Development of Miltefosine for the Leishmaniases

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Abstract: The leishmaniases consist of visceral and cutaneous syndromes present in > 30 endemic regions of the world. Miltefosine (hexadecylephosphocholine) is the first oral agent that is effective and tolerated for both visceral and cutaneous disease in several endemic regions, and represents a major advance in the treatment of these diseases.

Keywords: Miltefosine, visceral leishmaniasis, cutaneous leishmaniasis, efficacy, tolerance.

INTRODUCTION

Miltefosine was originally developed as an anticancer agent. The discovery that Leishmania metabolize miltefosine-like compounds led to the testing of miltefosine against the parasites. Clinical evaluation for visceral and also cutaneous leishmaniasis soon followed. These trials have led to the registration of miltefosine for visceral leishmaniasis in India and in Germany. As positive clinical data was generated, interest in the biochemical mechanisms of this compound has enhanced.

1. CLINICAL USE OF MILTEFOSINE FOR CANCER

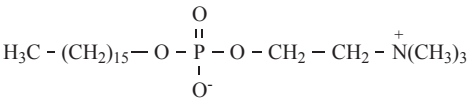
Lysophosphatidycholine was found to have immunomodulatory activity in the 1960s [summarized in reference 1]. More stable derivatives including ether-phospholipids and structurally related alkylphosphocholines were made in the 1970s and 1980s [2], and some inhibited multiplication of tumor cells. One such alkylphosphocholine was miltefosine [3] (Fig. **1a**). Because the combination of inefficacy and side effects in the cancer population were thought to preclude successful development as an oral anticancer drug [4-6], the drug was developed as a topical formulation for cutaneous cancers. In an open-label trial, a 6% miltefosine solution showed favorable results in approximately half of the 18 cutaneous lymphoma lesions [4]. In later work, 20 breast cancer patients with progression of skin metastases were treated open-label with a 6% miltefosine solution, administered daily during the first week and twice daily thereafter, with concomitant systemic therapy. Modest efficacy for the cutaneous lesions was seen [6]. In 1992, the 6% topical miltefosine formulation was registered in Germany as a treatment for skin metastasized breast cancer [1].

2. PRECLINICAL DEVELOPMENT OF MILTEFOSINE FOR THE LEISHMANIASES

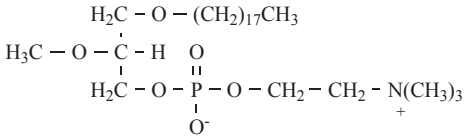
2.1. Efficacy Against Leishmania

Leishmaniasis is an arthropod-transmitted disease. Extracellular free-living flagellated *Leishmania* promastigotes in the gastrointestinal tract of female sandflies are injected into the mammalian host during the bite of the insect. The promastigotes rapidly adhere to and are ingested by mononuclear phagocytic cells. With the phagolysosome of the macrophage, the parasite undergoes a morphological change and because a non-flagellated amastigote. The amastigote multiplies within the phagolysosome, kills its host cell, and is released to infect other mononuclear phagocytes. It is remarkable that in the mammalian host, Leishmania survive and multiply only in one place, the phagocytic organelle designed to kill whatever is inside it. If subsequent TH1 immune reactions are sufficient, the activated mononuclear cell is able to destroy its intracellular parasite. In established visceral leishmaniasis, macrophage activation does not occur and the parasite ultimately kills the host. In cutaneous leishmaniasis, macrophage activation does occur and after some months the parasite is eliminated.

a. Miltefosine



b. Edelfosine



c. Ilmofosine

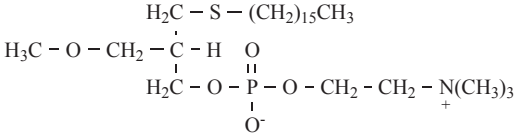


Fig. (1). Structures of Miltefosine and other phospholipids analogs [from reference 1].

