Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1768 (2007) 246–252
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbamem

Interaction of sitamaquine with membrane lipids of *Leishmania donovani* promastigotes

Ana Maria Dueñas-Romero [a], Philippe M. Loiseau [a], Michèle Saint-Pierre-Chazalet [a,b,*]

[a] *Groupe Chimiothérapie Antiparasitaire, UMR 8076 CNRS, Faculté de Pharmacie, Université Paris-Sud XI, rue Jean-Baptiste Clément, F-92290-Châtenay-Malabry, France*
[b] *Laboratoire de Biophysique Moléculaire Cellulaire et Tissulaire, UMR 7033 CNRS, Université Paris VI Génopole Campus 1, 5 rue Henri Desbruères, F-91030-Evry Cedex, France*

Received 2 June 2006; received in revised form 7 July 2006; accepted 7 July 2006
Available online 15 July 2006

Abstract

Sitamaquine is an 8-aminoquinoline which is active by the oral route for the treatment of life-threatening visceral leishmaniasis caused by *Leishmania donovani*, with an IC_{50} of 29.2 μM against the promastigote form in vitro. At high concentration (100 μM), sitamaquine affected parasite motility, morphology and growth in a way that was only partially reversible. As a first approach to determine its mechanism of action, we describe the interaction of sitamaquine with parasite membrane components, representing the first barrier to be crossed by the drug. Analysis of the physicochemical interactions of sitamaquine with monolayers of phospholipids and sterols at the air–water interface showed that these interactions only occurred in the presence of anionic phospholipids. Thus, electrostatic interactions between positively charged sitamaquine and the negative polar headgroups are a pre-requisite for subsequent hydrophobic interactions between the sitamaquine aromatic ring and the alkyl chains of phospholipids leading to drug insertion into the monolayer.

Keywords: Sitamaquine; Leishmania; Monolayer; Phospholipid

1. Introduction

Leishmaniases are tropical and sub-tropical diseases caused by protozoan parasites of the genus *Leishmania*. Three major clinical expressions are found in humans, depending on the *Leishmania* species involved: cutaneous, mucosal and visceral leishmaniasis [1–3]. Despite efforts developed to find a vaccine and to control the insect vector, chemotherapy remains the most efficient way to overcome leishmaniasis. The treatment of visceral leishmaniasis (VL) employs antimonials, drugs containing antimony, as the first-line treatment. In cases of antimonial resistance, amphotericin B and its liposomal formulations are used by the intravenous route. Recently, miltefosine (hexadecylphosphocholine or HePC), has been proposed for the treatment of visceral leishmaniasis by the oral route [4]. Sitamaquine (WR6026.2HCl), a 8-aminoquinoline, (Fig. 1) is in clinical development for the treatment of visceral leishmaniasis, also by the oral route [5–8]. Phase II trials in India and Kenya were encouraging [9,10]. In order to assure a long life for sitamaquine as anti-leishmanial drug, it is important to understand its mechanism of action; however, the information available remains fragmentary. Sitamaquine was described as being responsible for an alteration of parasite morphology [11], and the intracellular targets identified up to date are mitochondria [12] and acidocalcisomes [13]. Whatever the mechanism, sitamaquine has to cross the parasite membrane to reach its biological targets. We hypothesize that sitamaquine, as a cationic, amphiphilic molecule, could interact with membrane lipids. In order to confirm this hypothesis, we have studied the physico-chemical interactions between sitamaquine and lipid monolayers, which serve as biomimetic models of external monolayer of *Leishmania* plasma membrane.

In this paper, we describe the effect of sitamaquine on *Leishmania* promastigotes and we analyze the specific interactions of sitamaquine with the major lipid components

* Corresponding author. Chimiothérapie Antiparasitaire UMR 8076 CNRS, Faculté de Pharmacie, Université Paris XI, 5, rue Jean Baptiste Clément, 92296 Châtenay Malabry, France. Tel.: +33 1 46 83 55 55; fax: +33 1 46 83 55 57.
E-mail address: stpierre@ccr.jussieu.fr (M. Saint-Pierre-Chazalet).

0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.07.003

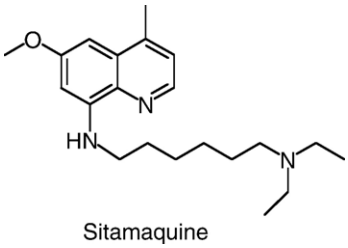

Fig. 1. Chemical structure of sitamaquine.

of the plasma membrane, mainly phospholipids, as a function of the charge of their polar head group and of their fluidity.

2. Materials et methods

2.1. Reagents

2.1.1. Sitamaquine

Sitamaquine [8-(6-diethylaminohexylamino)-6-methoxylepidine dihydrochloride], (WR6026.2HCl), is composed of two hydrophobic aromatic rings and a hydrocarbon carbon chain, containing two positively charge amine groups (Fig. 1). It was supplied by Glaxo Smith Kline (GSK, London, UK, batch No 1003). Solutions of sitamaquine were prepared daily in Millipore® water at an initial concentration of 10^{-3} M immediately before monolayer experiments.

