Repurposing Miltefosine for the Treatment of Immune-Mediated Disease?

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ABSTRACT

Miltefosine is an ether lipid that was initially developed for cancer treatment in the early 1980s. Miltefosine largely failed development for oncology, although it was approved for the topical treatment of breast cancer metastasis. It was subsequently discovered that miltefosine is a highly effective treatment of visceral Leishmaniasis, a parasitic disease that affects millions worldwide and causes an estimated 30,000 fatalities each year. Oral treatment with miltefosine is generally well tolerated and has relatively few adverse effects. The exact mechanism of action of miltefosine treatment is still under investigation. Its close resemblance to phospholipids allows it to be quickly taken up by cell membranes and affect related processes, such as lipid metabolism and signaling through lipid rafts. These processes play an important role in the immune response and it comes as no surprise that miltefosine has been successfully tested for the treatment of a number of immune-mediated diseases in preclinical models of disease. Drug repurposing of miltefosine for immune-mediated diseases may provide an opportunity to expand the limited number of drugs that are currently available for therapeutic use.

Introduction

Miltefosine, or hexadecylphosphocholine, was developed in the early 1980s as a potential cytostatic drug. Miltefosine largely failed development for oncology and is now best known as an oral treatment of visceral Leishmaniasis, a lethal disease that results from infection with the Leishmania parasite. Discovery of miltefosine’s potential as an antiparasitic drug in recent years has renewed scientific interest in the drug and its mechanism of action. Development of new drugs is a costly and time-consuming process with a high risk of failure at multiple steps along the road of preclinical and clinical development. This makes repurposing of existing drugs with established side effects for novel indications an attractive alternative to classic drug development. Miltefosine has been extensively investigated in large clinical trials and appears to be relatively safe with limited and reversible side effects. This review focuses on the potential to repurpose miltefosine as an anti-inflammatory drug for immune-mediated diseases. To note, this review does not cover perifosine, an alkyl-phospholipid developed to improve oral tolerability. Assuming that the mechanism of action of perifosine is similar to that of miltefosine, perifosine could be an alternative candidate for repurposing. However, the number of studies that address perifosine for applications other than cancer treatment is very limited.

Miltefosine (hexadecylphosphocholine) is an alkyl-phospholipid or ether lipid with structural resemblance to lysophosphatidylcholine (LPC), a type of phospholipid that is present in the cell membrane and comprises approximately 3% of the cellular membrane. LPCs have a very short half-life because they are quickly metabolized by phospholipases and acyltransferases. An acyl group is replaced by an alkyl group in the LPC analogs miltefosine, perifosine, and edelfosine (Fig. 1), which makes these compounds metabolically stable (Eibl and Unger, 1990). The first stable LPC analog was synthesized with an interest in enhancing macrophage-mediated antitumor immunity (Munder et al., 1976) because LPCs were found to increase the phagocytic capacity of peritoneal macrophages.

ABBREVIATIONS: Akt, protein kinase B; IBD, inflammatory bowel disease; IL, interleukin; LPC, lysophosphatidylcholine; NO, nitric oxide; PC, phosphatidylcholine; Th, T helper; TNF-α, tumor necrosis factor-α.
Burdzy et al., 1964). It was subsequently discovered that some of these alkyl-phospholipids possessed direct cytostatic properties, shifting the interest away from their immunomodulatory properties. Because the ability to inhibit cell growth appeared to be related to structural properties, researchers attempted to create the most minimal structure required. This resulted in a new group of molecules (alkylphosphocholines) and the discovery of miltefosine and edelfosine (Munder et al., 1979).

Screening of miltefosine in cancer cell lines suggested that miltefosine possesses potent antineoplastic properties. Miltefosine inhibited cell growth in various leukemic cell lines (Unger et al., 1989) and reduced tumor weight and size in dimethylbenzanthracene-induced mammary carcinomas and transplanted mammary tumors in rats in vivo (Scherf et al., 1987). However, miltefosine appeared to only be effective against some cell types, because no effect was observed in a variety of other cancer cell lines, such as rat DS-carinosarcoma, AH 13s sarcoma and L5222 leukemia cells, mouse L1210 and P388 lymphocytic leukemia cells, Lewis lung carcinoma cells, and B16 melanoma cells. The reason for this differential sensitivity of cancer cell lines to miltefosine has not been established.

