**INTRODUCTION**

Leishmaniases are tropical and sub-tropical diseases caused by the parasite protist belonging to the genus *Leishmania*. Two basic forms of leishmaniases occurs: i) visceral leishmaniasis (VL) or “Kala-azar” is caused by *Leishmania donovani* and *Leishmania infantum* (also known *L. chagasi* in South America), and ii) cutaneous leishmaniasis (CL) is caused by about 15 species of *Leishmania*, *L. tropica* (recidivan leishmaniasis) in the old world, and two possible forms in Latin America, diffuse CL (*L. guyanensis, L. amazonensis*) and mucocutaneous form with destruction of mouth mucosa, pharynx and facial tissue (*L. braziliensis*) (WHO, 2010). VL, the most severe form, is fatal without treatment. The leishmaniases are prevalent in about 88 countries: 350 million (M) people living in endemic aeras. The morbidity of about 12-14 M people and roughly 1.5-2 M new cases per year from whom 0.4-0.5 M for VL mainly in India, Nepal, Bangladesh, Brazil and Sudan (WHO, 2010). The global mortality is about 60,000 people (Desjeux, 2004). Conventional treatments include antimonial drugs (Glucantime® and Pentostam®), amphotericin B and its liposomal formulation (AmBisome®) which are used by parenteral route. A phosphorylcholine ester of hexadecanol, designated as miltefosine, originally developed as an anticancer drug (Muschiol et al., 1987) was shown to be the first oral drug against visceral (Jha et al, 1999, Sundar et al., 2002) and cutaneous (Soto et al., 2004; Sinderman & Engel, 2006, Soto & Toledo, 2007). However, it can be noticed that miltefosine (Impavido®) possesses a long half-life able to generate resistant *Leishmania* isolates and exhibits contra-indication in pregnancy because of adverse effects (Jha et al., 1999, Soto & Berman, 2006). Despite these limitations, miltefosine is now success-fully proposed in combination with AmBisome® in order to prevent drug resistance to both the drugs (Sundar et al., 2008).

Anyway, new compounds active by oral route should be developed in case of failure of this AmBisome®-miltefosine bitherapy in the future. Sitamaquine (WR-6026) is a 8-aminoquinoline analog (Fig. 1) discovered...
by the Walter Reed Army Institute of Research (WRAIR, USA) and in development with GlaxoSmithKline (UK) for the oral treatment of VL. Sitamaquine was first synthesized as part of the collaborative antimalarial program in the US (1944-1950) that led to primaquine (Elderfield et al., 1955). Sitamaquine is an orally active drug and appears as promising agent for treatment of VL both in India (Jha et al., 2005) and Africa (Wasunna et al., 2005).

**IN VITRO AND IN VIVO SITAMAQUINE ACTIVITIES ON LEISHMANIASIS MODELS**

Recent in vitro parasite evaluation confirmed the antileishmanial properties of sitamaquine dihydrochloride against a range of *Leishmania* species responsible for either cutaneous or visceral leishmaniasis, with ED50 values against amastigotes in a range from 2.9 to 19.0 microM (Garnier et al., 2006). In fact, the antileishmanial activity of 8-aminoquinoline was revealed more than fifty years ago when a series of 6-methoxy-8-alkylpiperazinoalkylaminoquinoline derivatives were shown to exhibit both a higher activity than pentavalent antimonials and oral availability against *L. donovani* in the hamster model (Beveridge et al., 1958). Later, a series of 4-methyl-6-methoxy-8-aminoquinolines called lepidines was shown to be several hundred times more active than pentavalent antimonials in a rodent model (Kinnamon et al., 1978). Structure-activity relationships of methoxy- and hydroxy-substituted compounds were further investigated in a *L. tropica*-macrophage model in vitro (Berman & Lee, 1983). Among them, primaquine exhibited a notable high activity and 8-[[6-(diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline or WR6026, now called sitamaquine was 708 times more active than meglumine antimoniate (Glucantime®) against *L. donovani* in hamsters (Kinnamon et al., 1978).

On *L. major* cutaneous lesions in BALB/c mice, different topical sitamaquine dihydrochloride formulations using topically acceptable excipients were evaluated in vivo without success since no reduction of the parasite burden and lesion progression was observed (Garnier et al., 2006).