2.1.2. Lipids

The different lipids: 1,2-Dipalmitoyl-sn-glycero-3 phosphatidylglycerol (DPPG), 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylinositol 4-monophosphate sodium salt extract of brain (b4-PIP), 1,2-Dipalmitoyl-sn-glycero-3-phosphatidyl-myo-inositol-4-monophosphate (DPPI-4P), 1,2-Distearoyl-sn-glycero-3-phosphatidylcholine (DSPC), 1-Palmitoyl-2 oleoyl-sn-glycero-3-phosphatidylcholine (POPC), 1-Palmitoyl-2 oleoyl-sn-glycero-3 phosphatidylethanolamine (POPE), cholesterol and ergosterol were purchased from Sigma (Saint-Quentin Fallavier, France). They were 99% pure, except for ergosterol (79% pure). These lipids were dissolved in a chloroform/ethanol 4/1 (v/v) mixture at a concentration of 10^{-3} M. All organic solvents were HPLC quality and purchased from VWR, (Strasbourg, France).

2.2. Methods

2.2.1. Antileishmanial activity

2.2.1.1. Parasite strains and culture. Promastigote forms of wild-type *Leishmania donovani* (MHOM/ET/67/HU3) clone were grown in M-199 medium supplemented with 40 mM HEPES, 100 μM adenosine, 0.5 mg/l hemin, 10% heat-inactivated foetal bovine serum (FBS) and 50 μg/ml gentamycin at 26 °C in a dark environment. The experiments were performed with parasites in their logarithmic phase.

2.2.1.2. IC_{50} determination on L. donovani promastigote forms in vitro [14]. The antileishmanial screening was performed in flat-bottomed 96-well plastic tissue-culture plates maintained at 27 °C in an atmosphere of 95% air/5% CO_2. Promastigote forms from a logarithmic phase culture were suspended to yield 10^6 cells/ml after hemocytometer counting. Each well was filled with 100 μl of the parasite suspension, and plates were incubated at 27 °C for 1 h before drug addition. Sitamaquine solutions were added so as to attain the desired concentrations of 100 μM and below; each in triplicate. After a 3-day incubation period at 27 °C in the dark and under a 5% CO_2 atmosphere, the viability of promastigotes was assessed using the tetrazolium-dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide or MTT colorimetric method, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent, a detergent solution was added to lyse the cells and dissolve the coloured crystals. The absorbance at 570 nm, was directly proportional to the number of viable cells, was measured using an ELISA plate reader. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_{50}) after a 3-day incubation period. HePC was used as antileishmanial reference compound and was added in the same way as sitamaquine.

2.2.1.3. Assessment of motility, morphology and growth. The effect sitamaquine at 100 μM was observed microscopically by cell counting and observation of morphology and motility as a function of the incubation time (1, 3, 6, 24, 48 and 72 h) and of the fetal calf serum (FCS) concentration within the medium (no serum, 10, 20 and 40%) in comparison with untreated control cultures. After each incubation time, parasites were washed three times in PBS and subcultured in drug-free M 199-medium for 72 h, after which the capacity of the parasites to multiply was assessed by cell counting in comparison with untreated controls.

2.2.2. Interaction of sitamaquine with phospholipids monolayers

Monolayers were prepared using a Teflon trough (6.2×26.3×0.5 cm) provided by Riegler (Riegler and Kirstein, Wiesbaden, Germany filled with Millipore® water (18 MΩ.cm, pH=5.6) as described elsewhere [15,16]. The lipid monolayers were obtained by spreading 20 μl of a 10^{-3} M organic solution of each lipid, at the air–water interface, except for DPPI-4P (50 μl of a 2×10^{-4} organic solution). The film was compressed 10 min after the evaporation of the solvent, and the compression isotherms (P/A) was recorded.

The surface pressure (P) was measured using the Wilhelmy method, by means of a filter paper plate [17]. The mean molecular area A was determined as A=S/N where S is the surface of the trough and N is the number of molecules spread. An electronic device enabled us to keep the surface pressure constant by monitoring the displacement of the barriers. The speed of movement of the barriers (3×10^{-2} cm s^{-1}) was kept constant during the experiments. All experiments were performed at 21±1 °C.

