demonstrated by the replica plating technique of Lederberg and Lederberg (1952).

Case reports

CASE 1

Mrs P. McK., a 48-year-old Jamaican resident in the UK for 10 years, presented with a short febrile illness associated with oedema, ascites, hypertension, and proteinuria. LE cell preparations were positive and a renal biopsy showed acute glomerulonephritis consistent with systemic lupus erythematosus. She improved with prednisone (80 mg daily, reducing to 20 mg daily) and diuretics. Azathioprine was later added. She remained hypoproteinaemic with proteinuria and an elevated blood urea (11.7 mmol/l) and subsequently developed a steroid-induced diabetes mellitus, controlled by diet and chlorpropamide.

A year later, during an episode of malaise and

dyspnoea, a mass was seen on chest x-ray in the lower lobe of the right lung (Fig. 1). Believed at the time to be neoplastic, this was presumably the primary cryptococcal lesion. It was still present, essentially unchanged, at the time of her last admission.

Two years after the onset of her illness she was admitted complaining of headache and drowsiness. She was pyrexial (40°C) but had no meningism or focal neurological signs. A clinical diagnosis of septicaemia was made and she was treated with broad-spectrum antibiotics. Because of the lack of clinical response a lumbar puncture was performed. The cerebrospinal fluid showed a pleocytosis and *C. neoformans* was isolated. Results of this and subsequent CSF examinations are summarised in Table 1. Cryptococcal antigen was initially present in the CSF to a titre of 1/400 but could not be detected in the serum.

In view of her renal lesion it was not thought desirable to treat her with amphotericin B, and so flucytosine was administered in a dose of 150 mg/kg per day (2 g six-hourly). This was well tolerated and satisfactory serum and CSF concentrations were obtained (Table 2), but there was little clinical improvement. At first there was a reduction in the number of organisms visible in, and cultured from, the CSF, but after 28 days of flucytosine treatment the numbers increased again, and cryptococci highly resistant to flucytosine were isolated.

Daily intravenous amphotericin B was then started, in increasing doses up to 1 mg/kg, and just before her death the intrathecal route was also used. Flucytosine was continued until it became clear that no synergistic activity between flucytosine and amphotericin B could be demonstrated *in vitro*.

There was gradual deterioration in the patient's condition with no change in the CSF findings and she died 56 days after the diagnosis of cryptococcal infection had been made, and 19 days after the start of amphotericin B treatment. Permission for necropsy was refused.

She received a total dose of 390 g of flucytosine orally and approximately 1.3 g of amphotericin intravenously. No toxic effects of flucytosine were observed but the introduction of amphotericin B was followed by hypokalaemia (plasma potassium 1.9 mmol/l) and the plasma urea rose terminally.

CASE 2

Mrs D. D. presented at age 52 with a polyarthritis affecting mainly her wrists. Her erythrocyte sedimentation rate was 44 mm in one hour. Her rheumatoid factor and antinuclear factor remained persistently positive but no LE cells were ever seen. She subsequently developed Sjögren's syndrome,

Fig. 1 Case 1. Radiograph of right lung showing cryptococcal lesion.

Table 1 Case 1. Cerebrospinal fluid findings

Day of treatment	Treatment in progress	CSF findings			
		Leucocyte count/mm ³	C. neoformans		
			Seen	Cultured	Flucytosine MIC (mg/l)
1	nil	310	++	++	1
4	flucytosine	110	++	++	1
7	flucytosine	14	+	+	1
12	flucytosine	23	±	±	1
19	flucytosine	18	+	+	100
26	flucytosine	1	++	++	> 1000
35	flucytosine				
	+ amphotericin	3	++	++	> 1000
42	amphotericin	52	++	++	> 1000
54	amphotericin	4	++	++	> 1000

MIC = minimum inhibitory concentration.