The suggestion that miltefosine might be effective against Leishmania may be viewed as first being suggested by the work of Gercken and colleagues in the 1980s. In 1982, Herrmann and Gercken found that the long-chain ether 1-O-octadecyl-glycerol was taken up and metabolized by

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Leishmania donovani promastigotes. At the high concentration of 25 uM, the agent was leishmaniacidal [7]. In 1987, Achterberg and Gercken incubated ether-lysophospholipds, including ones containing phosphocholine, with promastigotes [8, 9] Further, 1 –O-hexadecyl-glycerophosphocholine had the 2nd lowest 50% inhibitory constant [9].

Croft *et al.* followed in 1987 with a report of the *in vitro* efficacy of alkyl phosphorylcholines against *L. donovani* amastigotes in mouse peritoneal macrophages [10]. The ED50 for miltefosine was 5 ug/ml, the highest (worst) of the compounds tested. When administered at 100 mg/kg/day subcutaneously for 5 days against *L. donovani* in BALB/c mice, miltefosine eliminated 100% of organisms, but at the price of a 15% weight loss. At this point in time, miltefosine was simply one member of an attractive series of antileishmanial compounds.

In 1992, Kuhlencord *et al.* showed that miltefosine was active *via* the oral route in the BALB/c mouse model [11]. Croft *et al.* then reported in 1996 that the oral ED50 of miltefosine against *L. donovani* in mice was ~6 mg/kg/day x 5 days, with >97% of liver organisms being eliminated by 30 mg/kg/day x 5 days. The demonstration of oral efficacy in mice, for a compound already in phase 2 clinical trial as an oral treatment for cancer, justified a pilot efficacy trial of oral miltefosine for visceral leishmaniasis. This trial, published in 1998 by Sundar *et al.* in Lancet, was the report that led to the acceptance of miltefosine by TDR/WHO and its formal development [13].

Escobar, Croft *et al.* later reported the comparative *in vitro* efficacy of miltefosine against a range of *Leishmania* amastigotes within macrophages [14]. In passing it may be noted that miltefosine analogs tested by Escobar *et al.* [14] were later examined by Santa-Rita *et al.* [15] and by Azzouz *et al.* [15a]. One analog---edelfosine (Fig. **1b**)--- was generally somewhat more active than miltefosine for Escobar *et al.* In contrast, miltefosine, edelfosine, and ilmofosine (Fig. **1c**) had similar *in vitro* efficacies in the work of Azzouz *et al.* [15a]. As shown in Table **1**, *L. donovani* is the most sensitive and *L. major* is the least sensitive of the Leishmania species. On the other hand, topical 6% miltefosine [Miltex^R] reduced the *L. major* burden in the draining lymph nodes of cutaneously infected mice from 2, 000-to-59, 000 parasites in control animals to 10-to-30 parasites in drug treated animals [16].

2.2. Efficacy Against Related Protozoa

In addition to *in vitro* efficacy against the original target of tumor cells and against Leishmania, miltefosine and analogs have *in vitro* activity against the related Trypanosomids. Efficacy against *T. b. brucei*, *T. b. rhodesiense*, *T. b. gambiense*, and *T. cruzi* is generally worse than that against the *L. donovani* standard (Table **1**). The ED50 for typomastigote forms of the trypanosomes is 35 uM or higher, whereas the ED50 for *L. donovani* promastigotes was ~0.5 uM. However, *L. donovani* appears to be the most sensitive of the Leishmania. An additional complication is that more complex and clinically relevant models show widely differing results. For amastigotes within macrophages, *T. cruzi* was more sensitive than *L. donovani* (Table **1**). For infected mice, *L. donovani* is much more sensitive than any Trypanosome. The ED50 against *L. donovani* was approximately 30 mg/kg total dose, whereas 75-150 mg/kg total dose was generally inactive against *T. b. brucei* and *T. cruzi* .

