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Miltefosine — discovery of the antileishmanial activity of phospholipid derivatives

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Summary Miltefosine (hexadecylphosphocholine, Impavido™), a novel antiprotozoal drug used for the treatment of visceral and cutaneous leishmaniasis, was identified and evaluated independently in the early 1980s as a potential anticancer drug in Germany and as an antileishmanial drug in the UK. Although miltefosine is not the most active compound of its class against *Leishmania* parasites in vitro, the early demonstration of activity after oral administration in experimental models of visceral leishmaniasis helped to bring this compound to the attention of WHO TDR for further development in a unique collaboration model with the pharmaceutical industry (Zentaris GmbH). Miltefosine is active against most *Leishmania* species, including those that cause cutaneous disease.

1. Introduction

Miltefosine (hexadecylphosphocholine) is the first oral drug to be registered for the treatment of visceral leishmaniasis (VL) (Ganguly, 2002) and more recently for the cutaneous form of the disease. Although several oral drugs have been undergoing clinical trials for the treatment of VL, for example, allopurinol and sitamaquine (Croft and Yardley, 2002), no other has been successfully registered for this indication. The development of phospholipid derivatives as drugs, essentially based upon their antitumour activities, involves two closely related groups of compounds: the alkylglycerophosphocholines (AGPs), also termed ether-lipids, and the alkylphosphocholines (APCs) (Brachwitz and Vollgraf, 1995). The identification of miltefosine and other phospholipid derivatives as potential antileishmanials came in the late 1980s as a result of three independent lines of research.

The first pathway to miltefosine followed observations on the anticancer activity of the AGP rac-1-O-octadecyl-2-O-methyl-glycero-3-phosphocholine, also known as ET-18-OCH_3 and later called edelfosine, and a subsequent programme of medicinal chemistry. The second followed studies on the phospholipid metabolism of *Leishmania* promastigotes, which led to the identification of several AGPs, including ET-18-OCH_3, that were cytotoxic to promastigotes. The third pathway, which actually identified hexadecylphosphocholine (miltefosine) as a lead antileishmanial compound, came through a pharmaceutical company screening programme. These routes are described in the next sections of this review. A final section covers comparative studies on miltefosine against species of *Leishmania* and a consideration of other phospholipid drugs.

2. Discovery of miltefosine — the anticancer drug

The group of Westphal and Munder (Westphal, 1987) first described the in vitro and in vivo anticancer activity of

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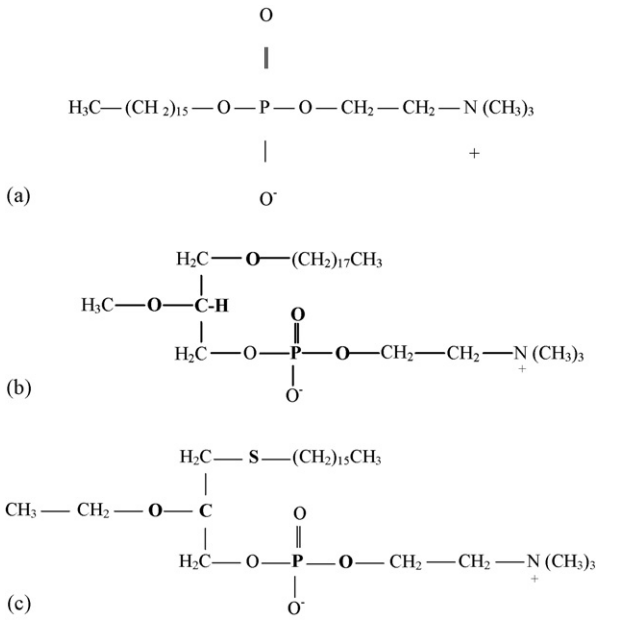

Figure 1 Phospholipid drugs with antileishmanial activity: (a) miltefosine, (b) edelfosine, (c) ilmofosine.

the new substance class, alkyl lysophospholipids (ALPs), which are structurally related to platelet aggregation factor (PAF) (Demopoulos et al., 1979). Edelfosine (Figure 1) (Munder et al., 1977, 1981) — the most prominent compound out of this class — was tested in clinical studies in cancer patients but did not reach the market for various reasons including but not limited to reduced oral bioavailability, lack of demonstration of clear anticancer activity and its weak PAF agonistic potency. Ilmofosine (Figure 1), another candidate from this class of alkyl lysophospholipids, was also tested clinically in cancer patients (Giantonio et al., 2004).