Table 2 Case 1. Serum and CSF concentrations of flucytosine

Day of treatment	Flucytosine concentrations (mg/l)			
	Serum (hours after dose)			CSF 2 h after dose
	2	4	6	
12	100		30	45
19	80	45		30
26	90		65	38

pericarditis, pleural effusions, and a mononeuritis multiplex. A diagnosis of systemic lupus erythematosus was made and she was treated with corticosteroids.

Six years after her first presentation she had an episode of headache and vomiting associated with papilloedema, retinal haemorrhages and exudates, and a blood pressure of 190/120 mmHg. She was known to have had an IgM monoclonal gammopathy since early in her illness and on this occasion her serum IgM level was 27 g/l. Plasma viscosity was 1.92 cP (ESR 52 mm in 1 h), and her blood urea was 9.8 mmol/l. Albuminuria was present. Her symptoms improved with plasmapheresis and chlorambucil for her macroglobulinaemia and hyperviscosity syndrome and control of her hypertension. The papilloedema resolved. She remained on large doses of corticosteroids.

One year later she returned with a short history of further headache, vomiting, and lassitude. Plasma protein electrophoresis showed that the macroglobulinaemia had not recurred. Her condition deteriorated over a three-week period and she became stuporose with meningism, bilateral papilloedema, and upgoing plantars. At lumbar puncture CSF pressure was greater than 40 cmH₂O, the CSF protein was 0.49 g/l, and there were no cells in the CSF except 2500 cryptococci/mm³. *C. neoformans*

was isolated from both CSF and blood (approximately 50 colonies per ml of blood).

Flucytosine was given orally twice daily because of impaired renal function (blood urea 7.9 mmol/l, creatinine clearance 20 ml/min/m²). On the basis of serum assays the dose was reduced from 3 g twice daily to 2 g twice daily; 100 mg flucytosine were administered intrathecally twice weekly. After our experience with case 1 we added amphotericin B to the regimen beginning with 0.25 mg/kg by intravenous infusion daily and gradually increasing to 1 mg/kg daily.

The patient's progress was unexpectedly good at first. Within two days she was alert and cheerful and free from headache. She continued to improve, surviving an episode of pneumococcal pneumonia with bacteraemia which was treated, before laboratory results were known, with ampicillin. Intrathecal flucytosine injections were reduced to once weekly and then stopped. After 20 days amphotericin infusions were given on alternate days and then twice weekly before being stopped after eight weeks. Oral flucytosine was continued.

Laboratory findings improved in parallel with the patient's clinical progress (Fig. 2). Three months after admission she was discharged home still taking flucytosine.

She was readmitted 10 days later having become drowsy and uncooperative. Signs of meningism were present but the papilloedema had not recurred. She was in left ventricular failure and this was successfully treated without halting a decline in her neurological state. At lumbar puncture CSF pressure was 12 cmH₂O, CSF protein was 0.84 g/l, and there were 16 leucocytes/mm³, of which 13 were lymphocytes. No cryptococci were seen. A second lumbar puncture on the day of her death three days later showed 127 leucocytes/mm³, of which 116 were lymphocytes. Permission for a necropsy was refused.

She had received 1260 g of flucytosine by mouth.

Fig. 2 Case 2. Serum and CSF results.

1 g by the intrathecal route, and approximately 1.2 g of amphotericin intravenously. The initial regimen gave rather high serum flucytosine concentrations but satisfactory results were obtained when she had been stabilised on the lower dose (Fig. 3). CSF concentrations of flucytosine ranged from 17 to 35 mg/l. Amphotericin was undetectable in all CSF specimens examined, but in the serum a concentration of 1.5 mg/l was reached during the period of daily infusions.