Given the disparate nature of the non-clinical models, conclusions about the rank order of Trypanosomid susceptibility to miltefosine should be made with caution. For the Leishmania, *L. donovani* would seem to be the most susceptible except that *in vivo*, *L. major* was very susceptible to topical drug. Trypanosomes would seem to be much less susceptible, except for one species of *T. cruzi* amastigotes within macrophages.

Table 1. Non-Clinical Efficacy of Miltefosine for Leishmania and Trypanosomes

Species	Promastigotes ED50 (uM) [14]	Amastigotes in macrophages ED50 (uM) [14, 35]	Amastigotes in liver of infected mice ED50 (mg/kg total dose) [12, 35]
L. donovani	0.5	3.9	30
L. infantum			<<100
L. tropica	1.1	8	
L. aethiopica	2.0	3.8	
L. panamensis	2.5	10	
L. mexicana	7	8	
L. major	9	34	
	Trypomastigote ED50 (uM) [12, 35]		
T. cruzi (Y)	55	0.5	>75
T. b. brucei	35		>150
T. b. rhodesiense	47		

3. DEVELOPMENT OF MILTEFOSINE FOR VISCERAL LEISHMANIASIS

3.1. Pilot Study

The clinical development of miltefosine for the leishmaniases began with the 1998 pilot study of Sundar *et al.* sponsored by ASTA Medica AG, a pharmaceutical firm that subsequently gave the miltefosine project to its offspring, Zentaris GmbH. In a dose ranging study with drug administered for 4 weeks, 5 patients were administered 50 mg every other day, 100 mg every other day, 100 mg daily, 150 mg daily, 200 mg daily, or 250 mg daily [13]. All patients exhibited initial parasitologic cure at the end of therapy. 3-to-4 of the 5 patients in each of the two lowest dose groups relapsed by 8 months after therapy. Only 1 patient relapsed in each of the four higher dose groups. However, the 200 mg per day dose was stated to be the maximum tolerated dose due to gastrointestinal side effects in this and especially the 250 mg per day dose. In a later study [17], the 200 mg/day dose was later found to be poorly tolerated.

Thus, regimens of 100 mg and 150 mg per day for 4 weeks were highly effective and well tolerated, whereas lower doses were ineffective and higher doses were poorly tolerated. The data from this pilot study suggests that 100-to-150 mg per day for 4 weeks would be an effective and tolerated regimen. Remarkably, in spite of the fact that the 1998 study utilized only 5 patients per dose group, the data of that study has been confirmed with very little alteration in approximately ~8 further studies with 500 further patients.

3.2. Phase 2 Dose Ranging Study in Adults

The next study to be performed was the first of several collaborative studies between Zentaris and TDR/WHO. Because the pilot study by Sundar *et al.* indicated that doses should be between 100 and 150 mg per day, a dose-ranging study with 4 dosage groups all within that range was devised [18]. The patients, adult by Indian clinical trial criteria ($\geq$ 12 years of age), had mild-moderate disease: a parasitology grade of 2.6 on a 1-to-6 log scale; spleen size of 8.1 cm below the left costal margin on a scale of 0-to-15, a white cell count of 3.1/mm3, and a platelet count of 130, 000/mm3. Because it was thought that adverse gastrointestinal side effects might be greater in the beginning of therapy, the first group received 50 mg per day for the first week and then 100 mg per day for weeks 2-4. Group 2 received 100 mg per day for 4 weeks. Group 3 received 100 mg a day for 1 week followed by 150 mg daily for weeks 2-4, and group 4 received 150 mg a day for 4 weeks. There were 30 patients per group. All patients were initially cured by parasitologic criteria soon after the end of therapy. 2 patients relapsed by the end of follow up 6 months after therapy in groups 1 and 2; 1 patient relapsed in groups 3 and 4. 62% of patients reported vomiting and/or diarrhea soon after dose administration on a mean of 8% of the days of therapy, but no patient discontinued therapy on the basis of gastrointestinal side effects. With these patient numbers, there was no correlation of gastrointestinal side effects with dose-regimen. Liver function tests were occasionally abnormal, creatinine values were rarely abnormal.