The monolayer of phospholipid was compressed up to 30 mN/m (the surface pressure of a biological membrane) [18,19]. This pressure was kept constant while aqueous solutions of 10^{-3} M sitamaquine were injected with a microsyringe under the monolayer at final concentrations of 0.2 to 3.5×10^{-5} M. If an interaction occurred between these molecules and the monolayer, the barriers were moved back to keep the pressure at 30 mN/m and the variation in the mean molecular area (ΔA) of the lipid was recorded as a function of time over 60 min (adsorption kinetics). The molecules remaining at the interface (lipids and any sitamaquine that had adsorbed and/or penetrated) were compressed and a new P/A isotherm was recorded. If an interaction (penetration and/or adsorption) of the drug with the monolayer had taken place, this isotherm was shifted to higher area compared to the initial pure lipid monolayer.

3. Results

3.1. Effect of sitamaquine on the parasites

3.1.1. Effect on parasite integrity, morphology and motility

The experiments were performed with an inoculum of 20×10^6 parasites/ml. This high parasite density allowed us to observe changes in parasite morphology microscopically under optimal conditions and to have sufficient parasites for subsequent subcultures. Parasites were incubated in the presence of 100 μM sitamaquine for 1, 3, 6, 24, 48 and 72 h, after which they were counted. Fig. 2 shows the percentage of parasite recovery as a percentage of untreated controls. After a 6-h incubation period, a large reduction of parasite number (60%) was observed. Since this period is too short for parasite multiplication, this decrease in parasite number can be ascribed

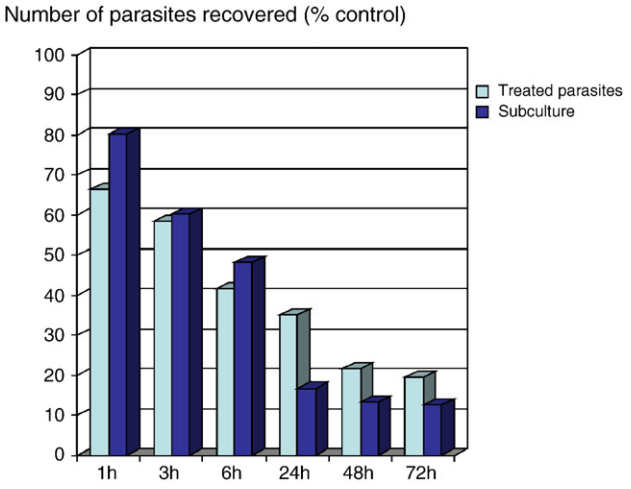

Fig. 2. *In vitro* effect of sitamaquine treatment on *Leishmania donovani* promastigotes. Inoculum: 20×10^6 parasites/ml. Parasites were incubated in the presence of sitamaquine (100 μM) for 1, 3, 6, 24, 48 or 72 h. They were counted at the end of each incubation time, then washed and subcultured for a further 72 h before counting in comparison with control subcultures.

to lysis rather than growth inhibition. A larger drop in parasite numbers was observed from 24 to 72 h, resulting from growth inhibition of the surviving parasites. Furthermore, the motility of about 90% of the surviving parasites was severely affected after 6 h of incubation. After a 24-h incubation period, most of promastigotes exhibited rounded-up forms with reduced motility, whereas untreated control parasites kept their slender form and were motile. In summary, sitamaquine affects parasite integrity, morphology and motility.

3.1.2. Reversibility of the sitamaquine effect

The reversibility of the action of sitamaquine on the parasites was evaluated after exposure to 100 μM for different periods of time. The treated parasites were washed and subcultured in sitamaquine-free medium for a further 72 h. Fig. 2 shows that the subcultured parasites always had a reduced capacity for multiplication compared to those that had not been exposed to the drug before subculture. Therefore, the action of sitamaquine was not reversible since the inhibition of parasite multiplication persisted in drug-free medium.

3.1.3. Dose dependence of the effect on parasite growth

The IC_{50} of sitamaquine against *L. donovani* promastigotes was found at 29.2±2.5 μM after a 72-h incubation period. The IC_{50} values for subcultures of parasites previously treated with 100 μM sitamaquine for 1 h or 6 h were twice as high as those of non treated parasites (58.4 and 29.2 μM, respectively) indicating that the sensitivity of the parasites to sitamaquine was significantly decreased after a first contact (Table 1). Parasites incubated with 100 μM sitamaquine for 24, 48 and 72 h and washed were not able to multiply enough to allow subsequent IC_{50} determination. These data confirm the non reversibility of the effect of sitamaquine on parasite growth.

Table 1
Effect of a sitamaquine pretreatment at 100 μM on the IC_{50} of sitamaquine on *Leishmania donovani* promastigotes subcultured in drug-free medium

Duration of sitamaquine pre-treatment	IC_{50} (μM±SD) of sitamaquine
1 h	55.2±6.0
6 h	58.4±6.2
No treatment (control)	29.2±2.5

3.1.4. Serum effect on sitamaquine activity

The medium for promastigote culture usually contains 10% fetal calf serum. However, sitamaquine could interact with serum proteins. Therefore, we evaluated the effect of the serum concentration in the medium on the antileishmanial effect of sitamaquine, using an inoculum of 8×10^6 parasites/ml. Fig. 3 shows that sitamaquine activity was reduced as a function of the serum concentration in the culture medium, suggesting that sitamaquine, a positively charged molecule, probably interacts with negative charges on plasma proteins.