**Development of Miltefosine for Oncology**

Because intravenous administration of miltefosine is hemolytic, clinical applications have been limited to topical and oral treatments. A phase II dose-finding study in cancer patients confirmed previous phase I data showing that the major dose-limiting toxicity effects were nausea and vomiting and suggested a maximum dose of 150 mg/d split into three doses (Verweij et al., 1992). Oral treatment was subsequently tested in a number of oncological trials, including a phase II study for treatment of soft tissue sarcomas (Verweij et al., 1993b), a phase II study in advanced nonsmall cell lung cancer (Berdel et al., 1992), a phase II study in advanced colorectal cancer (Planting et al., 1993), a phase II study in advanced breast cancer (Unger et al., 1993), and a phase II study for squamous cell head and neck cancer (Verweij et al., 1993a).
Leishmaniasis

Leishmaniasis is a disease caused by infection of macrophages by a protozoan parasite belonging to the genus *Leishmania*. The parasites are transferred by a type of sandfly that only lives in (sub)tropical regions, restricting the disease to those areas. The infection can lead to cutaneous, mucocutaneous, or visceral disease (also called kala-azar). Visceral Leishmaniasis is characterized by bouts of fever, weight loss, hepatosplenomegaly, and anemia, and is lethal if left untreated (Chappuis et al., 2007). Unfortunately, the incidence efficacy was observed in the latter trial, with 2 of 24 patients achieving a complete response of the skin lesions (0 of 27 for placebo) and 6 of 24 showing a partial response (1 of 27 for placebo).

ImmunoModulatory Effects of Miltefosine

The development of alkyl-phospholipids initially started with the aim to create an immunomodulator that would increase antitumor phagocytic capacity of tumor-associated macrophages. Miltefosine exhibits immunomodulatory properties that are similar to the originally developed compounds.

Stimulatory Effects on the Innate Immune Response

There are a number of reports suggesting that miltefosine may stimulate multiple aspects of monocyte- and macrophage-mediated immunity. Such effects may explain the potent clearance of *Leishmania* parasites that have their niche inside macrophages. For example, Balb/c mouse monocytes and macrophages treated with miltefosine showed a more potent response to LPS, with enhanced secretion of tumor necrosis factor-α (TNF-α) and release of nitric oxide (NO) (Eue et al., 1995; Zeisig et al., 1995; Ghosh et al., 2013). In a similar experiment with macrophages isolated from C57BL/6 mice, there was no increase in NO release, but miltefosine did increase their phagocytic capacity. Not only did sensitized macrophages phagocytize more *Saccharomyces cerevisiae* after miltefosine treatment, but miltefosine also increased the number of macrophages that participated in the process (Ponte et al., 2012).

One report investigated the effect of miltefosine on circulating monocytes and cytokine levels in patients suffering from cutaneous Leishmaniasis (Mukhopadhyay et al., 2011). The percentage of CD14+ monocytes decreased in 12 patients that completed 4 months of miltefosine treatment (100 mg/d), whereas CD16+ monocytes increased. This shift indicated that miltefosine treatment may have triggered maturation of the monocyte population toward a more proinflammatory phenotype. Levels of monocyte-associated proinflammatory cytokines, such as TNF-α, interleukin (IL)-6, IL-1β, and IL-8, were significantly increased compared with these levels at the time of disease presentation. Of course, it cannot be excluded in this study that some of these effects were indirect and a systemic response to the eradication of the *Leishmania* parasites.

Tissue biopsies were taken from the lesional area in a case study of one patient suffering from cutaneous Leishmaniasis treated with miltefosine. Quantitative analysis of mRNA expression showed a significant decrease of TNF-α, IL-10, and transforming growth factor-β levels, whereas comparisons before and after treatment showed that interferon-γ and CD40 were significantly increased (Ansari et al., 2008). CD40 is a costimulatory molecule found on antigen-presenting cells that helps to stimulate T helper (Th) cells, which, in turn, produce interferon-γ-stimulating macrophages. Again, this case study comprised a single patient; therefore, it is difficult to judge whether the changes in expression are the direct result of the miltefosine treatment or alternatively result from the eradication of parasite-laden macrophages.