Pre-clinical concerns about ophthalmologic toxicity were unsupported in this study: there were no eye abnormalities relatable to drug treatment.

3.3. Phase 3 Pivotal Study in Adults

Although any of the 4 regimens could have been the subject of a phase 3 trial, the regimen of 100 mg/day for 4 weeks (2.5 mg/kg/day for these 40 kg patients) was most attractive on the basis of dose-simplicity and efficacy. The phase 3 trial compared miltefosine, at a targeted dosage of 2.5 mg/kg/day for 4 weeks in ~300 patients, to the standard of care amphotericin B, 1 mg/kg every other day for a total of 15 injections over 4 weeks in ~100 patients [19]. To approximate this target, actual dosing was 50 mg/day for patients up to 25 kg bodyweight, and 100 mg/day for patients >25 mg bodyweight. Again, mild-moderate disease was being treated: the patients had a mean parasite grade of 2.9 and a mean spleen size of 6.9 cm.

At the end of therapy, all patients who submitted to repeat splenic aspiration were parasitologically-negative and demonstrated initial cure. At 6 months follow-up 9 miltefosine patients (3%) and no amphotericin B patients (0%) had relapsed parasitologically. 3% of miltefosine patients were lost prior to 6 month follow up, so the intent-to-treat final cure rate in the miltefosine group was 94% and the per-protocol final cure rate in that group was 97%. Approximately $^{1}/_{4}$ of miltefosine patients had previously failed treatment with pentavalent antimony, and can be regarded as clinically resistant to antimony. The cure rate in the previously-treated group was equal to that in the naïve group.

Miltefosine tolerance in the VL population could be well assessed with the ~300 patients of this trial, with the data from the ~100 amphotericin patients being available to correct for side effects due to the disease itself. Vomiting was seen in 38 % of miltefosine patients (*vs* 20% of amphotericin B patients). In $^{3}/_{4}$ of cases, vomiting lasted 1-to-2 days. All episodes were Common Toxicity Criteria grades 1 and 2 (1 and 2-5 episodes per day, respectively). Diarrhea was seen in 20% of miltefosine patients (*vs* 6% for amphotericin B). Again $^{3}/_{4}$ of episodes lasted 1-2 days, and all but one episode was CTC grades 1 or 2 (an increase of 2-3 stools per day and 4-6 stools per day, respectively).

Liver function tests (ALT and AST) increased somewhat during the 1st week of therapy, before falling during the second week and then decreasing further as the disease resolved. Mean values of renal function tests (BUN and creatinine) did not change significantly, although 1 patient had a sizeable creatinine elevation most likely due to drug administration.

Because of the preclinical concern about male reproductive capacity, in this study, male patients were followed to determine the number of live and healthy births to their sexual partners. In the miltefosine group, there were 48 healthy births to the partners of 80 male patients (0.6 births per patient). In the amphotericin B group, there were 12 healthy births to 20 such partners (0.6 births per patient).

The phase 3 study established the efficacy and tolerability of miltefosine, 2.5 mg/kg/day for 4 weeks under

supervised clinical circumstances, for ≥ 12 year old Indian VL patients with mild-moderate disease including those that were clinically resistant to antimonials therapy.

Clinical issues related to visceral leishmaniasis that remained after the Indian phase 3 study were the efficacy and tolerability of this regimen for patients < 12 years of age, efficacy of courses shorter than 4 weeks, efficaciousness in less-supervised circumstances of general outpatient use, efficacy against more severe disease, efficacy in other endemic regions, and efficacy in the HIV-coinfected population whose immunological response to Leishmania antigens will not return.

