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Transport of fluoroalkyl dihydroartemisinin derivatives across rat intestinal tissue

Federica Bigucci, Tayou Kamsu-Kom, Constance Cholet, Madeleine Besnard, Danièle Bonnet-Delpon and Gilles Ponchel

Abstract

Artemisinin and its derivatives represent an important class of antimalarials. In order to obtain new derivatives with a longer half-life and better bioavailability, the development of fluorinated analogues has received increasing attention. The purpose of this study was to investigate the permeation of artemisinin and of two fluoroalkyl derivatives of dihydroartemisinin (DHA), namely 10β-(trifluoropropyloxy)dihydroartemisinin (F_1-DHA) and 10-trifluoromethyl-16-[2-(hydroxyethyl)piperazine] (F_2-DHA), across rat intestine using Ussing diffusion chambers. Further, the saturation solubility and partition coefficient of the compounds were determined in order to determine whether the substitution of hydrogen atoms by fluorine can induce great changes in these molecular properties. Artemisinin and F_2-DHA permeability coefficients of 27.5 ± 1.6 and 23.2 ± 1.2 ($\times 10^{-6}$, cm s^{-1}), respectively, are predictive of good oral absorption. This indicates that the introduction of a fluoroalkyl group in a compound such as artemisinin in order to prolong its half-life does not constitute an obstacle for its absorption after oral administration. Moreover, the introduction of a polar substituent into the DHA structural scaffold increased the aqueous solubility of F_2-DHA relative to artemisinin. F_1-DHA permeability measurements showed low transepithelial diffusion across the intestinal mucosa. This indicates that the introduction of a fluorinated substituent at the α-methylene carbon of DHA ethers in order to provide protection against oxidative processes constitutes an obstacle for the absorption after oral administration.

Department of Pharmaceutical Sciences, Bologna University, via S. Donato 19/2, 40127 Bologna, Italy

Federica Bigucci

Physicochimie-Pharmacotechnie-Biopharmacie, UMR CNRS 8612, Faculty of Pharmacy, University of Paris-Sud, 5 rue Jean-Baptiste Clément, 92296 Châtenay-Malabry, France

Tayou Kamsu-Kom, Madeleine Besnard, Gilles Ponchel

BIOCIS-CNRS, Faculty of Pharmacy, University of Paris-Sud, rue Jean-Baptiste Clément, 92296 Châtenay-Malabry, France

Constance Cholet, Danièle Bonnet-Delpon

Correspondence: Federica Bigucci, Department of Pharmaceutical Sciences, Bologna University, Via S. Donato 19/2, 40127 Bologna, Italy. E-mail: federica.bigucci@unibo.it

Introduction

Isolated in 1972 from the Chinese medicinal plant *Artemisia annua* L. (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials 1979; Klayman 1985), artemisinin (Figure 1) is a potent antimalarial with a sesquiterpene lactone structure containing an internal endoperoxide linkage, which is essential for its activity (Gu et al 1980; China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982a). It is a fast-acting blood schizonticide with a short parasite clearance time, leading to rapid symptomatic relief of malarial infection. It is also effective against multidrug-resistant strains of *Plasmodium falciparum* and produces rapid recovery even in patients with cerebral malaria. However, artemisinin has low aqueous solubility, resulting in poor and erratic absorption upon oral administration (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982b). This, together with its short half-life and high first-pass metabolism, might lead to incomplete clearance of the parasites, resulting in recrudescence (Titulaer et al 1991). In order to obtain more effective and soluble drugs, a number of ether derivatives of dihydroartemisinin (DHA) have been synthesized. These compounds had greater solubility than artemisinin in water (sodium artesunate) and in oil (artemether and arteether), but they had a shorter half-life in plasma (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982c, d). Although knowledge of their pharmacokinetic properties is incomplete, it is apparent that the main pathway of metabolism of ether derivatives of DHA is the

