In a trial of the use of miltefosine to treat HIV-related cryptosporidiosis in Zambian adults, extreme metabolic disturbances contribute to high mortality

E. SINKALA^{*}, M. KATUBULUSHI^{*}, S. SIANONGO^{*}, A. OBWALLER[†] and P. KELLY^{*,‡}

^{*}*Tropical Gastroenterology and Nutrition Group, University of Zambia School of Medicine, Nationalist Road, Lusaka, Zambia*

[†]*Orphanidis GmbH, Wilhelminenstrasse 91/II, 1160 Vienna, Austria*

[‡]*Blizard Institute of Cell and Molecular Science, Barts and The London School of Medicine, Queen Mary University of London, Turner Street, London E1 2AD, U.K.*

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There is still no effective treatment for cryptosporidiosis even though the disease has a significant impact on HIV-infected adults and children. Following evidence of the drug's promising efficacy *in vitro*, a phase-1–phase-2 study of miltefosine (given at 2.5 mg/kg for 14 days, with the dose capped at 100 mg/day) was recently initiated among Zambian adults with HIV-related cryptosporidiosis. Seven patients were recruited before the trial was terminated prematurely because of lack of efficacy and the development of severe adverse events. The latter may have been entirely drug-related or the result of extreme metabolic abnormalities already present in the patients enrolled in the trial. In future trials of miltefosine, attention will have to be paid to the possibility of metabolic abnormalities in the subjects investigated.

Human cryptosporidiosis is a severe intestinal infection that is associated with considerable morbidity and mortality in the tropics (Mor and Tzipori, 2008). In their randomized controlled trial in Zambian children with cryptosporidiosis, Amadi *et al.* (2002) found that nitazoxanide only benefitted the HIV-negative patients. They also recorded high levels of mortality (17% within 8 days in the placebo group), which they attributed to their patients being gravely ill on admission to the trial, with severe undernutrition and metabolic abnormalities (Amadi *et al.*, 2002). Patients with AIDS-related diarrhoea usually have advanced

malnutrition and show high levels of mortality — around 20%/month, depending on the specific microbiological cause (Amadi *et al.*, 2001). Much work has been done on the opportunistic gastro-intestinal pathogens found in AIDS patients but surprisingly little is known about the metabolic consequences of HIV infection in Africans (Kaile *et al.*, 2008).

Although several substances have been evaluated for the treatment of cryptosporidiosis and some have shown encouraging results in pilot studies, the beneficial effects seen in controlled clinical trials have always been modest, especially in the context of HIV (Abubakar *et al.*, 2007). A fully effective treatment for cryptosporidiosis awaits discovery. Paromomycin and (most recently) nitazoxanide are already used but these antiparasitic drugs are, at best, moderately

Reprint requests to: P. Kelly, Blizard Institute of Cell and Molecular Science, Barts and The London School of Medicine, Queen Mary University of London, Turner Street, London E1 2AD, U.K.
E-mail: m.p.kelly@qmul.ac.uk.

effective and appear to offer no benefit to AIDS patients (Hewitt *et al.*, 2000; Rossignol *et al.*, 2001; White *et al.*, 2001, 2003; Rossignol, 2006). There have been very few relevant, double-blind clinical trials and those that have been conducted have had only small numbers of participants (Cacciò and Pozio, 2006; Abubakar *et al.*, 2007). Neither spiramycin nor azithromycin are effective against cryptosporidiosis, despite early promise (Gentile *et al.*, 1991; Dionisio *et al.*, 1998; Kadappu *et al.*, 2002). Clarithromycin and rifabutin, alone or in combination, have been reported to have some efficacy as prophylactic agents and to reduce the risk of symptomatic cryptosporidiosis when used in this manner (Holmberg *et al.*, 1998; Fichtenbaum *et al.*, 2000). Such an effect of rifamycins, if confirmed, might explain the observation that anti-tuberculous chemoprophylaxis reduces the incidence of diarrhoea in Zambian AIDS patients (Mwinga *et al.*, 2002).

In a collaborative study between the Institute of Parasitology of the Veterinary University in Leipzig, Germany, and an Austrian firm, Orphanidis, it was demonstrated that miltefosine inhibited the multiplication of *Cryptosporidium parvum* *in vitro* — by 97.3% when used at a concentration of 10 µg/ml and by 96.5% when used at 5 µg/ml (A. Obwaller, unpubl. obs.). As these data indicate that miltefosine is as effective, *in vitro*, against *C. parvum* as against *Leishmania donovani* (Azzouz *et al.*, 2005), it was hoped that the drug would have similar *in-vivo* efficacy against the two species of parasite.