In conclusion, we have observed that treatment of promastigotes with n high concentration of sitamaquine (100 μM) affected parasite integrity, morphology, motility and growth.

The lytic effect observed in the first hours of treatment could be the result of a destabilizing drug–membrane interaction. In addition, the electrostatic properties of sitamaquine could explain its interaction with charged serum proteins. All these observations prompted us to investigate whether sitamaquine interacts with membrane lipids and particularly with negatively charged phospholipids.

3.2. Monolayer results

Firstly, the tensioactive properties of sitamaquine alone were studied at the air–water interface. The interactions of sitamaquine with the principal lipids of plasma membrane of *Leishmania donovani* promastigotes were also investigated.

The choice of lipids used in this study was based on our recently published results describing the nature of the polar head groups and alkyl chains of phospholipids in plasma membranes

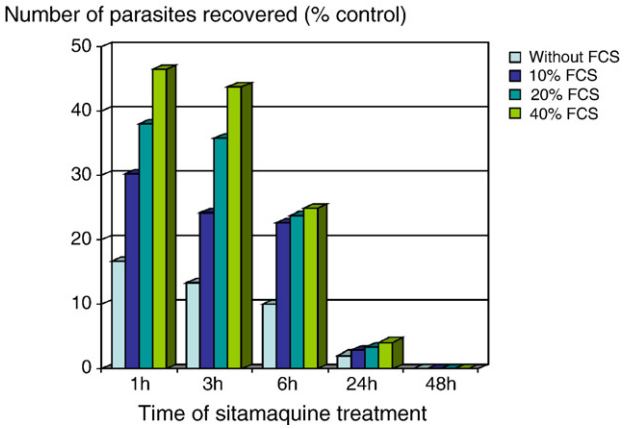

Fig. 3. Sitamaquine activity at 100 μM against *L. donovani* promastigotes as a function of Fetal Calf Serum (FCS) concentration. Inoculum: 8×10^6 parasites/ml.

from *L. donovani* promastigotes [20]. The principal lipid chains are octadecanoic acid C18:0 (28%), 9-octadecenoic C18:1 (17%) and hexadecanoic C16:0 (11%). Head group determinations indicated that the major polar lipids of surface membrane from *Leishmania* are phosphatidylcholine PC (47%), phosphatidylethanolamine PE (26%), and phosphatidylinositol PI (7%). The neutral lipids comprise mainly sterols (60%) and diglycerides (30%), with some mannose and galactose-containing glycolipids [21–24].

3.2.1. Properties of sitamaquine alone

Different concentrations of sitamaquine were injected directly in the aqueous subphase. No variation of the pressure surface was obtained over 45 min, indicating that sitamaquine, a soluble amphiphilic molecule, is not tensioactive.

3.2.2. Interaction of sitamaquine with phospholipids

3.2.2.1. Isotherms of phospholipids alone. The isotherms of principal lipid components of the plasma membrane of *Leishmania donovani* promastigotes [20] recorded at 21 ± 1 °C are shown in Fig. 4. We observed that, at a pressure of 30 mN/m, monolayers of ergosterol, DPPE, DPPG, DPPC and DPPI-4P were in the liquid condensed (LC) and POPE, b4-PIP (extract of brain) and POPC were in the liquid expanded (LE) states. These results are consistent with those previously reported [17,20,25–29]. The isotherms of DPPI-4P and b4-PIP were not similar although the two phospholipids have the same polar head with three negatives net charges. The difference is due to the composition of alkyl chains: only saturated chains (2 × C16) for DPPI-4P and a mixture of saturated and unsaturated chains for b4-PIP. Therefore, the fluidity of b4-PIP (LE phase) is higher than DPPI-4P.