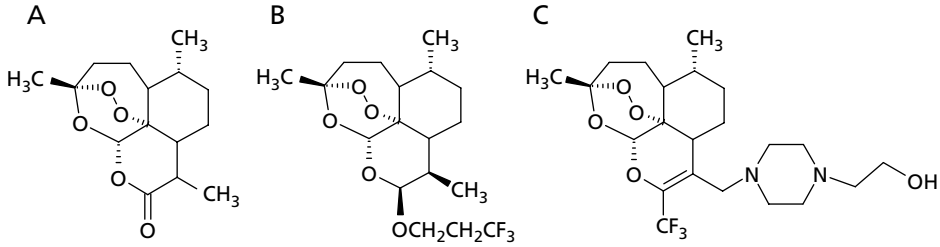

Figure 1 Chemical structures of artemisinin (A), 10β-(trifluoropropyloxy)dihydroartemisinin (B) and 10-trifluoromethyl-16-[2-(hydroxyethyl)piperazine] (C).

rapid hydroxylation by cytochrome P-450 enzymes to generate a hemiketal intermediate (Lee & Hufford 1990; Chi et al 1991; Leskovac & Theoharides 1991a, b). This intermediate decomposes to produce the active compound DHA, which is cleared within hours from the circulation (Song & Zhao 1989; Hien & White 1993).

One method to prolong the half-life of artemisinin derivatives is to design new ethers that are poorer substrates for cytochrome P-450 than reported DHA ethers, or new hemiacetals that are more stable than DHA (Bèguè & Bonnet-Delpon 2007). For this purpose, Bèguè & Bonnet-Delpon (2007) introduced a fluorinated substituent at the α-methylene carbon of DHA ethers that provides protection against oxidative processes (10β-(trifluoropropyloxy)dihydroartemisinin; F_1-DHA) or an electron-withdrawing triflurometh group at the hemiacetal carbon of DHA to stabilize the hemiketal (10-trifluoromethyl-16-[2-(hydroxyethyl)piperazine]; F_2-DHA) (Figure 1).

The aim of this study was to investigate the effect of the introduction of these fluoroalkyl substituents on intestinal permeability. The apparent permeability coefficients (P_{app}) of these new fluorinated derivatives were studied across rat intestinal tissues mounted in Ussing chambers and compared with the P_{app} of artemisinin. The rationale for selecting this in-vitro model is that it can provide a rapid method for evaluating P_{app} values of drugs across the intestinal epithelia and allows prediction of the human in-vivo permeability of passively transported compounds with a particularly high degree of accuracy (Lennernäs 1997). Moreover, the amounts of drug required are relatively small (milligram quantities) and the collected samples are analytically clean, which facilitates quantitative analysis. We also determined some basic physicochemical properties such as the aqueous solubility (S_0) and *n*-octanol/Ringer's solution partition coefficient ($P_{oct/Ringer}$) of artemisinin and fluoroalkyl DHA derivatives in order to evaluate whether the substitution of hydrogen atoms by fluorine can induce great changes in these molecular properties, as with those of solubility and lipophilicity.

Materials and Methods

Materials

The artemisinin used for this study was obtained commercially from Sigma-Aldrich (Lyon, France) and the fluoroalkyl derivatives of dihydroartemisinin were synthesized at the Molécules Fluorées BIOCIS CNRS, Faculté de Pharmacie, Châtenay-Malabry, France. ^{14}C Mannitol (specific activity 315 μCi mg^{-1}) was purchased from Amersham Pharmacia (Buckinghamshire, UK). Ultima Gold liquid scintillation fluid was from Perkin Elmer Life Science Products (Boston, MA, USA). Sodium pentobarbital was obtained from Sanofi Santé Animale (Libourne, France). Agar was purchased from ICN Biomedicals (Aurora, OH, USA). Carbogen (95% O_2 and 5% CO_2) was provided by Air Liquide (Gentilly, France). NaCl, KCl, $NaHCO_3$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $K_2HPO_4 \cdot 6H_2O$ and KH_2PO_4 were purchased from Prolabo (Fontenay sous Bois, France). All the solvents used were of analytical reagent grade and from Carlo Erba (Val de Reuil, France).