Miltefosine is a member of the alkylphosphocholines, a group of compounds, each consisting of phosphocholine esterified to a long-chain aliphatic alcohol, that exhibit antineoplastic activity both *in vitro* and *in vivo* (Unger *et al.*, 1989; Eibl and Unger, 1990). Although miltefosine appeared to have no major effect on the blood and bone marrow of rats or dogs, cancer patients on miltefosine treatment showed elevated leucocyte and thrombocyte counts (Pronk

et al., 1994). The drug seems to have no effect on male fertility (Sundar *et al.*, 2003) but does have teratogenic activity (Sundar and Oliaro, 2007) and is therefore contraindicated in pregnancy. Cancer patients given 150 mg miltefosine/day showed signs of renal toxicity but this was usually reversible, with good hydration recommended (Verweij *et al.*, 1992). According to the DRUGDEX® database, miltefosine has been associated with reversible increases in serum aminotransferases in <10% of patients, and with retinal changes during prolonged therapy (www.thomsonhc.com/hcs/librarian).

The main aim of the present study, a phase-1–phase-2 trial, was to assess the safety of miltefosine when used against cryptosporidiosis in HIV-positive Zambian adults.

PATIENTS AND METHODS

Over a period of 6 months, 41 patients with AIDS-related diarrhoea were screened at the University Teaching Hospital in Lusaka. The seven patients who satisfied the inclusion criteria (age ≥18 years, diarrhoea for ≥2 weeks, seropositive for HIV, and able and willing to give informed consent) and were not excluded [because they were pregnant, had a Karnofsky score of ≤20, had a pre-existing hepatic or renal dysfunction (indicated by serum concentrations of the relevant markers that were more than double the upper limit of 'normal'), were enrolled in another trial, and/or had taken another drug intended to eradicate *Cryptosporidium* in the previous 2 weeks] were enrolled.

Most of the patients enrolled where found to have oocysts of *C. parvum* or *C. hominis* in stool samples before they were treated. As the primary endpoint of the trial was safety, however, patients with HIV-related diarrhoea who provided baseline stool samples in which no *Cryptosporidium* oocysts were found were not excluded. For the same reason, no attempt was made to distinguish between *C. parvum* and *C. hominis* infections.

Approval for the trial was obtained from the Research Ethics Committee of the University of Zambia School of Medicine, and from the Zambian Pharmaceutical Regulatory Authority in Lusaka.

The purpose of the study was primarily to evaluate safety, and secondarily to assess the efficacy of miltefosine as a treatment for cryptosporidiosis in immunocompromised adults, with a view to the drug's further evaluation at higher doses and in children. Miltefosine (Impavido; Aeterna Zentaris, Quebec, Canada) was administered orally at 2.5 mg/kg for 14 days, with the dose capped at 100 mg/day. Follow-up was for 28 days. Patients were observed closely, around the clock, as in-patients in a dedicated side ward, with liver- and renal-function tests and full blood counts at least three times/week. The primary endpoint was safety (i.e. the absence of any clinically relevant adverse events) and the secondary endpoint was efficacy (i.e. clearance of oocysts from at least two consecutive stool samples, and reduction in the number of loose stools to one or none passed in 24 h). The planned second phase of the study, to be conducted in children once safety had been demonstrated in adults, was never carried out.

RESULTS

Of the seven adult patients enrolled, five patients had stool-positive cryptosporidiosis at enrolment, one had stool-positive isosporiasis at enrolment and one was found stool-negative for intestinal protozoa at enrolment although stool-positive for *Cryptosporidium* oocysts 2 days earlier.

When enrolled, all seven patients were malnourished and ill, with Karnofsky scores of 40–80 and hypokalaemia (Table 1). Of the five patients who had their serum concentrations of phosphate and magnesium measured, all five had hypophosphataemia and four had hypomagnesaemia (Table 1). Patients with the most extreme hypokalaemia

had electrocardiographic changes consistent with hypokalaemia, confirming the validity of these extreme results. The blood-chemistry abnormalities probably account for the profound muscle weakness experienced by all seven patients. Oral potassium, magnesium and phosphate supplements were given as soon as these abnormalities were detected. Intravenous potassium and magnesium were also given but parenteral phosphate was not available. Fluid management was crucial as the patients had persistent diarrhoea with some dehydration.