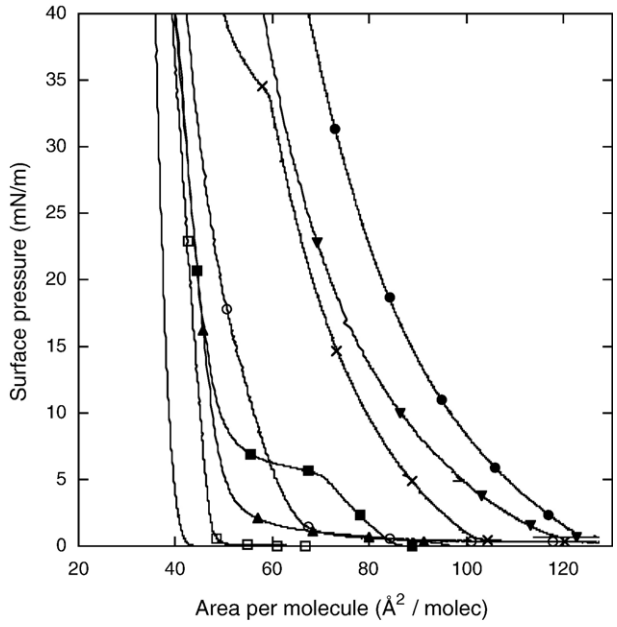

Fig. 4. Isotherms of lipids and phospholipids at $T = 21 \pm 1$ °C (subphase distilled water at pH = 5.6) (—) ergosterol, (□) DPPE, (▲) DPPG, (■) DPPC, (○) DPPI-4P, (✖) POPE, (▼) b4-PIP, (●) POPC.

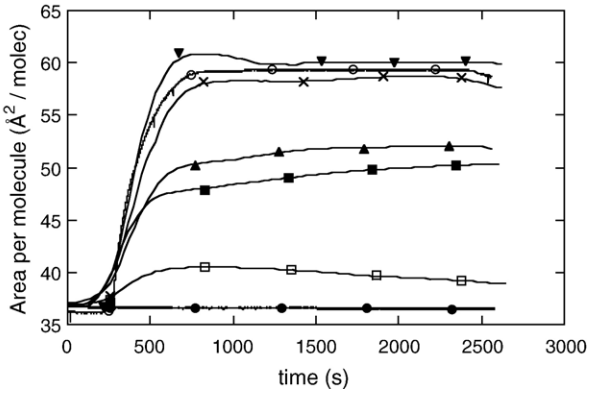

Fig. 5. Adsorption kinetics of sitamaquine injected under a DPPG monolayer (ΔA versus time) at $P = 30$mN/m. (subphase $T = 21 \pm 1$ °C and pH = 5.6). Concentration of sitamaquine : (●) 0 μM, (□) 5 μM, (■) 10 μM, (▲) 12 μM, (○) 16 μM, (▼) 18 μM, (✖) 65 μM.

3.2.2.2. Role of the charge of the polar head group of phospholipids on interactions with sitamaquine. Sitamaquine was injected at different concentrations under zwitterionic phospholipid (DSPC, DPPE, DPPC, POPE, and POPC), anionic phospholipids (DPPG and b4-PIP) and sterol (ergosterol and cholesterol) monolayers. Its adsorption kinetics was recorded over 45 min at 30 mN/m (ΔA versus time). The results in the presence of DPPG are reported in Fig. 5. The area per molecule increased rapidly to reach a maximum value (ΔAmax). These values are plotted as a function of the sitamaquine concentration in Fig. 6. The highest values of ΔAmax observed in presence of anionic phospholipids (DPPG and b4-PIP) were 21 and 25 Å^2/molecule, respectively. Generally the variation of the mean molecular area of phospholipid monolayer obtained at high pressure (30 mN/m) fall within a range of a few angstrom [15,16]. The high values obtained suggest no only an adsorption but also an insertion of sitamaquine into the monolayer of anionic phospholipids. The concentration of drug which saturated DPPG and b4-PIP monolayers respectively was 1.6×10^{-5} M and 1.2×10^{-5} M. The difference of the net charge of these anionic phospholipids could explain these results (one negative charge for DPPG and

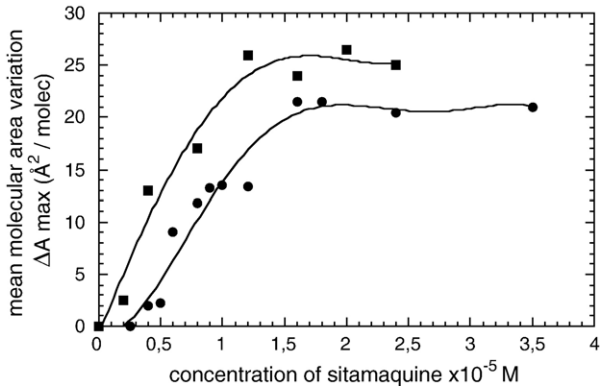

Fig. 6. Maximum variation of the mean molecular area (ΔAmax) of DPPG (●) and b4-PIP (■), as a function of sitamaquine concentration injected under the monolayer at $P = 30$ mN/m. (subphase distilled water pH = 5.6, $T = 21 + 1$ °C).

three negative charges for b4-PIP). Indeed, the electrical field due to the presence of the polar heads of b4-PIP at the interface is stronger than those induced by DPPG, therefore the b4-PIP would have a stronger attractive effect on sitamaquine.

The values of ΔAmax reached in presence of the different phospholipids are summarized in Table 2. The «zero» values obtained in presence of zwitterionic and neutral phospholipids monolayers indicate that no interaction with sitamaquine occurred. furthermore, no direct interaction between sitamaquine and sterols was detected.