All transport studies were carried out in a Ringer's solution with the following composition (mM): 115 NaCl; 24 K_2HPO_4; 4 KH_2PO_4; 25 $NaHCO_3$; 24 $MgCl_2 \cdot 6H_2O$; 24 $CaCl_2 \cdot 2H_2O$. The buffer solution was oxygenated with carbogen and pH was adjusted to 7.4.

Chromatography

Concentrations of artemisinin, F_1-DHA and F_2-DHA in the samples from the permeability measurements, solubility studies and partitioning studies were determined by high-performance liquid chromatography (HPLC) using UV absorbance detection. The HPLC system (Waters, Milford, MA, USA) consisted of a system controller (model 600E), a mobile phase delivery pump (model 501), an auto-sampler (model 712 WISP), a tunable absorbance UV-vis detector (model 486) and a data module (model 746). Artemisinin separation was achieved at ambient temperature on a hypersphere 5 C18 column (250 × 4.6 mm, 5 μm packing; Interchim, Montluçon, France), which was eluted with a mobile phase consisting of 10 mM phosphate pH 4.5 and MeOH (50:50, v/v) at a flow rate of 1 mL min^{-1}; F_1-DHA separation was achieved at ambient temperature on a Symmetry C18 column (75 × 4.6 mm, 3.5 μm packing; Waters), which was eluted with a mobile phase consisting of CH_3CN/H_2O (60:40, v/v) at a flow rate of 0.5 mL min^{-1}; F_2-DHA separation was achieved at ambient temperature on a Symmetry C18 column (75 × 4.6 mm, 3.5 μm packing; Waters), which was eluted with a mobile phase consisting of CH_3CN/H_2O (75:25, v/v) at a flow rate of 1 mL min^{-1}. Before injection, all samples were filtered through a 0.22-μm filter (Millex-GV; Millipore, MA, USA). Calibration curves were obtained as follow: adequate

aliquots of artemisinin or F_1-DHA or F_2-DHA stock solution over ethanol (2 mg mL^{-1}, freshly prepared) were diluted with Ringer's solution to produce standards over the range 0.1–100 μg mL^{-1}. The final concentration of ethanol was 5%. Standards were treated according to a sample preparation procedure mentioned above.

Sample preparation

Concentrations of artemisinin were measured according to a slight modification of the method of Zhao (1987). Samples (100 μL) were alkalinized with 200 μL of 0.4% NaOH. After 5 min at 50°C, samples were acidified with 50 μL of 1 M acetic acid (in 50:50 MeOH/H_2O). The resulting degradation product showed a UV maximum at 260 nm and could be analysed using HPLC. Concentrations of F_1-DHA were measured according to a modification of the method of Idowu et al (1989) for arteether. Samples (100 μL) were treated with 100 μL of 5 M aqueous HCl at 53°C for 15 min. The resulting degradation product showed a UV maximum at 254 nm and could be analysed using HPLC. Samples of F_2-DHA were directly analysed using HPLC with UV detection at 215 nm.

Intestinal permeation experiments

Preparation of tissue segments

Male Sprague–Dawley rats (Iffa Credo, Saint Germain sur l'Arbresle, France), 200–300 g, were used in this study and were received at least 1 week before use. The rats had free access to food and water before they were killed. Under anaesthesia with sodium pentobarbital (100 μL/100 g, i.m.), the ileum was removed, carefully washed with cold Ringer's solution, put in a beaker with Ringer's solution on ice, which was continuously gassed with carbogen. For the experiments, the ileum was then divided into four 3-cm long segments. Each segment was opened along the mesenteric border and the serosa was carefully removed. Care was taken to avoid Peyer's patches. After preparation, tissues were mounted in the Ussing diffusion chamber with an exposed intestinal area of 1 cm^2. The mucosal and serosal reservoirs were immediately filled with 4.750 mL of Ringer's solution that was continuously oxygenated and maintained at 37°C by a water jacket connected to a recirculating water bath (Polystat; Fischer Bioblock Scientific, Illkirch, France). Before an experiment, the electrical parameters of the intestinal segments were allowed to stabilize for 30 min to allow the tissue to recover from the preparation and to equilibrate to the temperature. The rats used in this study were purpose-bred. They were housed according to the French and European Laws, Guidelines and Policies for Animal Experiments, Housing and Care, in the Central Animal Facilities of the university. These facilities have the obligatory accreditation of the authorized French ministry. Approval for this project was granted by the institutional Ethical Committee for Animal Experimentation.