3.4. Trials in Children

In a pilot pediatric study [20], 39 children <12 years of age were treated with oral Miltefosine daily for 28 days: 21 patients received 1.5 mg/kg/day and 18 patients received 2.5 mg/kg/day. All patients were parasitologically negative by the end of therapy. 2 patients in each treatment groups relapsed by the end of the 6 month follow-up. The per protocol final cure rate was 19/21 = 90% in the 2.5 mg/kg/day group and 15/17 = 88% in the 1.5 mg/kg/day group.

Because these efficacy rates did not differ, the pivotal childhood trial employed the adult dose, 2.5 mg/kg/day, for 28 days [21]. There were 80 patients of 2-to-11 years of age. One patient died early due to intercurrent pneumonia. The other 79 patients were initially cured. 3 patients relapsed by the end of 6 months of follow up and the final cure was 75 of 79 evaluable patients (95%). As per the adult studies, miltefosine treatment occasioned mild-moderate vomiting and diarrhea each in $^{1}/_{4}$ of patients, and modest elevations of liver function tests early in treatment.

As a result of the phase 3 adult trials and these pediatric trials, miltefosine was registered in India for visceral leishmaniasis for patients ≥ 2 years of age in 2002 [22].

3.5. Shorter and Less Supervised Courses of Therapy

A large phase IV post registration trial of miltefosine, 2.5 mg/kg/day for 4 weeks, is underway in India. The entrance criteria have been expanded to include more severely ill, although not clinically moribund, patients, and treatment is being given in an outpatient setting in which drug administration is not strictly monitored. Both the increased severity of disease and the outpatient setting may lead to decreased effectiveness. A prior report however gives some reassurance that if some drug is not taken, efficacy will still be high. In a small study, when patients were administered 100 mg (~2.5 mg/kg/day) for 2 weeks and 3 weeks, the cure rate was 89% and 100%, respectively [23]. In addition, the long half-life of the product (approximately 1 week) gives some assurance that if a few doses are missed, efficacy will remain high.

3.6. Patients Coinfected with HIV

HIV-coinfected patients have been receiving miltefosine on a named-person basis in Europe. The combined experience has been reported by the company at the end of 2004 [24]. 39 patients of mean weight 59 kg received initial treatment of 100 mg per day for a mean of 55 days. Most of the patients had previously failed other antileishmanial therapies, including amphotericin B. This dose of miltefosine was chosen because it had been well investigated in Indian adults. Of the 25 patients who showed initial cure or improvement, 22 received a 2nd course of therapy lasting a mean of 48 days. Of the 15 who responded to the 2nd course of therapy, 9 patients received a 3rd course and 4 patients received a 4th course. These results suggest that miltefosine provides initial responses in many patients, but in conformity with the relapses that are seen when patients are treated with standard antileishmanial agents, most of the miltefosine patients relapse. Because the dose used for the HIV patients was developed for 40 kg patients in India, it may be possible to increase the dose for European HIV patients and sustain longer initial responses.

4. DEVELOPMENT OF MILTEFOSINE FOR CUTANEOUS LEISHMANIASIS

An initial open-label dose-ranging study in Colombia utilized a range of dosages in approximately 15 patients per group [25]. The two highest dose levels, 133 mg per day for 3 weeks and 150 mg per day for 4 weeks, had per protocol cure rates of 100% and 89% respectively. Because the Colombia patients weighed approximately 60 kg, a dose of 150 mg/day is equivalent to a dose of 100 mg/day in the 40 kg Indian adults that were the subject in the phase 3 VL trial. The dose of 2.5 mg/kg/day for 4 weeks therefore appeared appropriate for leishmaniasis in general.

In the subsequent cutaneous trial [26], 133 patients were randomized between miltefosine (89 patients) and placebo (44 patients) for disease in both Colombia and Guatemala. In essence, two separate studies were performed: one against *L. panamensis* disease in Colombia, one against combined *L. braziliensis* and *L. mexicana* disease in Guatemala. The median number of skin lesions was 1 per patient and the mean size was about 175 mm2.