The main result was that this cationic drug molecule only interacted with the negatively phospholipids, presumably by electrostatic interactions.

3.2.2.3. Effect of phospholipid fluidity. The data in Table 2 show that when interactions occur, the physical state of the monolayer (condensed or expanded) influences the value of ΔAmax. To investigate this, the following experiments were performed: after 45 min adsorption of sitamaquine ($C=1.6\times10^{-5}$) into monolayers of anionic phospholipid, the monolayer was compressed again and, new isotherms were recorded (Fig. 7). At high pressure (40 mN/m), a large shift of the isotherm compared with that of the lipid alone indicated that sitamaquine stays inserted into the lipid monolayer. However, the ΔAmax obtained for b4-PIP (25 Å^2/molecule) was higher that that for DPPG (21 Å^2/molecule) due to the higher fluidity of b4-PIP (Fig. 4). Indeed, sitamaquine could penetrate more easily into the monolayer of b4-PIP than into those of DPPG. In order to study the fluidity parameter alone, the adsorption kinetics with another anionic phospholipid, DPPI4P (LC phase) were determined. Since DPPI-4P phospholipid is very expensive, only one concentration of sitamaquine (1.6×10^{-5} M) was studied. As shown in Table 2, the maximal variation of mean molecular area (ΔAmax) value depended on the fluidity of the lipid monolayer: 21 Å^2/molecule in the presence of DPPI-4P and DPPG in the liquid condensed phase and 25 Å^2/molecule in the presence of b4PIP in the liquid expanded phase, confirming that the insertion of sitamaquine was favoured by fluid-phase phospholipids. These results indicate that after electrostatic interaction, the aromatic cycles of sitamaquine could penetrate between the phospholipid alkyl chains by a hydrophobic effect.

Table 2
Interaction of sitamaquine with lipid monolayers at the air–water interface (subphase: $T=21\pm1$ °C, pH=5.5, $P=30$ mN/m)

Lipid	Net charge	State of lipid monolayer at $P=30$ mN/m	ΔAmax Å^2/molec	Interaction with sitamaquine
ERGOSTEROL	neutral	condensed	0	no
CHOLESTEROL	neutral	condensed	0	no
DSPC	zwitterionic	condensed	0	no
DPPE	zwitterionic	condensed	0	no
DPPC	zwitterionic	condensed	0	no
DPPG	anionic	condensed	21	yes
DPPI-4P	anionic	condensed	21	yes
POPE	zwitterionic	fluid	0	no
b 4-PIP	anionic	fluid	25	yes
POPC	zwitterionic	fluid	0	no

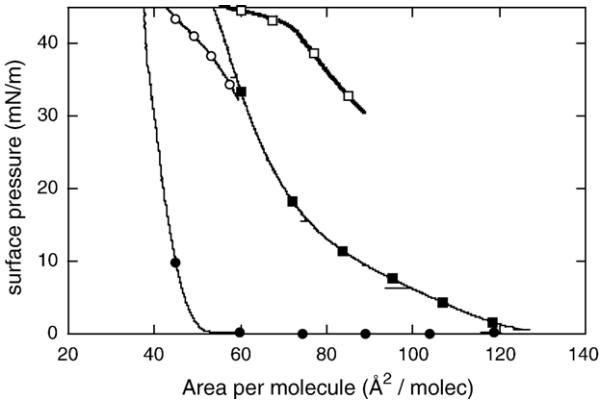

Fig. 7. Compression isotherms (pressure versus area per molecule) for DPPG and b4-PIP recorded before and after 45 min of sitamaquine adsorption ($C=1.6\times10^{-5}$) at $P=30$ mN/m at $T=21\pm1$ °C. DPPG before (●) after (○), b4-PIP (before (■) and after (□).

4. Discussion

Sitamaquine is a 8-aminoquinoline in development as an antileishmanial agent; however, little is known about its mechanism of action. We report in this study that sitamaquine has significant effects on *Leishmania donovani* morphology, motility and growth. We chose a high concentration (100 μM) in order to monitor significant biological effects more easily. The change in parasite morphology could be the result of an interference with proteins of the parasite cytoskeleton. The rapid lysis observed could be ascribed, for example, to pore formation within the plasma membrane leading to ion leakage and osmotic stress. The absence of reversibility of the sitamaquine action suggests severe damage to the target(s) that cannot be corrected by the parasite. A previous study has reported that sitamaquine provoked a fast and extensive alkalization of *Leishmania donovani* acidocalcisomes, which are acidic internal vacuoles containing sodium, calcium, magnesium, zinc and phosphorus [12,13] . This indicates that sitamaquine is able to cross the plasma membrane of the parasite. Our present results showing that sitamaquine binds to serum proteins suggest that it could probably also bind to parasite membrane proteins. Since sitamaquine is a positively charged and amphiphilic molecule, it could interact with both negatively charged proteins and phospholipids at the level of the membrane.