Transport studies

Bidirectional (mucosal to serosal; serosal to mucosal) transport studies were performed across rat intestinal tissue. At the beginning of the experiments (t_0), 250 μL artemisinin (MW = 282.34 g mol^{-1}) or F_1-DHA (MW = 380.41 g mol^{-1}) or F_2-DHA (MW = 462.51 g mol^{-1}) ethanolic solution and 250 μL ethanol were added to the donor and receiving compartment, respectively (drug concentration in the donor reservoir: 0.25 mM; 70.59 μg mL^{-1} artemisinin, 95.10 μg mL^{-1} F_1-DHA, 115.63 μg mL^{-1} F_2-DHA). Artemisinin, F_1-DHA and F_2-DHA were added to the donor compartment after solubilization by an additive. In fact, for evaluation of intestinal membrane permeability of poorly water-soluble compounds, it is important to completely dissolve the compounds and prevent precipitation during the experiment. For the additives, polyethylene glycol 600, dimethylsulfoxide and ethanol were examined at concentrations that did not affect the membrane integrity and permeability (5% and 10%) (Watanabe et al 2000). On the basis of the solubilizing ability, ethanol/Ringer's solution mixture (5:95) was selected to study the transport of artemisinin and fluoroalkyl derivatives in the chamber. Thereafter, 250-μL samples were taken from the receiving compartment at 30-min intervals for up to 120 min for artemisinin and 150 min for fluoroalkyl derivatives. To maintain a constant volume, 250 μL of Ringer's solution containing all the ingredients of the original bathing solution were added after each sample. The effect of dilution was taken into account for the calculation of fluxes. Permeability measurements were performed under 'sink' condition. P_{app} (cm s^{-1}) was calculated according to the following equation:

$$P_{app} = (dQ/dt) \times (1/AC_0)$$

where dQ/dt represents the steady-state flux of artemisinin or fluoroalkyl derivatives (μg s^{-1}) on the receiving compartment. The flux was determined by calculating the slope from the linear portion of the Q versus time plot. A is the surface area of the intestinal segment (cm^2) and C_0 is the initial concentration of the compounds (μg mL^{-1}) on the donor side.

No significant adsorption of artemisinin or F_1-DHA or F_2-DHA to the diffusion chambers was observed in pre-study validation experiments (data not shown). No metabolites or other degradation products were observed in the HPLC analysis.

Assessment of tissue integrity

Tissue integrity was checked in two ways: electrophysiological measurements and flux of the paracellular flux marker, mannitol.

The electrical parameters, potential difference (PD) and short-circuit current (I_{sc}) were recorded simultaneously during the experiments with agar/Ringer-bridges connected through 3 M KCl to either calomel electrodes (positioned not further than 5 mm from the tissue surface) for measurement of PD, or to silver electrodes (positioned at the far end of the chamber) for measurement of I_{sc}. The electrodes were connected to an amplifier with a voltage-clamp facility (MDVC-2C; Titis Business Corporation, Paris, France). I_{sc} was recorded by passing a brief period of alternating current through the tissue to reduce the PD to zero at appropriate time intervals. The electrical set up was calibrated for resistance in the Ringer's solution and for differences in electrodes prior to each experiment. The resistance of the tissue segments was calculated from the PD and I_{sc} values according to Ohm's law.