Infusions of amphotericin gave rise to sweating and backache which were controlled by salicylates. Her plasma urea before treatment was 7.9 mmol/l, and rose to 14.2 mmol/l while amphotericin was being given daily, but fell again to its original levels after amphotericin was discontinued. Her creatinine clearance was unaltered after treatment. Hypokalaemia was not a problem although one serum potassium value of 2.9 mmol/l was recorded. Her haemoglobin before the start of treatment was 10.9 g/dl and fell to 7.5 g/dl, with a leuko-erythroblastic appearance of the peripheral blood film. Marrow examination revealed hypocellularity with normal cell precursors, and no evidence of infiltration. It was not possible to distinguish the effect of the antifungal agents from that of the previous chlorambucil treatment. Liver function tests were normal before treatment and did not change during treatment except for a slight transient reduction in

Fig. 3 Case 2. Serum flucytosine concentrations.

albumin. She suffered persistent diarrhoea, which was probably due to the flucytosine treatment.

Mycological results

CASE 1

The isolations of *C. neoformans* from CSF and the appearance of resistance to flucytosine are shown in Table 1. All isolates from case 1 had a MIC of amphotericin of approximately 0.25 mg/l. No synergy between amphotericin and flucytosine was demonstrated against flucytosine-sensitive and flucytosine-resistant isolates.

Fig. 4 Case 1. Velvet pad replica plating of plate culture of original cryptococcal isolate on yeast morphology agar with (right) and without (left) 100 mg/l flucytosine.

Fig. 5 Effect of incubation of cryptococcal isolates in supplemented yeast nitrogen base (YNB) with and without flucytosine: (a) original isolate from case 1; and (b) original isolate from case 2.

The pre-existence of resistant mutants (MIC more than 1000 mg flucytosine/l) among sensitive cells in the original isolate was demonstrated by the replica plating method. Figure 4 shows the result of velvet pad transfer from a plate culture of the first *C. neoformans* isolate from case 1 on to yeast morphology agar, with and without 100 mg/l flucytosine. Colonies of resistant cryptococci can be seen on the flucytosine-containing medium. Repeated subculture from the flucytosine-free plates from the exact area corresponding to the position of the mutant colonies on the flucytosine-containing plates provided a resistant culture which had not previously been exposed to flucytosine.

Growth of the original isolate in supplemented yeast nitrogen base with and without 100 mg/l flucytosine is shown in Figure 5a. The viable count was at first decreased by the presence of the flucytosine, but the resistant mutants survived and grew up to counts similar to those achieved earlier in the medium without flucytosine. Cells from the flucytosine-containing culture at five days were uniformly highly resistant to flucytosine.

CASE 2

The main laboratory findings are summarised in Figure 2. Although cryptococci continued to be visible in the CSF for four months, none was cultured after 10 days of treatment, and all isolates obtained were sensitive to flucytosine (MIC 2 mg/l) and to amphotericin (MIC 0.25 mg/l). No synergy between the agents was demonstrated. Cryptococcal antigen in serum and CSF fell in titre from over 1/600 to 1/20 after six months, and cryptococcal antibody became detectable at that time.

As in case 1, resistant mutants (MIC more than 1000 mg flucytosine/l) could be obtained in the laboratory from the original isolate of case 2. Figure 5b shows the results of growth of this isolate in supplemented yeast nitrogen base with and without 100 mg/l flucytosine. Resistant mutants, however, were not isolated from the patient during combined therapy.

Discussion

Amphotericin B is still generally regarded as the standard chemotherapy for cryptococcal meningitis, and successful treatment has been reported in cases associated with adrenocorticosteroid-treated systemic lupus erythematosus (Collins *et al.*, 1972; Khan and Sbar, 1975). Nevertheless, penetration of amphotericin B into many body sites, including the CSF, is poor; and the symptomatic and objective side-effects of this drug may be severe, renal impairment in particular being almost invariable with effective