Over the course of the 14 days of treatment, there was some evidence of clinical improvement, with clinical response (defined as cessation of diarrhoea) in two patients by day 7, three by day 14, five by day 21, and three (of the five surviving patients) by day 28. There was no evidence of parasitological clearance.

Patient 3 developed frank renal failure with oliguria, and Patient 1 developed transient renal impairment. Five patients (numbers 1, 2, 3, 4 and 5) developed abnormal serum concentrations of liver aminotransferases. Patient 6 developed colonic infarction on day 6, with intestinal obstruction; he was managed conservatively because of his poor clinical state but died on day 27. Patient 5 also died, on day 22. Patient 4 developed mild impairment in visual acuity. The fluid requirements of the patients were very considerable because of the ongoing diarrhoea (Table 2).

DISCUSSION

In the present trial, miltefosine, despite the good evidence of its in-vitro activity against *C. parvum*, did not eradicate *Cryptosporidium* from any patient found to be excreting *Cryptosporidium* oocysts when he or she was enrolled. Evidence of clinical effect was difficult to assess, as only five patients were able to complete the treatment course. Although there was some suggestion of reduced diarrhoea in some patients, the

TABLE 1. Baseline clinical characteristics of the seven patients enrolled in the trial

No.	Age (years)	Gender	Weight (kg)	Karnofsky score	Body mass index (kg/m ²)	Duration of diarrhoea (days)	Serum concentration (mmol/litre)				
							K ⁺ (mmol/litre)	PO ³⁻ (mmol/litre)	Mg ²⁺ (mmol/litre)	Creatinine (µmol/litre)	Alanine aminotransferase (IU/litre)
1	39	Female	59	60	20.1	28	3.00	ND	ND	112.9	24.0
2	28	Female	32	40	18.3	84	2.70	<0.21	0.72	72.0	39.0
3	40	Male	49	70	16.1	336	2.10	0.22	0.40	91.0	32.0
4	30	Male	41	80	14.1	56	1.50	0.39	1.04	86.0	16.3
5	34	Female	40	40	14.3	21	2.00	0.29	0.43	65.0	43.0
6	32	Male	38	40	12.5	112	1.80	<0.21	0.59	100.0	16.3
7	20	Female	40	80	16.6	28	3.00	ND	ND	68.0	21.8

ND, Not determined.

fluid requirements in those with continuing diarrhoea remained disappointingly high. The observed adverse events were also worrying. Renal impairment and impaired visual acuity are known to be associated with miltefosine (Theischen *et al.*, 1993; Sundar and Oliaro, 2007), and the manufacturer's data-sheet makes it clear that maintenance of good hydration status is important. In the present study, difficulty in correcting hydration status may have contributed to the renal impairment seen in two patients. While renal dysfunction, hepatic dysfunction, intestinal infarction and death are all likely

outcomes in severely ill patients with AIDS-related diarrhoea in Africa, the miltefosine treatment given in the present trial could not be associated with any improvement in outcome. The high incidence of hepatic dysfunction was particularly worrying and was a major factor in the decision not to proceed to the second stage of the trial.

The management of extreme metabolic disturbances, such as those observed among the patients investigated in the present trial, is very difficult. Although, in the trial, oral potassium, magnesium and phosphate supplements were given as soon as these abnormalities were detected, it was not clear whether such supplements were sufficient, given the presence of diarrhoea. While intravenous potassium and magnesium were therefore also given, no parenteral phosphate was available.

In future trials among AIDS patients in Zambia (and, presumably, other regions of Africa), more intensive treatment of such metabolic abnormalities, which were found to be of unanticipated severity in the present study, will be required. Given the drug's lack of efficacy in terms of parasite clearance, and the high frequency of adverse events, further work with miltefosine in the treatment of diarrhoeal disorders is not recommended.

TABLE 2. Total amount of fluids received by each patient, during the 28 days of follow-up

Patient no.	Intravenous fluids (ml)	Oral fluids (ml)
1	27,200	20,385
2	29,000	35,830
3*	52,000	123,635
4	22,500	62,540
5	56,500	28,120
6	74,000	30,620
7†	Nil	22,470

*Patient 3 developed an intestinal obstruction and later died of septicaemia following a bowel infarct (diagnosed at autopsy).

†Patient 7 did not receive any intravenous fluids as her hydration status was considered satisfactory.

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