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ORIGINAL PAPER

Antiprotozoal activity of ferroquine

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Abstract Ferroquine (FQ, SSR97193) is a synthetic compound currently in development for the treatment of malaria. The use of a single compound to treat several parasitoses would be very convenient for multi-infected patients and also for financial considerations. In this work, the activity of FQ was investigated on three other Protista parasites: Kinetoplastidae (*Leishmania* and *Trypanosoma*) and the cosmopolite parasite *Trichomonas vaginalis*. FQ exhibited a significant *in vitro* activity on *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense*, the agents of African trypanosomiasis in a range from 0.2 to 3.1 μM. In vivo, intraperitoneally administered FQ demonstrated a weak but significant trypanocidal activity at 100 μmol/kg, which is however higher than the maximum tolerated dose. The drop of the parasitemia of the treated mice was significantly related to the amount of injected FQ. Furthermore, this organometallic compound was responsible for a delay in the appearance of bloodstream parasites at 50 μmol/kg. However, it was not able to cure infected mice. Although no synergy was identified in vitro between FQ and pentamidine, these results justify further investigations by evaluating analogues in this chemical series.

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Introduction

Ferroquine (FQ, SSR97193; Fig. 1) is a compound in clinical development as antimalarial drug candidate (Biot et al. 2005; Mombo-Ngoma et al. 2011; Supan et al. 2012). Especially, this molecule is active against quinoline-resistant *Plasmodium falciparum* and its mechanism of action, in infected red blood cells, has been shown to be multifactorial: lipid targeting, inhibition of hemozoin formation, and reactive oxygen species generation (Henry et al. 2008; Dubar et al. 2008, 2012a, b). It is therefore of special interest to estimate the spectrum of antiparasitic activities of this antimalarial compound against other Protista parasites since the use of a single compound to treat several parasitoses would be very convenient for multi-infected patients. In this work, FQ activity was investigated against two Kinetoplastidae (*Leishmania* sp. and *Trypanosoma* sp.) since, in the past, the first organometallic drugs used in Kinetoplastids chemotherapy were arsenicals against Human African Trypanosomiasis (HAT) and antimonials against leishmaniasis (Nok 2003; Frézard et al. 2009). FQ was also evaluated against the cosmopolite parasite *Trichomonas vaginalis*.

Leishmaniasis and HAT are neglected tropical diseases for which the treatments, such as arsenicals and pentamidine for HAT and antimonials and amphotericin B for leishmaniasis, are toxic and generate drug resistance (Baneth et al. 2008; Simarro et al. 2011; Alvar et al. 2011; WHO 2012).

Urogenital trichomoniasis is a sexually transmitted infection (STI) affecting humans worldwide (Kirkcaldy et al. 2012). Metronidazole is the compound mostly used against the agent of this STI, *T. vaginalis*. Resistance against this drug is however currently growing, showing the need to find new trichomonacide drugs.

Overall, in the context of these parasitoses, it is necessary to develop new drugs to overcome the issues of resistance or

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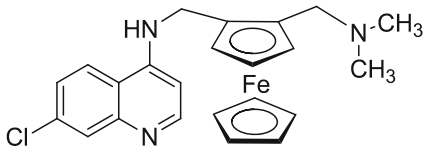

Fig. 1 Chemical structure of ferroquine, FQ (SSR97193)

toxicity of the current treatments. In the field of these diseases, the development of drugs able to target several parasites would also be useful for financial considerations.

Materials and methods

Trypanocidal efficacy

FQ was evaluated both in vitro and in vivo on the *Trypanosoma brucei brucei* CMP strain, responsible of trypanosomiasis of cattle in Africa, and which provokes mortality in mice within 3–4 days, according to the protocols previously described by Loiseau et al. (2000). Briefly, the bloodstream forms of *T. brucei brucei* were maintained in vitro for 48 h in the dark at 37 °C in a 5 % CO_2 atmosphere, in minimal essential medium (Gibco BRL) including 25 mM Hepes and Earle's salts and supplemented with 2 mM L-glutamine, 1 g/l of glucose, 10 ml/l of minimum essential medium non-essential amino acids (100×, Gibco BRL), 0.2 mM 2-mercaptoethanol, 2 mM sodium pyruvate, 0.1 mM hypoxanthine, 0.016 mM thymidine, 15 % heat-inactivated horse serum (Gibco BRL), and 50 μg/ml gentamycin. In vitro, the minimum effective concentration (MEC) was defined as the minimum concentration for which no viable parasite was observed microscopically after 24 h of drug–parasite co-incubation, and for which naive mice infected with treated parasites remained aparasitemic 30 days post-infection. Pentamidine di-isethionate, melarsamine dihydrochloride, and chloroquine diphosphate were used as reference compounds.