The initial development of alkyl-phospholipids as compounds that stimulate macrophage phagocytosis, the potent parasite clearance by Leishmania-infected macrophages together with some of the experiments above suggest that treatment with miltefosine potentiates monocyte and macrophage-mediated innate immunity.
Inhibitory Effects on the Adaptive Immune Response

Because miltefosine potently inhibits T-cell activation, miltefosine and other alkyl-phospholipids may have a potential role in immune-mediated diseases (Bäumer et al., 2010; Verhaar et al., 2013). This effect was verified in animal models in which inflammation was successfully reduced using miltefosine. We investigated the effect of miltefosine in a CD4CD45RB\textsuperscript{high} transfer mouse model of inflammatory bowel disease (IBD). In this model, SCID mice, which lack T and B cells, are transplanted with CD45RB\textsuperscript{high} T cells resulting in chronic inflammation of the gut that develops over the course of several weeks. Miltefosine, given twice weekly, greatly reduced colonic inflammation in this model. Miltefosine improved clinical parameters such as weight loss, significantly improved the pathology score, and reduced the expression of proinflammatory cytokines il-6, tnf-\alpha, and ifn-\gamma to levels that were no longer significantly different from control mice without colitis (Verhaar et al., 2013). These findings may make miltefosine an attractive candidate for the treatment of IBD. Although the cause of IBD remains unclear, there are many indications that suggest that a reduced function of the innate immune system predisposes to an excessive response of the adaptive immune response to intestinal microbiota (Marks et al., 2006; Hayee et al., 2010; Uhlig, 2013). Given the results discussed above, miltefosine may have a dual therapeutic effect in IBD by both stimulating macrophage–mediated innate immunity and reducing excessive activation of T cell–mediated adaptive immunity.

Bäumer et al. (2010) investigated miltefosine treatment in a number of animal models, addressing its effect on Th1- or Th2-related inflammatory responses and feasibility for use in atopic dermatitis. The models used ear thickness as a measure of inflammation. In an ovalbumin-induced delayed-type hypersensitivity model, miltefosine was significantly reduced ear swelling. Likewise, in the more Th2-orientated mouse model of toluene diisocyanate–induced hypersensitivity, treatment with miltefosine significantly reduced ear swelling and proinflammatory cytokine expression, both when administered systemically as well as topically (Bäumer et al., 2010).

Topical application of a 6% miltefosine solution was compared with 1% hydrocortisone in a small clinical trial of 16 patients (Dölle et al., 2010). Patients with at least two different target lesions were treated topically with one drug on each lesion for 3 weeks. Both treatments effectively reduced the severity of the disease and reduced the number of infiltrating lymphocytes in the lesions. Patient follow-up revealed a more sustained effect of miltefosine treatment. Interestingly, a closer examination revealed a local increase in the number of FoxP3\textsuperscript{+} regulatory T cells in the miltefosine-treated lesions, whereas hydrocortisone reduced the number of FoxP3\textsuperscript{+} cells. An important potential advantage of miltefosine over hydrocortisone was that, in contrast with hydrocortisone, miltefosine did not affect the proliferation of cells in the basal layer of the epidermis. Indeed, in contrast with hydrocortisone, no reduction in epidermal thickness was observed with miltefosine.

Miltefosine and Allergic Disease

A potential role for miltefosine in allergic disease was suggested when it was shown that miltefosine can reduce histamine release from rat primary mast cells (Grosman 1990). Mast cells contain large granules with inflammatory mediators such as cytokines, proteases, histamine, serotonin, and eicosanoids. Mast cells release their granules in response to activation by pattern recognition receptors or cross-linking of the IgE receptor. Degranulation causes redness of the skin as well as swelling and itching.

Miltefosine was also shown to inhibit histamine release in human mast cells (Weller et al., 2009; Batista et al., 2010, 2011). Ten minutes of pretreatment with 25 \(\mu\)M miltefosine reduced anti-IgE–induced histamine release by more than 50%. These findings were translated into a small study in which the effect of miltefosine was tested on the allergic response to a standard skin prick test. Five allergic patients were treated on both arms with either placebo or a 6% miltefosine solution. The inflammatory response was measured by the diameter of the resulting weal and erythema. Miltefosine was able to significantly reduce the inflammatory response that resulted from injection with an allergen. A control condition in which the patients were injected with histamine showed no effect (Weller et al., 2009).