It was Hansjoerg Eibl from the Max-Planck-Institute for Biophysical Chemistry in Goettingen who suggested replacing the glycerol backbone with a simple alkyl chain; this retained the antitumour activity and led to the discovery of miltefosine (hexadecylphosphocholine, D-18506) (Figure 1) as a new type of anticancer agent (Eibl and Unger, 1990). In molecular modelling studies and calculations of isopotential surfaces, we were able to demonstrate that alkylphosphocholines no longer retained PAF agonistic activities. Figure 2 shows the putative binding of PAF-acether, edelfosine, ilmofosine and miltefosine to the PAF-receptor model proposed by Godfroid and co-workers (Braquet and Godfroid, 1986; Dive et al., 1989; Godfroid and Braquet, 1986; Godfroid et al., 1991; Lamotte-Brasseur et al., 1991). According to the model there are two attractive potential areas of the receptor (red cylinders) with which the negative electrostatic potential surfaces of the ligands may interact. In contrast to the other molecules, miltefosine does not have a side chain at position 2 of the glyceryl backbone, and since it has only one negative electrostatic potential surface around the phosphate group, it can interact with only one of the attractive potential areas of the receptor. Furthermore, miltefosine does not interact with the putative hydrophobic pocket (green cylinder) inside the receptor.

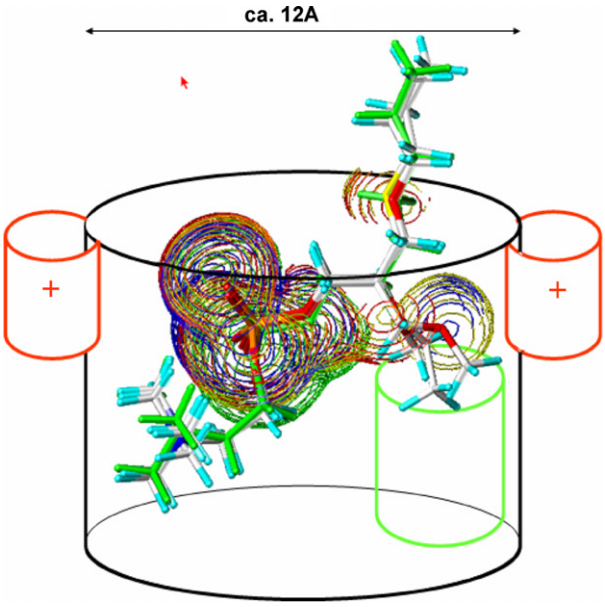

Figure 2 Putative binding of PAF-acether, edelfosine, ilmofosine and miltefosine into the PDF-receptor model proposed by Godfroid and co-workers (see references in text). Miltefosine is coloured green to distinguish it from the other molecules which are coloured by atom type. The electrostatic isopotential surfaces for PAF (yellow) edelfosine (red) ilmofosine (blue) and miltefosine (green) are shown at −30 kcal/mol. The red cylinders indicate areas of the receptor with which negative electrostatic potential surfaces of the ligands may interact; the green cylinder indicates a putative hydrophobic pocket.

The antitumour activity of miltefosine was proven in a variety of in vitro and in vivo models including authochtonous tumour models, such as the nitrosomethylurea and dimethylbenzanthracene-induced mammary carcinoma of the rat, by various groups (Hilgard et al., 1988; Unger et al., 1989). Miltefosine was selected for clinical development for oral use against solid tumours and for the topical treatment of cutaneous metastases in breast cancer patients. After successful clinical development the topical treatment was approved as Miltex® in several countries in Europe (Burk et al., 1994).

In phase II studies of oral miltefosine dose-limiting gastrointestinal side effects were described. Because of rather low plasma levels — not in the expected range of in vitro antitumour activity — development for this indication was abandoned. Through structure—activity relationship analysis, perifosine was identified with an enhanced gastrointestinal tolerability and increased anticancer activity (Hilgard et al., 1997). This new alkylphosphocholine is now in several phase II studies in Europe and the USA as the first oral protein kinase B inhibitor in clinical development.

3. Basis for activity of alkyl-glycerophosphocholines

In the early 1980s, a group in the Department of Biochemistry, University of Hamburg, Germany, began to study ether lipid biosynthesis in *L. donovani* promastigotes. Initial studies (Herrmann and Gercken, 1982) showed that