FQ was then evaluated in vitro in combination with pentamidine on the *T. brucei brucei* CMP strain in order to point out a potential synergy action between both compounds. The compounds were associated at the MEC/2 and successive dilutions of the mixture solution were performed using the isobologram method.

FQ was also evaluated in vitro on *Trypanosoma brucei gambiense* (strain FéoITMAP/1893), according to a similar protocol except the medium composition (Bourjot et al. 2010). The medium composition consisted of prepacked Iscove's modified Dulbecco's medium (Gibco, BRL) supplemented with 36 mM $NaHCO_3$, 1 mM hypoxanthine, 0.05 mM bathocuproine, 0.16 mM thymidine, 0.2 mM 2-mercaptoethanol, 1.5 mM L-cysteine, 10 % heat-inactivated foetal bovine serum, 100 IU penicillin and 100 μg/ml streptomycin. In this evaluation, the activity was expressed in concentration inhibiting the parasite growth by 50 % (IC_{50}) and the reference drugs used were pentamidine di-isethionate and melarsonyl potassium.

In vivo, Swiss mice were infected intraperitoneally with 10,000 trypanosomes (*T. brucei brucei* CMP strain) and then randomly allocated to groups and treated by intraperitoneal route with the compounds to be evaluated at 100, 50, 20, 10, and 5 μmol/kg at a single dose. Ten mice were used per batch. Control mice were treated with the reference compounds (pentamidine and melarsamine). The follow-up of parasitemia was monitored at days 2, 3, 4, and 30. The efficiency was evaluated with the survival time in comparison with control mice (mean survival time, 3.5±0.5 days).

Antileishmanial efficacy

The *in vitro* antileishmanial evaluation was carried out using intramacrophage amastigotes of *Leishmania donovani* LV9 strain as described by Peyron et al. (2005). The activity was expressed as IC_{50}. The reference drug used was amphotericin B. In addition, the cytotoxicity on murine peritoneal macrophages was observed microscopically and was expressed as maximum tolerated concentration (MTC).

Antitrichomonal efficacy

The antitrichomonal activity of FQ was assessed as described by Camuzat-Dedenis et al. (2001). Briefly, the metronidazole-sensitive CMP strain of *T. vaginalis* (Châtenay-Malabry Parasitology) was isolated from a woman in 1987 and stored as stabilate in liquid nitrogen with 6 % dimethyl sulfoxide. Culture tubes with fresh trypticase–yeast extract–maltose medium and filtered horse serum 10 % alone or with (in triplicate) the tested compound, were inoculated with 10^4 protozoa per milliliter. The tubes were cultivated in anaerobic conditions for 48 h at 35 °C and the number of parasites per milliliter in each tube determined with a haematocytometer (Kova slide 10, Boeringer). The results were estimated as the percentage of growth inhibition with untreated controls. The IC_{50} values were interpolated from the corresponding dose–response curve. Metronidazole (8823 R.P.) was the reference compound.

Statistical analysis

The mean number of parasites per microliter in treatment groups and controls were compared using Kruskal–Wallis analysis of variance and Mann and Whitney non-parametric tests for comparing two groups.

Results and discussion

Since the discovery of trypanocidal activity of arsenicals, some organometallic compounds have been found to have

Table 1 In vitro activity of ferroquine on *T. brucei brucei* and *T. brucei gambiense*. (n=3)

Compound	*T. brucei brucei* MEC (μM)	*T. brucei gambiense* IC_{50} ± SD (μM)
Ferroquine	3.1	0.20±0.01
Chloroquine	>25	–
Pentamidine di-isethionate	0.4	0.01±0.002
Melarsamine dihydrochloride	0.006	–
Melarsonyl potassium	–	0.03±0.001

In the three experiments, the same MEC value was obtained, according to the "Materials and methods" section

an activity on Trypanosomatids (Loiseau et al. 1992, 2001; Mbongo et al. 1997). FQ is a new organometallic drug in development for the treatment of malaria. In order to assess the spectrum of activity of this drug, we checked its potential against other protists.

Trypanocidal activity

Table 1 shows that FQ exhibited considerable in vitro trypanocidal activity with a MEC value at 3.1 μM on *T. brucei brucei*, whereas chloroquine, used as reference analogue compound, was inactive at 25 μM. It is not common to find compounds having a MEC value less than 5 μM on this strain and this promising result prompted us to search for a synergy action between FQ and pentamidine. Thus, trypanosomes were treated in vitro for a 24-h period according to the protocol described above with FQ and pentamidine in association but no synergy action was found. However, an in vitro trypanocidal activity of FQ was also observed on the agent of HAT, *T. brucei gambiense*, with an IC_{50} of 0.2 μM (Table 1). Altogether, these in vitro results show that FQ is active on both *T. brucei brucei* and *T. brucei gambiense*. Especially, *T. brucei gambiense* is more sensitive to FQ than *T. brucei brucei*. As for *Plasmodium*, the activity of FQ on *Trypanosoma* sp. could be ascribed to the ferrocene structure involved in the production of reactive oxygen species leading to membrane damages (Chavain et al. 2008; Dubar et al. 2011). The lysis of trypanosomes observed at low concentrations may reflect parasite membrane damage which may have occurred although no alteration of parasite morphology was seen.