Miltefosine was also tested in a number of other mast cell–related conditions. One such condition, mastocytosis, involves the accumulation of mast cells in various organs, most commonly in the skin. This rare condition affects mostly children and skin manifestations cause symptoms related to mast cell mediator release such as pruritus and flushing. A topical 6% miltefosine solution and the glucocorticoid clofetosol (0.5 mg/ml) were tested in a double-blind, placebo-controlled trial with 39 adult patients for 2 weeks. Although it seems that miltefosine may have reduced weal formation in this study, none of the differences reached statistical significance and it seems the study was underpowered to detect a meaningful difference (Hartmann et al., 2010).

Chronic urticaria is a type of idiopathic rash that is relatively common, affecting up to 1% of the population in the United States. In most cases, the underlying cause is unclear but the rash is the result of activated mast cells. Urticaria can be treated with antihistamines; however, most patients with chronic urticaria respond poorly and are often resistant. Miltefosine was tested as an oral drug in a multicenter, randomized, double-blind, placebo-controlled study that included 54 patients with antihistamine-resistant chronic urticaria (Magerl et al., 2013). Patients took increasing dosages of miltefosine (up to 150 mg/d unless intolerable adverse effects) daily for 4 weeks. At the end of the treatment period, miltefosine-treated patients showed substantial improvement, as was clear from a specific urticaria activity score and a decrease in the number of wheals. Miltefosine treatment did not reduce the intensity of pruritus.

Collectively, these data suggest that miltefosine could be a potential drug for mast cell–related conditions.

Side Effects

Miltefosine has few side effects; however, the existing effects may hamper long-term use. From the phase II trials for the oral use of miltefosine with cancer patients, it has become clear that daily dosages of 150 mg and higher cause potentially severe gastrointestinal side effects resulting in loss of appetite, nausea, and vomiting (Verweij et al., 1992). This can be solved by combining miltefosine treatment with
antiemetics; however, the side effects are dose limiting in some cases. Development of a better-tolerated formulation for oncological indications was suspended due to the discovery of perifosine, a better-tolerated oral anticancer drug (Crul et al., 2002).

Miltefosine treatment may have teratogenic effects. Studies on embryonic development and organogenesis of rats suggested embryotoxic, fetotoxic, and teratogenic risks (Sindermann and Engel, 2006). The same was reported from studies in rabbits, although with the exception of the teratogenic effect. Due to these findings, and because of a lack of human study results, use of miltefosine during pregnancy is contraindicated. This is especially a problem due to its long half-life. Six months after treatment, miltefosine can still be measured in serum and it is therefore advisable to use contraception for 6 months after stopping treatment.

In some patients, the renal function is affected and a rise in creatinine levels is observed. In most cases, this effect is reversible. Studies in dogs showed no lasting effect of miltefosine on the kidney (Bianciardi et al., 2009). Miltefosine might have a direct effect on renal function, but a change in fluid balance due to gastrointestinal effects might contribute. Patients with Leishmaniasis who are treated with miltefosine sometimes show elevated serum creatinine levels during episodes of vomiting and dehydration (Sindermann and Engel, 2006), which supports this hypothesis.

In summary, a treatment schedule of 50–100 mg/d miltefosine is generally well tolerated and rarely leads to serious or treatment-limiting adverse effects. Studies on the miltefosine treatment of visceral Leishmaniasis clearly indicate that such a treatment schedule can be maintained for at least 4 weeks. These data indicate that the potential of miltefosine as a maintenance treatment of IBD could be investigated.

**Mechanism of Action**

The precise mechanism of action of miltefosine is lacking and may be different for different cell types. Because miltefosine was originally selected as a cytostatic drug, most of the current research is focused on explaining how miltefosine induces apoptosis in cancer cells. Although the effect of miltefosine on macrophages, lymphocytes, and mast cells appears to be different, it may involve similar pathways.