In Colombia, the per-protocol cure rates were 91% for miltefosine and 38% for placebo (Table **3**). These values were similar to historic values for the antimony standard of care and for placebo, respectively. In Guatemala, the per protocol cure rates were 53% for miltefosine and 21% for placebo (Table **3**).

The disparate efficacy results indicated that miltefosine is a useful oral agent against cutaneous leishmaniasis due to *L. v. panamensis* at least in Colombia, but not against *L. v. braziliensis* at least in Guatemala. The fact that the *P panamensis* data derived from 2 studies, whereas the *L. braziliensis* data derived from 1 study, suggests that efficacy or lack of same against *L. braziliensis* should continued to be investigated in further clinical trials.

Cutaneous leishmaniasis patients suffer only from a skin ulcer and are systemically normal. Because this was the first blinded trial of miltefosine in an essentially "normal population", the trial permitted determination of the inherent clinical tolerance of this drug. Nausea was reported by 27% more miltefosine patients than placebos (Table **3**). Vomiting but not diarrhea was also specifically attributable to miltefosine, and was experienced by 32% of patients *vs* 5% in placebo (Table **3**). Approximately $^{3}/_{4}$ of miltefosine patients who vomited had only 1-2 episodes during the 28 day course of therapy, and no patient stopped therapy for this reason.

Table 2. Salient Clinical Data from Visceral Leishmaniasis Trials

Trial [ref]	Regimen For 28 days	Efficacy Data (PP cure rate 6-8 Months after RX)	Tolerance Data
Pilot [13]	50 mg qod	2/5 = 40%	
	100 mg qod	1/5 = 20%	
	100 mg/d	5/5 = 100%	
	150 mg/d	4/5 = 80%	
	200 mg/d	5/5 = 100%	Maximum tolerated dose
	250 mg/d	4/5 = 80%	Not well tolerated
Phase 2 [18]	50 mg/d (x 6 wks)	28/30 = 93%	
	50 mg/d x 1 wk, then 100 mg/d x 3 wk	28/30 = 93%	
	100 mg/d x 4 wk	29/30 = 97%	
	100 mg/d x 1 wk, then 150 mg/d x 3 wk	29/30 = 97%	
Phase 3 [19]	100 mg/d	282/291=97%	Vomit: 38% of patients Diarrhea: 20% of patients Rigors: 1% of patients AST: 17% rise in wk 1 ALT: 1% rise in wk 1 Creatinine: -1% rise in wk 1 Healthy births: 48/60
	Amphotericin B	96/96 =100%	Vomit: 20% of patients Diarrhea: 6% of patients Rigors: 90% of patients AST: 1% rise in wk 1 ALT: 18% rise in wk 1 Creatinine: 35% rise in wk 1 Healthy births: 12/20
Pediatric [21]	2.5 mg/kg	75/79 = 95%	Vomit: 26% of patients Diarrhea: 25% of patients AST: 19% rise in wk 1

Creatinine was more frequently elevated in the miltefosine group compared to the placebo group, but almost all creatinine elevations were mild. AST and ALT were not more frequently elevated in the miltefosine group compared to controls. The mild changes in laboratory parameters suggests that in the cutaneous leishmaniasis population, in contrast to visceral leishmaniasis patients who have systemic disease, routine recording of laboratory parameters need not be performed.

5. MECHANISMS OF ACTION

Because miltefosine is active against tumor cells and Trypanosomids as well as Leishmania, the putative mechanisms of action against Leishmania derive from studies against these other cell types as well as against Leishmania itself.

Phosphatidylcholine (PC) represents 50% of phospholipids in Leishmania [27]. Phosphatidylcholine can be synthesized from choline (Kennedy pathway). After choline is transported into the cell, it is phosphorylated by choline kinase before being transferred onto CDP by CTP phosphocholine cytidylyltransferase [28]. CDP-choline reacts with 1, 2 diacylglycerol *via* diacylglycerol cholinephosphotransferase to yield phosphatidylcholine. [28]. In HepG2 tumor cells, miltefosine did not inhibit either choline kinase nor diacyglycerol cholinephosphotransferase, but did inhibit CTP phosphocholine cytidylyltransferase [28].