Tissue integrity was also monitored by measuring transport of the paracellular permeability marker mannitol. At the beginning of the experiments (t_0), 100 μL mannitol aqueous solution (50 mM) was added to the mucosal and serosal bathing solutions. Then, 1 mL ^{14}C-labelled mannitol Ringer's solution (50 μCi mL^{-1}) was added to the mucosal bathing solution. Thereafter, 100-μL samples were taken from the serosal bathing solution at 30-min intervals for up to 120 min, added to 4 mL of scintillation fluid and counted using a Beckman LS 6000 TA scintillation system (Beckman Instruments Inc., Fullerton, CA, USA).

Solubility studies

An excess of artemisinin or F_1-DHA or F_2-DHA was added to 10 mL of water. Equilibrium was reached by gentle agitation over 2 days at 37 ± 0.5°C. After equilibration, the solution was centrifuged (ALC 4222 centrifuge; Thermo Fisher Scientific, Waltham, MA, USA) at 4000 rev min^{-1} (2057 *g*) for 15 min, filtrated through a 0.20-μm filter (Albet-Jacs) and analysed by HPLC.

Partitioning studies (n-octanol/Ringer's solution)

Artemisinin or F_2-DHA were dissolved in Ringer's solution (pH 7.4) to give final concentrations of 100 μM; for F_1-DHA a solution of the drug in the Ringer's solution (pH 7.4) at the saturated point was used. A total of 3 mL of the aqueous solution and 3 mL of n-octanol were shaken for 3 h at 37 ± 0.5°C. After separation of the two phases by centrifugation at 4000 rev min^{-1} (2057 g) for 10 min, the compound concentration was determined by HPLC in the aqueous layer and the concentration in the octanol phase was calculated from the initial and final concentrations in the aqueous phase. Transport medium and n-octanol were mutually saturated before the experiment.

Statistical analysis

After performing an analysis of variance using the non-parametric Kruskal–Wallis test with the significance level set at $P < 0.05$, a multiple comparisons test (Dunn's test) was performed to test the null hypothesis of no difference between each of the effects of the different conditions and the control conditions.

Results and Discussion

Analytical methods

The availability of selective and sensitive methods for the quantification of artemisinin and fluoroalkyl derivatives of dihydroartemisinin in Ussing chamber medium was a primary prerequisite for the transport studies. Artemisinin does not possess any sensitive spectrophotometric characteristics: only weak absorption bands at short wavelengths are obtained in UV light and so there is a need to prepare derivatives of the compound that possess suitable UV-vis absorption characteristics. Artemisinin has been determined in this work by a slight modification of the method of Zhao (1987), based on its decomposition in an alkaline medium to give a UV-absorbing derivative. With regard to F_2-DHA, reports on the synthesis of some ether derivatives of artemisinin indicate that these compounds are stable to alkali (Lin et al 1987). Moreover, a method for the determination of arteether, a similar synthetic derivative of artemisinin, using HPLC followed by UV detection has been reported (Idowu et al 1989). This involves an acid decomposition with hydrochloric acid to produce a UV-absorbing product, an α,β-unsaturated decalone (8-methyl-5-(2-propanalyl) decalin-4-ene-3-one). Examination of arteether chemical structure suggests that F_2-DHA would be expected to undergo this reaction when incubated with acid under the same conditions. Finally, F_1-DHA was quantified by UV detection at 215 nm without any basic or acid decomposition. Representative chromatograms of the compounds at a concentration of 5 μg mL^{-1} are shown in Figure 2. The artemisinin, F_1-DHA and F_2-DHA peaks eluted at 8.70, 3.74 and 2.62 min, respectively. Calibration curves were linear over the range 0.1–100 μg mL^{-1} for artemisinin and fluoroalkyl DHA derivatives, with correlation coefficients of greater than 0.998. The minimum detectable concentration of artemisinin, F_1-DHA and F_2-DHA corresponding to a peak 3-times baseline noise at 0.01 aufs was 5 ng mL^{-1}, 18 ng mL^{-1} and 12 ng mL^{-1}, respectively.