**MECHANISM OF ACTION**

Although the sitamaquine effects on the parasite have been visualized via alterations in their morphology (Langreth et al., 1983), the molecular targets of sitamaquine are still unknown. However, the sequential steps of interactions of sitamaquine with parasites are now well documented. Sitamaquine entry into *Leishmania* does not involve a transporter (López-Martín et al., 2008). As a lipophilic weak base, the sitamaquine accumulation into *Leishmania* promastigotes occurs along an electrical gradient involving two steps: first, the positively charged sitamaquine interacts with the anionic polar head groups of membrane phospholipids, and second, the sitamaquine insertion into the parasite plasma membranes results of a subsequent hydrophobic interaction between acyl chains of phospholipids and the hydrophobic quinoline ring leading to a deeper insertion of the drug into the lipid monolayer (Dueñas-Romero et al., 2007). This process is energy- and sterol-independent (Goïmbra et al., 2010). However, this affinity of sitamaquine for membranes is transitory since the main sitamaquine location was found into the cytosol (Goïmbra et al., 2010). In contrast, an energy-dependent efflux has been evidenced but the nature of the protein involved in this efflux remains to be elucidated (Goïmbra et al., 2010). NMR study of motile lipids showed that sitamaquine does not affect lipid trafficking in *Leishmania* (Goïmbra et al., 2010). Once internalized, sitamaquine rapidly accumulates into acidic compartments, mainly acidocalcisomes [acid vacuoles containing most of the cellular calcium] (López-Martín et al., 2008). This accumulation in acidocalcisomes allows to their alkalization (Vercesi et al., 2000). A rapid collapse of the mitochondrial inner-membrane potential was also observed (Vercesi et al., 1992). However, the antileishmanial action of sitamaquine is not related to its level of accumulation in acidocalcisomes (López-Martín et al., 2008). Proteomic analysis are running now to identify the sitamaquine targets.

**BIOAVAILABILITY**

Pharmacokinetics data in humans showed that sitamaquine has a short elimination half-life (about 26 hr) in contrast to miltefosine half-life (150-200 hr) (Theoharides et al., 1987). The metabolism of sitamaquine was studied in a rat hepatic microsomal system (Theoharides et al., 1985). Two metabolites were found: 8(6-diethylaminohexylamino)-6-methoxy-lepidine and 8(6-diethylaminohexylamino)-6-methoxy-4-hydroxy-methyl-quinoline (Yeates, 2002). The formation of both metabolites was NADPH-dependent. The formation of both metabolites seems to be
catalyzed by different cytochrome P450 isozymes. No more data are so far available to understand the importance of metabolites in the sitamaquine action.

**CLINICAL TRIALS ON VISCERAL LEISHMANIASIS**

First phase II assays performed in Kenya on 16 patients were encouraging enough to be continued further (Sherwood et al., 1994). In phase II assays in India with 120 VL patients (Jha et al., 2005), and in Kenya with 95 VL patients (Wasunna et al., 2005), sitamaquine was well tolerated with the doses ranging from 1.5 to 3 mg/mg/day, with vomiting and abdominal pains (about 10 %), headache (also about 10 %). Cyanosis (3 %) in India was reported to be due to methemoglobinemia, a recognized side effect of 8-aminoquinolines for individuals with glucose-6-phosphate deshydrogenase (G6PD) deficiency (Jha et al., 2005). Methemoglobinemia was not reported in the Kenyan study (Wasunna et al., 2005). Renal adverse effects (nephritic syndrome 3 % and glomerulonephritis 2 % in India) were observed only for