PC can also be synthesized from phosphatidylethanolamine (Greenberg pathway). Phosphatidylethanolamine itself can be synthesized *via* decarboxylation of phosphatidylserine. Phosphatidylethanolamine is then methylated *via* phosphatidylethanolamine methyltransferases to yield PC.

Zufferey and Mamoon studied choline transport in *L. major* promastigotes [27]. Transport was carrier mediated with a Vmax of 5-10 uM which corresponds well to physiologic choline concentrations of 10-40 uM. Transport of 10 uM choline was approximately 90% inhibited with 10 uM miltefosine. However, the parasites grew well in media

Table 3. Salient Clinical Data from Cutaneous Leishmaniasis Trials

Trial	Regimen For 28 days	Efficacy Data (PP cure rate 6-8 Months after RX)	Tolerance Data for both sites
Cutaneous In New World [26]	2.5 mg/kg	Colombia: 40/44 = 91%	Vomit: 32% of patients
		Guatemala: 20/38 =53%	Nausea: 36% of patients
			Diarrhea: 6% of patients
			AST: 8% of patients
			ALT: 10% of patients
			Creat: 33% of patients
	Placebo	Colombia: 9/24 = 38%	Vomit: 5% of patients
		Guatemala:4/19 = 21%	Nausea: 9% of patients
			Diarrhea:2% of patients
			AST: 18% of patients
			ALT: 11% of patients
			Creat: 9% of patients

lacking choline and ethanolamine, which indicates that *L. donovani* can synthesize PC *via* the CDP-DAG-serine pathway.

Miltefosine induces apoptosis in tumor cell lines [29] and also in *Leishmania donovani* promastigotes [30]. At least in the tumor cells, inhibition of PC synthesis was not linked to this physiologic endpoint [29].

Urbina and colleagues have investigated the efficacy and mechanism of miltefosine against *T. cruzi* [31]. The IC50 for miltefosine was 1 uM against axenic epimastigotes and intracellular amastigotes within Vero cells were 24% reduced by 0.1 uM of drug. In epimastigotes, phosphatidylcholine decreased and phosphatidyserine / phosphatidylethanolamine increased at effective drug concentrations. Coupled with the finding that radioactivity was incorporated from labeled methionine but not from labeled choline, it was suggested that in these organisms, PC is synthesized from phosphatidylserine-phosphatidylethanolamine rather than from CDP-choline and that this pathway is inhibited by miltefosine.

The effect of miltefosine on ether-lipid biosynthesis and remoldeling was studied by Lux *et al.* [32]. The initiating steps in ether-lipid metabolism catalyzed by dihydroxyacetonephosphate acytransferase, and acyl and alkyl lyso-glycerol-3-phosphocholine transferases, were not inhibited by the drug. Although a remolding enzyme (alkyl-specific-acyl CoA acyltransferase) was inhibited in a dose dependant manner, the inhibitory constant was 50 uM which may be too high for physiologic meaning.

Leishmania donovani have been made 15-fold resistant to miltefosine by *in vitro* drug pressure. Gene amplification was not seen in the resistant parasites [33]. Drug influx was drastically diminished, whereas binding of drug to the plasma membrane and drug efflux from preloaded cells was not inhibited [34]. These results suggested that inward translocation of short chain phospholipids occurs in *Leishmania* promastigotes and is deficient in the miltefosine-resistant strain. However, *in vitro* drug pressure may not mimic clinical drug pressure, where resistance is unlikely to be to concentrations that differ by more than 2-fold.