One aim of this study was to determine whether sitamaquine might be able to enter the *Leishmania* cell independently of any membrane transporter. We recently found that miltefosine (hexadecylphosphocholine or HePC), another new antileishmanial drug, was able to interact with plasma membrane and enter the parasite even in HePC-resistant parasites for which the LdMT (*Leishmania donovani* Miltefosine Transporter) is inactivated [20,30]. To date, no study has yet attempted to identify a sitamaquine transporter; therefore we decided to investigate the ability of sitamaquine to enter the parasite by

direct interaction with the plasma membrane using a simplified model consisting of a monolayer of phospholipids, mimicking the external face of the plasma membrane.

The first result obtained with the monolayers is that sitamaquine has no tensioactive properties and therefore cannot adsorb alone at the air/water interface. However, it can interact directly with phospholipid spread at the air/water interface. We have observed that these interactions occur only between the positively charged sitamaquine and anionic phospholipids, presumably by electrostatic interaction. Indeed, the presence of anionic phospholipid PI and PG in plasma membrane of WT parasites has been demonstrated in previous work [21]. Moreover, in the first part of this study, it was shown that activity of sitamaquine decreased in the presence of FCS, possibly by interactions with negatively charged proteins. This result argues for a high affinity of sitamaquine for anionic molecules. These electrostatic interactions would be a prerequisite for membrane–drug interaction.

The second important result is that, after the electrostatic attraction, insertion of sitamaquine into a phospholipid monolayer occurs if the monolayer is in a fluid state. This association increases with the fluidity of the monolayer and is probably due to the insertion of the aromatic part of the sitamaquine between the alkyl chains of the phospholipids. As it has been shown previously that the plasma membranes of *Leishmania donovani* promastigote are in the fluid state, this phenomenon could take place in vivo. So, these results allow us to propose a mechanism of sitamaquine insertion within the external monolayer of a biological membrane by a two step-interaction: an electrostatic interaction with negative head groups of phospholipids followed by a hydrophobic interaction allowing a deeper insertion of sitamaquine within the lipid monolayer. This monolayer study allows us to understand how sitamaquine could cross over the membrane. Thus, the following step will be to study the passage of sitamaquine across a phospholipid bilayer by using vesicles.

Acknowledgment

We are grateful to Dr. Mark Felton, GSK, London, UK, for providing us with sitamaquine.