Intestinal permeation experiments

Transport studies

Although a variety of factors ultimately contributes to the oral activity of a molecule, in many instances, lack of intestinal permeability is the rate-limiting factor. Thus, in studies designed to evaluate the potential oral activity of a new molecule, measurement of intestinal permeability will provide an initial selection for evaluation in-vivo. In this work, transport of F_1-DHA and F_2-DHA was studied across rat intestine by the Ussing chamber technique. These molecules were selected for the presence of fluoroalkyl groups that can decrease the metabolism rate and increase the lipophilicity of the compounds. Transport of these molecules was further compared with the transport of artemisinin, which is reported to be completely absorbed by the passive mechanism. Artemisinin is not a substrate for rat jejunal P-glycoprotein, nor does artemisinin appear to be an inducer of P-glycoprotein. The low oral bioavailability is probably a result of high hepatic first-pass metabolism and/or solubility problems in the gastrointestinal tract, because artemisinin jejunal permeability is high and its absorption is not affected by efflux by P-glycoprotein (Augustijns et al 1996; Svensson et al 1999). We chose artemisinin as a control because it is a readily available and economic natural material. Moreover, pharmacokinetic and biological data for this compound are easily available and analytical methods have been developed for its determination.

Figure 3 shows P_{app} values of artemisinin and F_2-DHA. These compounds had the same P_{app} in the mucosal-to-serosal and serosal-to-mucosal directions, excluding the possibility of active transport or active efflux. Generally, high permeability coefficients ($P_{app} > 10^{-6}$ cm s^{-1}) are predictive of good oral absorption, whereas compounds with low permeability coefficients ($P_{app} < 10^{-7}$ cm s^{-1}) are probably

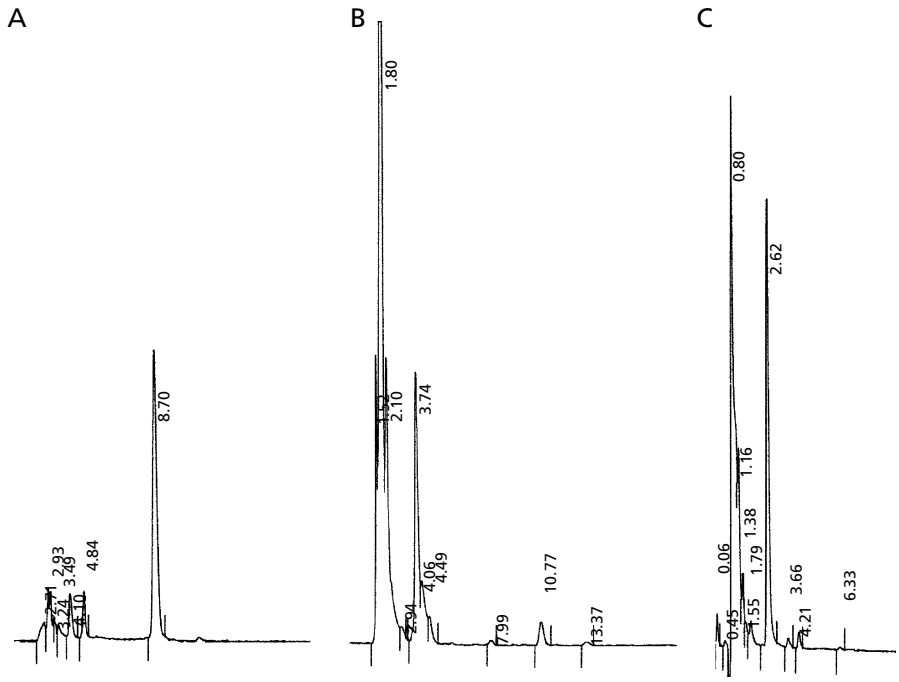

Figure 2 Representative chromatograms of artemisinin (A), 10β-(trifluoropropyloxy)dihydroartemisinin (B) and 10-trifluoromethyl-16-[2-(hydroxyethyl)piperazine] (C).