treatment (Bennett, 1974a). Flucytosine is better tolerated and less toxic (Cartwright, 1975) and penetrates well into most body spaces, and successful cures have been reported in the literature (Utz *et al.*, 1969). Failures of treatment, and relapse after apparently successful treatment, are common with both drugs (Utz *et al.*, 1975). The main problem with flucytosine is the emergence during treatment of cryptococci resistant to the drug (Block *et al.*, 1973) as occurred in our case 1. Flucytosine-resistant mutants occur *in vitro* at a rate of approximately 1 in 4×10^6 in most fungal cultures tested (Scholer, 1976). They vary in their degree of resistance and growth rate in the presence of flucytosine but include clones indifferent to high concentrations of the drug (Normark and Schönebeck, 1972). Such mutants could be demonstrated in both the original isolates from the patients reported here and in several other clinical isolates tested by us. It is easy to see that selective growth of mutants may occur *in vivo* when large populations of cryptococci, not exposed to effective body defences, are treated with flucytosine. In this respect the situation may parallel the treatment of cavitary tuberculosis with a single antibiotic.

This leads to the rationale for the use of the two drugs in combination. The presence of amphotericin may prevent the multiplication of surviving flucytosine-resistant mutants; while immediate high dosage of flucytosine produces anticryptococcal activity throughout the body, without toxic effects, during the time that maximal amphotericin activity is gradually obtained, in serum, over a number of days. It may be that the lower dosage of amphotericin can be used in this situation, but we felt that in view of the patients' underlying disease we should give full dosage, although we stopped the amphotericin in case 2 earlier than we should have done if we had been using it alone. The low concentrations of amphotericin obtained in body fluids other than serum probably do not fairly represent the antifungal activity in extravascular sites where the amphotericin may be bound to cell membranes (Bennett, 1974a); but it has been suggested that amphotericin should be given by the intrathecal route while large numbers of cryptococci are present in the CSF itself (Tobias *et al.*, 1976).

In addition, synergy between the two drugs has been demonstrated in the laboratory (Medoff *et al.*, 1971) although we were unable to show this with the isolates from these two patients. The effectiveness of combination treatment has been shown in animal experiments (Block and Bennett, 1973) and the first encouraging results with human cryptococcosis are beginning to appear (Utz *et al.*, 1975). Medoff and Kobayashi (1975) suggest that amphotericin's stimulating effect on host immunity may play a part

in the effectiveness of the combination *in vivo*. They warn, however, that the reverse effect may be achieved in some situations, and that there is tissue-culture evidence that the combination may show synergistic toxicity. The effects of flucytosine on the liver and bone marrow (Record *et al.*, 1971) would be more likely to occur when amphotericin had caused renal impairment and accumulation of flucytosine (Bennett, 1974b). It is necessary to adjust the dose of flucytosine in cases of renal impairment (Schönebeck *et al.*, 1973) and to assay the concentrations achieved. In our case 2, adjustment of the dosage produced satisfactory flucytosine concentrations; it was difficult to distinguish toxic effects from the background illness, but there was no definite evidence of marrow or liver impairment caused by the drug.

Both of these patients had many of the presenting features associated with failure of treatment of cryptococcosis (Diamond and Bennett, 1974)—adrenocorticosteroid treatment, high CSF pressure, less than 20 leucocytes/mm³ in the CSF, cryptococci seen in CSF film, cryptococci isolated from other sites, high cryptococcal antigen titre—and indeed both patients died. The terminal episode in case 2 is obscure and may have been the result of active cryptococcal disease. On the other hand, her progress with the combination therapy was strikingly better than that of the first patient who received flucytosine and amphotericin consecutively, in whom resistance emerged and the progress of the disease to death was almost uninterrupted. On the basis of our experience in these and other cases, and the results from other centres, we feel justified in recommending combination treatment for patients in this country with cryptococcosis intervening in severe predisposing disease, and we await with interest the results of the multicentre trial which has been set up to study this problem (Utz *et al.*, 1975).

We thank Professor A. E. Read and Dr D. R. Coles for permission to publish these case reports, Dr Mary P. English and Dr L. W. Greenham for confirming the identity of the isolates, and the Mycological Reference Laboratory for the estimations of cryptococcal antigen and antibody.

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