Miltefosine shows a resemblance to the membrane phospholipid lysophosphatidylcholine. Its structural properties allow miltefosine to integrate into the cellular membrane (Rakotomanga et al., 2004). From there, it redistributes to the endoplasmic reticulum as well as the Golgi and nuclear and mitochondrial membranes. How this happens is unclear; however, the process appears to be partly ATP-dependent in Caco-2 colorectal cancer cells, suggesting active transport. The normal membrane recycling process may be responsible for the remainder of the intracellular transport of the drug (Ménez et al., 2007). Because alkyl-phospholipids, such as miltefosine, are more resistant to metabolizing enzymes, they accumulate in the membranes and possibly affect the continuous turnover of endogenous phospholipids.

Inhibition of phosphatidylcholine (PC) synthesis is believed to be one of the most important effects of miltefosine incorporation into the cell membrane. PC is the most abundant phospholipid in cellular membranes of eukaryotic cells and interference with its production may predispose the cell to undergo apoptosis (Wieder et al., 1998; van der Luit et al., 2002). Miltefosine affects PC synthesis by inhibiting CTP:phosphocholine cytidylyltransferase, the rate-limiting enzyme in the PC biosynthesis pathway at the endoplasmic reticulum. This is supported by the fact that apoptosis can be prevented by supplementing miltefosine-treated cells with exogenous lysoPC, an alternative precursor for the synthesis of PC. Because apoptosis induced by other triggers could not be rescued by adding lysoPC, this is a strong suggestion that inhibition of PC synthesis, indeed, is the mechanism of miltefosine-induced apoptosis (Boggs et al., 1995; Baburina and Jackowski, 1998; van der Luit et al., 2003).

In addition to inhibition of PC synthesis, miltefosine also impairs signaling molecules through inhibition of phospholipases. Phospholipases hydrolyze phospholipids and inhibition results in a decrease in breakdown products, including the important second messengers, diacylglycerol and phosphatidic acid. Further downstream, alkyl-phospholipids may impact proliferation and cell survival through effects on protein kinase B (Akt) (Song et al., 2005). Under normal conditions, Akt can be activated once it is recruited and positioned at the plasma membrane. Although this has not been described for miltefosine, studies on perifosine suggest that treatment affects the recruitment of Akt to the plasma membrane and displacements of its ligands phosphatidylinositol-(3,4,5)-trisphosphate or phosphatidylinositol-(3,4)-bisphosphate.

All of these effects could be caused by the disturbance of membrane integrity and the functionality of lipid rafts (Kondapaka et al., 2003). Miltefosine increases the membrane fluidity in macrophages (Ghosh et al., 2013), an effect that was reported to decrease antigen presentation and stimulation of T cells (Chakraborty et al., 2005). On the other hand, miltefosine has an affinity for sterols and is known to stabilize sterol-rich areas, such as lipid rafts, which increase antigen presentation (Jiménez-López et al., 2010). Indeed, macrophages treated with miltefosine show an enhanced ability to stimulate T cells, resulting in higher production of IL-2, IL-12, TNF-α, and NO (Ghosh et al., 2013). The effect of miltefosine on membrane integrity could also explain why mast cells are inhibited in their IgE-dependent histamine release. Upon activation, IgE receptors cluster in organized microdomains (Weller et al., 2009; Silveira E Souza et al., 2011). Likewise, T-cell activation is dependent on the formation and organization of T-cell receptor microclusters and formation of the immunologic synapse (Kabouridis and Jury, 2008). Signaling molecules that are implicated as targets for miltefosine treatment in cancer (e.g., Akt, Ras/Raf-1, and PLC) and second messengers (e.g., phosphatidic acid and diacylglycerol) are also important mediators in cells of the immune system. For example, inhibition of even one of these molecules could explain the inhibition of lymphocyte proliferation. Of course, future investigations are needed to shed more light on this.

**Conclusions**

Miltefosine is an alkyl-phospholipid that was initially tried unsuccessfully in several phase II trials in oncology and hematology. This class of compounds was originally identified for its stimulatory effect on macrophage phagocytosis and several groups have observed that miltefosine may stimulate myeloid cell–mediated immunity. At the same time, miltefosine
shows profound inhibition of T-cell activation and miltefosine was successfully applied in preclinical models of atopic dermatitis and IBD. Given the well established and relatively limited side effects and oral route of administration, miltefosine may be a candidate for drug repurposing for immune-mediated diseases that are in dire need for novel therapeutic options such as IBD.

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References


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