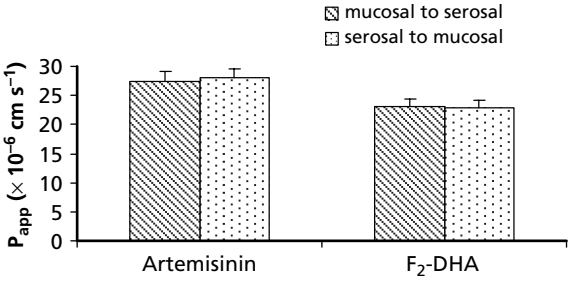

Figure 3 Apparent permeability coefficients (P_{app}) of artemisinin and 10-trifluoromethyl-16-[2-(hydroxyethyl)piperazine] (F_2-DHA) in rat ileum (mean $\pm$ s.d., n = 4).

incompletely absorbed (Artursson et al 2001). The observed artemisinin and F_2-DHA P_{app} values of 27.5 ± 1.6 and 23.2 ± 1.2 ($\times 10^{-6}$, cm s^{-1}), respectively, are indicative of good oral absorption. Moreover, these data indicate that the introduction of a fluoroalkyl substituent on DHA in order to prolong the half-life of the compound does not constitute an obstacle for the absorption after oral administration. F_2-DHA exhibited strong antimalarial activity in-vitro (IC50 = 15.2 ± 6.7 nM; *Plasmodium falciparum* W2 strain) and in-vivo (ED50 < 10 mg kg^{-1}; reduction of parasitaemia of 100% at D4 at 10 mg kg^{-1}). Pharmacokinetic studies performed on compound F_2-DHA exhibited good bioavailability in rats. C_{max} and T_{max} values for F_2-DHA, determined from the oral dosing studies, were 607.9 ng mL^{-1} and 60 min, respectively. At a 50 mg kg^{-1} oral dose, oral bioavailability was 34.6% (Grellepois et al 2004). This oral bioavailability may reflect a high metabolic stability imparted by the presence of the trifluoroalkyl group.

F_1-DHA permeability measurements showed low transepithelial diffusion across the intestinal mucosa. In fact, the maximum serosal reservoir concentration of the compound was very low (0.40 μg mL^{-1}) and was attained within 30 min. Moreover, a great decrease in F_1-DHA mucosal reservoir concentration was observed at the end of the experiment (4.18 μg mL^{-1} after 150 min). Probably this hydrophobic compound dissolves very easily into the lipidic phase of the membrane and shows poor partition from the membrane to the serosal reservoir. These data indicate that the introduction of a fluorinated substituent at the α-methylene carbon of DHA ethers in order to provide protection against oxidative processes constitutes an obstacle for absorption after oral administration. Knowledge of the biological data of fluoroalkyl ethers of DHA is incomplete. Most fluoroalkyl ethers of DHA exhibited strong antimalarial activity in-vitro and in-vivo, with only a small influence of the structure of fluorinated chains (Thanh Nga et al 1998). Antimalarial activity and pharmacokinetic data for F_1-DHA are essential in order to understand its behaviour after oral administration.

Assessment of tissue integrity

Electrical parameters were monitored in the diffusion chambers setup to characterize the mucosa and to assess the viability of the excised intestinal tissue. After equilibration (30 min), the resistance was $40.3 \pm 5.1\ \Omega\ cm^2$ and remained constant over the time course of the experiments.

Mannitol transport appeared to be unaffected by the conditions used in this study. Mannitol was transported

in a time-dependent manner and showed a P_{app} value of 1.46 ± 0.2 ($\times 10^{-6}$, cm s^{-1}) (n = 4). This value is in agreement with those reported in previous studies (Wu-Pong et al 1999).