6. SUMMARY

Basic science discoveries in the 1960s through the 1980s led to the development of miltefosine as an anticancer agent. Recognition that Leishmania might have biochemical similarities to cancer cells with respect to ether-phospholipid metabolism led to *in vitro* and then *in vivo* (mouse) determination of efficacy of miltefosine against Leishmania. The leap from early preclinical efficacy evaluation in the early 1990s to clinical phase 2 evaluation in the middle 1990s occurred with remarkable speed. The fundamental reason was that the animal toxicology and clinical phase 1 data was already available from the anticancer investigations, and therefore did not have to be generated for the indication of leishmaniasis. This is particularly fortunate given the orphan-drug nature of leishmaniasis in the developed world, whose medical requirements typically direct funding decisions.

The small pilot study in visceral leishmaniasis indicated that doses of 100-150 mg/day (2.5 to 4 mg/kg per day) were highly effective and also reasonably tolerated for Indian visceral leishmaniasis. It is remarkable that those observations have not been altered in spite of >500 other patients being entered over the next 5 years in a variety of trials.

Miltefosine has been registered as of 2002 for visceral leishmaniasis in immunocompetent patients of 2 years or greater in India, and as of Nov 2004 for visceral leishmaniasis patients in Germany. The German approval includes immunocompromised patients, and permits a high dosage for patients of high weight: 150 mg/day for patients >67 kg.

Oral miltefosine may be compared to other agents for visceral leishmaniasis with respect to efficacy, tolerance, and feasibility/cost of administration. Standard therapy for visceral leishmaniasis is pentavalent antimony, or in antimony-resistant regions such as India, amphotericin B. Antimonials have the disadvantages of at least 40% clinical resistance in certain regions of India of long-time use, and moderate toxicity is common. Common adverse effects are myalgia, arthralgia, anorexia, hyperamylasemia, and rises in liver function enzymes. Uncommon adverse effects are

significant declines in leucocytes and platelets, and death due to arrhythmia. Amphotericin B is generally 100% effective, but has well-known adverse effects of fever/chills and elevations of kidney function tests. Liposomal amphotericin B is highly effective and tolerated, but its cost precludes widespread use even in developed countries.

With a >95% cure rate in India, the efficacy of miltefosine compares well with all other agents, including amphotericin B and AmBisome, in this endemic region. The side effects of miltefosine are generally tolerable, and compare favorably to all agents other than AmBisome. The striking advantage of miltefosine is that it is administered orally.

Outstanding clinical problems with respect to visceral disease are gastrointestinal side reactions which might limit drug administration in outpatient settings, efficacy in visceral leishmaniasis regions in which *L. donovani* is not present, and efficacy of the 150 mg/day top dose in >> 60 kg patients, since this would result in a daily dose <<2.5 mg/kg.

A separate issue is the utility of miltefosine for the myriad of cutaneous syndromes. *In vitro* data, and the disparate efficacy against *L. panamensis* and *L. braziliensis*, suggest a range of susceptibility of Leishmania to miltefosine, which may convey to the clinic. It is ironic that although miltefosine was rejected as a systemic treatment for cancer partially because of side effects, careful clinical experimentation now indicates that for one Leishmania indication—cutaneous disease—very few safety precautions need to be taken. In this systemically normal population, clinically significant laboratory abnormalities have not so far been seen. In essence, the monitoring of cutaneous leishmaniasis patients can rely on clinical data. If the lesions respond clinically and the patient does not have vomiting, the treatment was a success.

Because the development of miltefosine for cancer and then Leishmaniasis was first driven by basic science findings, some interest in miltefosine mechanisms has been present from the beginning. Nevertheless, the speed with which miltefosine progressed from *in vitro* efficacy studies to advanced clinical studies has meant that the generality of basic science investigations have succeeded rather than preceded clinical investigation and registration. It must be said that our understanding of miltefosine mechanisms is modest. Leishmania and related Trypanosomids apparently synthesize phosphatidylcholine from phosphatidylserine-phosphatidylethanolamine rather than from choline. Miltefosine may inhibit conversion of PE to PC, and also inward translocation of short chain phospholipids.

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