| Chemical formula                   | C₂₁H₃₃N₃O.2HCl (dihydrochloride) |
|------------------------------------|---------------------------------|
| Physical properties                | Octanol/water partition coefficient: LogP = 5.84 |
|                                   | Molecular weight: 342.51 g       |
|                                   | 415.43 g (dihydrochloride)      |
| Solubility                         | Dihydrochloride: water soluble (> 100 mg/ml at 25 °C) |
|                                   | Ethanol soluble                  |
| Chemical characteristics           | Weak base pKa ∼ 4.2 (quinoline nitrogen) |
|                                   | 10.4 (amine side chain)         |
| Behaviour in biological fluids     | Affinity for proteins (Duenas et al., 2007) |
| Interaction with host cell / parasite | Affinity for negative phospholipids (Duenas et al., 2007) |
|                                   | Morphology alteration of *Leishmania* (Langreth et al., 1983) |
| Uptake and accumulation in Leishmania donovani promastigotes | Electrical gradient diffusion (Duenas et al., 2007) |
|                                   | No affinity for sterols (Soares et al., 2010) |
|                                   | No transporter suspected (López-Martín et al., 2008) |
|                                   | Energy dependent efflux (Soares et al., 2010) |
| Intracellular targets              | Accumulation in acidocalcisomes (Vercesi et al., 2000) |
|                                   | Rapid collapse of the mitochondrial inner-membrane potential (Vercesi et al., 2002) |
|                                   | Sitamaquine susceptibility not related to accumulation into acidocalcisomes (López-Martín et al., 2008) |
| Bioavailability                    | Plasma half-life: 26.1 hr |
|                                   | 4-CH₂OH as major urinary metabolite (Theoharides et al., 1987) |
| Clinical trials Phase II           | High efficacy rate at doses 1.5-3 mg/kg/day × 28 by oral route |
|                                   | Trials in India (Jha et al., 2005) |
|                                   | Trials in Kenya (Wasunna et al., 2005) |
| Toxicity / adverse effects (%)      | Vomiting |
|                                   | Abdominal pains |
|                                   | Headache (10 %) |
|                                   | Methemoglobinemia (3 %) |
|                                   | Cyanosis (3 %) |
|                                   | Renal adverse effects: if doses > 2.5 mg/kg |
|                                   | Nephritic syndrome (3 %) |
|                                   | Glomerulonephritis (2 %) |
|                                   | No methemoglobinemia in Kenya (Wasunna et al., 2005) |
| Resistance                         | At risk: obtained *in vitro* (Bories et al., 2008) |

Table I. – Physico-chemical and biological properties of sitamaquine, an antileishmanial agent active against visceral leishmaniasis.
doses ≥ 2.5mg /mg/day (Jha et al., 2005), but effects on kidney need further investigation.

Another phase II clinical trial including dose-escalating safety and efficacy study was carried out in L. chagasi infected patients in Brazil (Dietze et al., 2001). Cure rates were not successful since a lack of increased efficacy was observed with increased dosing above 2 mg/kg/day × 28. Nephrotoxicity was observed at 2.5 mg/kg/day in two patients and in the single patient administered 3.25 mg/kg/day (Dietze et al., 2001).

On cutaneous leishmaniasis, because of the lack of activity on the in vivo models, no clinical development was performed with sitamaquine (Garnier et al., 2006).

RISK OF DRUG RESISTANCE

The short elimination half-life of sitamaquine in mammals is in favour of a low probability of resistance emergence. However, in order to evaluate the risk of sitamaquine resistance in the field, a L. donovani promastigote line resistant to 160 µM sitamaquine was selected by in vitro drug pressure and some characterististics of this resistant line were studied (Bories et al., 2008). The resistant line was infective for murine peritoneal macrophages in vitro as its parent wild-type line but less infective for Balb/C mice, suggesting that a low transmission of resistant parasites could occur in the field. The sitamaquine IC_{50} on the resistant line was about five and three times higher than those of the wild-type line on promastigote and intramacrophage amastigote forms, respectively. No cross-resistance with other antileishmanial agents was observed, allowing to use another antileishmanial drug in case of sitamaquine resistance. However, this resistance was stable when parasites were subcultured in drug-free medium for a long time or after in vivo passage, suggesting that a maintenance at a constant level in the parasite populations. These considerations, apparently speculative, could be indicative from an epidemiological point of view.

CONCLUSION

Few chemical series reach the clinical development in the field of leishmaniasis because the antileishmanial screening and toxicity bottlenecks are selective. Sitamaquine is the second orally active antileishmanial drug after miltefosine. The development of sitamaquine is slow because time is needed to ensure the safety of the drug, mainly at the level of methemoglobinemia and nephrotoxicity. Recent data show that resistance is at risk. However, the level of resistance obtained by in vitro drug pressure corresponds to a loss of susceptibility of 5-fold, that would be compatible with higher dosages if sitamaquine was not toxic. The major advantages of sitamaquine are its administration route and pharmacokinetics characteristics. Thus, its bioavailability is better than those of miltefosine. From all these data gathered in Table I, it is now probable that GSK company, the developer, will take a decision concerning the marketing of sitamaquine in the next future.

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