Solubility studies

The physicochemical characteristics of the compounds, including molecular weight, S_0 and the log $P_{oct/Ringer}$ values, are summarized in Table 1. For absorption to occur, a drug must dissolve in the aqueous media of the gastrointestinal contents and partition into the lipid membrane of the intestinal wall. Thus, both aqueous and lipid solubility at an acceptable level are prerequisites to absorption. As can be seen, artemisinin was sparingly soluble in water (S_0= 99.1 μg mL^{-1}). The presence of the fluoroalkyl group in F_1-DHA drastically decreased its solubility (S_0= 1.00 μg mL^{-1}) compared with artemisinin. On the contrary, F_2-DHA solubility (S_0= 472.1 μg mL^{-1}) was greater than artemisinin due to the presence of the hydrosoluble function (2-(hydroxyethyl)piperazine group), which counterbalances the presence of the trifluoromethyl group.

Partitioning studies

In this work the octanol/water partition coefficient (Table 1) was used as a predictor of the lipophilicity of the compounds. In fact, it describes the ability of a molecule to partition into a lipophilic phase which is assumed to have lipophilicity comparable with that of a cell membrane. Typically, permeability in the intestinal epithelium increases roughly with the lipophilicity of the drug molecule until it reaches a plateau at a log P_{oct} value of approximately 2 (Martin 1981). Drugs displaying log P_{oct} values close to 2 are generally predicted to be completely absorbed in humans. For log $P_{oct} > 4$ the permeability starts to decrease since very hydrophobic drugs generally have low aqueous solubility and partition at a slower rate from the cell membranes to the extracellular fluids (Raub et al 1993; Wils et al 1994). The F_1-DHA *n*-octanol/pH 7.4 Ringer's solution partition coefficient (log $P_{oct/Ringer}$ = 4.50) with its low aqueous solubility suggested a slow partition rate from the cell membranes to the extracellular fluids and allowed us to classify this substance as a low permeability compound. F_2-DHA showed a higher partition coefficient (log $P_{oct/Ringer}$ = 2.55) than artemisinin (log $P_{oct/Ringer}$ = 1.89). This result showed that increased hydrophobicity does not necessarily increase the ability of the molecule to be absorbed (F_2-DHA P_{app} is not very different from artemisinin P_{app}) and confirmed that the principal physicochemical properties (hydrogen-bonding properties, molecular size and shape, polarity, flexibility, charge/ionization properties) as a whole, rather than just hydrophobicity, are better predictors of the absorption potential.

Table 1 Physicochemical parameters for artemisinin and fluorinated derivatives (n = 3)

Compound	Molecular weight (Da)	S_0 (μg mL^{-1})	log $P_{oct/Ringer}$
Artemisinin	282.34	99.1 ± 3.3	1.89
F_1-DHA	380.41	1.00 ± 0.03	4.50
F_2-DHA	462.51	472.1 ± 10.1	2.55

F_1-DHA, 10β-(trifluoropropyloxy)dihydroartemisinin; F_2-DHA, 10-trifluoromethyl-16-[2-(hydroxyethyl)piperazine]; S_0, water solubility; log $P_{oct/Ringer}$, *n*-octanol/Ringer's solution partition coefficient. S_0 values represent the mean $\pm$ s.d.

Conclusions

Low transepithelial diffusion across the intestinal mucosa of F_1-DHA allowed us to classify this substance as a low permeability compound. This indicates that the introduction of a fluorinated substituent at the α-methylene carbon of DHA ethers, in order to provide protection against oxidative processes, constitutes an obstacle for absorption after oral administration. On the contrary, the high permeability of F_2-DHA and artemisinin across intestinal membrane indicates that the introduction of a fluoroalkyl group on a compound such as artemisinin in order to prolong its half-life does not constitute an obstacle for its absorption after oral administration. Moreover, the introduction of a polar substituent into the DHA structural scaffold increased the aqueous solubility of F_2-DHA relative to artemisinin. Solubility is likely to be even higher upon ionization in the acidic medium of the stomach.

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