In Swiss mice model, FQ was toxic at 100 μmol/kg whereas no toxicity was found at 50 μmol/kg (Table 2). FQ did not clear infection but at 50 and 20 μmol/kg, the appearance of parasites within the bloodstream was significantly delayed and the number of trypanosomes was reduced. However, the number of parasites suddenly increased at day 4 leading to a survival period on average 4.4 days longer than in the controls. This slight in vivo activity of FQ could be an added value of treatment of malaria/HAT with this drug, especially in endemic areas where both *Plasmodium* sp. and *Trypanosoma* sp. cohabit. In these double-infected patients, the use of a

Table 2 In vivo trypanocidal activity of ferroquine on the *T. brucei brucei* CMP strain/Swiss mice model

Compound	Dose		Parasitemia (number of parasites/μl blood) ± SD at				Mean survival time vs controls (days ± SD)	Number of mice cured/ total number of mice	Cure rate (%)	Toxicity: number of dead mice [day of death post-treatment]
	μmol/kg	mg/kg	Day 2	Day 3	Day 4	Day 30				
Ferroquine	100	50.60	0	0	20.6±3.4$^{*, **}$	–	4.4±1.1	2/10	20***	3[Day 3]; 1[Day 4]
	50	25.30	0	0	700±35$^{*, **}$	–	4.1±1.3	0/10	0	No toxicity
	20	10.12	0	26±3	1,300±169$^{*, **}$	–	2.9±0.5	0/10	0	No toxicity
	10	5.06	6.2±1.3	34±3	2,800±325$^{*, **}$	–	2.2±0.4	0/10	0	No toxicity
	5	2.53	10.4±1.0	40±2	4,000±507$^{*, **}$	–	1.4±0.6	0/10	0	No toxicity
Chloroquine diphosphate	100	51.5	20.7±3.1	400±28	40,000±3,603	–	0	0/10	0	No toxicity
Pentamidine di-isethionate	100	59.6	0	0	0**	0	> 30	10/10	100***	No toxicity
Melarsamine dihydrochloride	100	49.9	0	0	0**	0	> 30	10/10	100***	No toxicity
Controls	–	–	22.1±4.5	400±34	41,000±3,109	–	0[a]	0/10	0	No toxicity

[a] Mean survival time of control: 3.5±0.5 days. Results are expressed as mean survival times after death of controls

*p<0.001, parasitemia at day 4 of treated mice vs control

**p<0.001, parasitemia at day 4 between mice treated at 100, 50, 20, 10, 5 μmol/kg of ferroquine

***p<0.05, **p<0.01, cure rate of treated mice vs control

single molecule to treat both malaria and HAT would be very convenient.

Antileishmanial activity

FQ was not active in vitro at 20 μM against the *L. donovani* intramacrophage amastigotes, whereas chloroquine exhibited an IC_{50} value of 0.5 μM, and amphotericin B an IC_{50} value of 0.1 μM. The MTC on macrophages was 5 μM for FQ and 1 μM for chloroquine, therefore FQ appeared to be better tolerated by murine macrophages than chloroquine. The MTC of the chloroquine being close to its IC_{50}, the analysis of the antileishmanial activity of this molecule is not considered meaningful. Furthermore, the absence of in vitro antileishmanial activity of ferroquine was shown and thus in vivo evaluation appears dispensable.

Antitrichomonal activity

Since some quinolines exhibit slight anti-trichomonal activity, it was interesting to evaluate the contribution of a metallic moiety to a quinoline scaffold (Martinez-Grueiro et al. 2005). However, no activity of FQ was found against *T. vaginalis* even at 500 μM. Although the mechanism of action of quinoline on Kinetoplastids and Trichomonads remains unclear, such a result could be related to differences in biochemical pathways between Kinetoplatidae and Trichomonads.

Conclusion

FQ exhibited a significant trypanocidal activity in vitro on *T. brucei gambiense* as well as both in vitro and in vivo on *T. brucei brucei*. In vivo, a delay in parasite multiplication was observed but FQ did not allow to cure infected mice. In addition, no activity was found against *L. donovani* and *T. vaginalis*. Despite the limited activity observed, analogues and metabolites of FQ are worth of evaluation against African trypanosomes.

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