References

[1] P. Desjeux, Worldwide increasing risk factors for leishmaniasis, Med. Microbiol. Immunol. (Berl.) 190 (2001) 77–79.
[2] P. Desjeux, The increase in risk factors for leishmaniasis worldwide, Trans. R. Soc. Trop. Med. Hyg. 95 (2001) 239–243.
[3] P. Desjeux, Leishmaniasis Public health aspects and control, Clin. Dermatol. 14 (1996) 417–423.
[4] S.L. Croft, G.H. Coombs, Leishmaniasis–current chemotherapy and recent advances in the search for novel drugs, Trends Parasitol. 19 (2003) 502–508.
[5] R. Dietze, S. Carvalho, L. Valli, J. Berman, T. Brewer, W. Milhous, J. Sanchez, B. Schuster, M. Grogl, Phase 2 trial of WR6026, an orally administered 8-aminoquinoline, in the treatment of visceral leishmaniasis caused by *Leishmania chagasi*, Am. J. Trop. Med. Hyg. 65 (2001) 685–689.
[6] J.A. Sherwood, G.S. Gachihi, R.K. Muigai, D.R. Skillman, M. Mugo, J.R. Rashid, K.M. Wasunna, J.B. Were, S.K. Kasili, J.M. Mbugua, et al., Phase 2 efficacy trial of an oral 8-aminoquinoline (WR6026) for treatment of visceral leishmaniasis, Clin. Infect. Dis. 19 (1994) 1034–1039.
[7] A.D. Theoharides, H. Chung, H. Velazquez, Metabolism of a potential 8-aminoquinoline antileishmanial drug in rat liver microsomes, Biochem. Pharmacol. 34 (1985) 181–188.
[8] S. Sundar, Drug resistance in Indian visceral leishmaniasis, Trop. Med. Int. Health 6 (2001) 849–854.
[9] M.K. Wasunna, J.R. Rachid, J. Mbui, G. Kirigi, D. Kinoti, H. Lodenyo, J.M. Felton, A.J. Sabin, M.J. Albert, J. Horton, A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya, Am. J. Trop. Med. Hyg. 74 (2006) 185.
[10] T.K. Jha, S. Sundar, C.P. Thakur, J.M. Felton, A.J. Sabin, J. Horton, A phase II dose-ranging study of sitamaquine for the treatment of visceral leishmaniasis in India, Am. J. Trop. Med. Hyg. 73 (2005) 1005–1011.
[11] S.G. Langreth, J.D. Berman, G.P. Riordan, L.S. Lee, Fine-structural alterations in *Leishmania tropica* within human macrophages exposed to antileishmanial drugs *in vitro*, J. Protozool. 30 (1983) 555–561.
[12] A. Vercesi, R. Docampo, Ca2+ transport by digitonin-permeabilized *Leishmania donovani*. Effects of Ca2+, pentamidine and WR-6026 on mitochondrial membrane potential *in situ*, Biochem. J. 284 (1992) 463–467.
[13] A. Vercesi, C. Rodrigues, R. Castisti, R. Docampo, Presence of a Na(+)/H(+) exchanger in acidocalcisomes of *Leishmania donovani* and their alkalization by anti-leishmanial drugs, FEBS Lett. 473 (2000) 203–206.
[14] C. Peyron, R. Benhida, C. Bories, P.M. Loiseau, Synthesis and in voto antileishmonial acivity of 5-substituted-2′-deoxyurudine derivatives, Bioorg. Chem. 33 (2005) 439–447.
[15] O. Berdycheva, B. Desbat, M. Vaultier, M. Saint-Pierre-Chazalet, Interaction of natural polyamines and dimethylsilylanalogues with a phospholipid monolayer: a study by Brewster angle microscopy and PM-IRRAS, Chem. Phys. Lipids 125 (2003) 1–11.
[16] M. Rakotomanga, P.M. Loiseau, P.M. Saint-Pierre-Chazalet, Hexadecylphosphocholine interaction with lipid monolayers, Biochim. Biophys. Acta 1661 (2004) 212–218.
[17] G.L. Gaines, Insoluble Monolayers at Liquid–Gas Interfaces, Interscience, New York, 1966 Prigogine editor.
[18] M. Pilon, W. Jordi, B. de Kruijff, R.A. Demel, Interactions of mitochondrial precursor protein apocytochrome c with phosphatidylserine in model membranes. A monolayer study, Biochim. Biophys. Acta 902 (1987) 207–216.
[19] A. Seelig, Local anesthetics and pressure: A comparison of dibucaine binding to lipid monolayers and bilayers, Biochim. Biophys. Acta 899 (1987) 196–204.
[20] M. Rakotomanga, M. Saint-Pierre-Chazalet, P.M. Loiseau, Alteration of fatty Acid and sterol metabolism in miltefosine-resistant *Leishmania donovani* promastigotes and consequences for drug–membrane interactions, Antimicrob. Agents Chemother. 49 (2005) 2677–2686.
[21] M. Wassef, T. Fioretti, D. Dwyer, Lipid analyses of isolated surface membranes of *Leishmania donovani* promastigotes, Lipids 20 (1985) 108–115.
[22] D. Dwyer, M. Gottlieb, The biochemistry of *Leishmania* surface membranes, Leishmaniasis, Elsevier Science Publishers B.V. (Biomedical Division), 1985, pp. 31–47.
[23] D. Beach, G. Holz, G. Anekwe, Lipids of *Leishmania* promastigotes, J. Parasitol. 65 (1979) 201–216.
[24] J. Urbina, Lipid biosynthesis pathways as chemotherapeutic targets in kinetoplastid parasites, Parasitology 114 (1997) 91–99.
[25] A.F. Mingotaud, in: C. Mingotaud, L.K. Patterson (Eds.), Handbook of Monolayers, vol. 1, no. 3, Academic Press, San Diego, CA, 1993, pp. 796–892.
[26] O. Domenech, F. Sanz, M.T. Montero, J. Hernandez-Borrell, Thermodynamic and structural study of the main phospholipid components comprising the mitochondrial inner membrane, Biochim. Biophys. Acta 1758 (2006) 213–221.
[27] J. Minones Jr., P. Dynarowicz-Latka, O. Conde, J. Minones, E.

Iribarnegaray, M. Casas, Interactions of amphotericin B with saturated and insaturated phosphatidylcholines at the air/water interface, Colloids Surf., B Biointerfaces 29 (2003) 205–215.
[28] D. Vollhardt, V.B. Fainerman, S. Siegel, Thermodynamic and textural characterization of DPPG phospholipid monolayers, J. Phys. Chem., B 104 (2000) 4115–4121.
[29] M. Saint-Pierre-Chazalet, C. Thomas, M. Dupeyrat, C.M. Gary-Bobo, Amphotericin B/sterol complex formation and competition with egg-phosphatidylcholine: A monolayer study, Biochim. Biophys. Acta 944 (1988) 477–486.
[30] F.J. Perez-Victoria, F. Gamarro, M. Ouel lette, S. Castanys, Functional cloning of the miltefosine transporter, a novel P-type phospholipid translocase from *Leishmania* involved in drug resistance, J. Biol. Chem. 278 (2003) 49965–49971.