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European Journal of Pharmacology 484 (2004) 41–48
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Inhibition of hERG K^+ currents by antimalarial drugs in stably transfected HEK293 cells

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Received 16 September 2003; received in revised form 27 October 2003; accepted 4 November 2003

Abstract

Several antimalarial drugs are known to produce a QT interval prolongation via a blockade of the rapidly activating delayed rectifier K^+ current (I_{Kr}), encoded by the *human-ether-a-go-go-related gene* (hERG). We investigated the influence of lumefantrine and its major metabolite desbutyl-lumefantrine, as well as halofantrine, chloroquine, and mefloquine, on wild type hERG K^+ channels in stably transfected human embryonic kidney cells (HEK293) using the whole cell patch-clamp technique. All of the tested antimalarial drugs inhibited the hERG K^+ channels in a concentration- and time-dependent manner. Only halofantrine blocked hERG tail currents voltage-dependently. The ranking of the half-maximal inhibitory concentrations (IC_{50}) of the antimalarials was: halofantrine (0.04 μM) < chloroquine (2.5 μM) < mefloquine (2.6 μM) < desbutyl-lumefantrine (5.5 μM) < lumefantrine (8.1 μM). Lumefantrine and desbutyl-lumefantrine showed a slower inhibition of I_{Kr} than the other tested antimalarials. In conclusion, lumefantrine and desbutyl-lumefantrine inhibited significantly the hERG tail current with a higher IC_{50}-value than mefloquine, chloroquine and halofantrine. This, together with the calculated cardiac safety indices, suggests that lumefantrine and desbutyl-lumefantrine have a weaker proarrhythmic potential than their comparator compounds.

Keywords: hERG K^+ channel; Electrophysiology; Antimalarial drug; IC_{50}

1. Introduction

Malaria is one of the most widespread reasons for disease and death in tropical and subtropical areas of the world. Unfortunately, there has been a growing body of evidence that certain antimalarial drugs induce cardiotoxicity with respect to QT prolongation which may lead to life-threatening cardiac arrhythmias like Torsade de Pointes (Nosten and Price, 1995; Touze et al., 2002). For example, the widely used antimalarial drug halofantrine has been associated with a QT interval prolongation in both clinical trials (Nosten et al., 1993; Wesche et al., 2000; Abernethy et al., 2001) and in in vitro assays (Tie et al., 2000). Although the structural similarity between lumefantrine and the aryl-amino alcohol group of antimalarials (in particular halofantrine) has raised concerns over cardiotoxicity, there has been no evidence of QT interval prolongation and Torsade de Pointes in humans so far (Bakshi et al., 2000).

The QT interval of the electrocardiogram is the time period needed for depolarization and repolarization of ventricular myocytes and its prolongation is mainly caused by delayed repolarization. Human cardiac ventricular myocytes are mainly repolarized by the activity of outward K^+ currents. One of the major currents is the delayed rectifier K^+ current I_K, which consists of a rapidly and a slowly activating component (I_{Kr} and I_{Ks}; Sanguinetti and Jurkiewicz, 1990). The human ether-a-go-go-related gene (hERG) encodes the α-subunit of the voltage gated K^+ channels underlying the native current I_{Kr} (Sanguinetti et al., 1995; Trudeau et al., 1995). When transfected into heterologous systems such as human embryonic kidney cells (HEK293), hERG expresses a K^+ channel with properties similar to I_{Kr} (Zhou et al., 1998). Inhibition of the hERG current (I_{hERG}) can lead to a prolongation of the action potential duration and consequently of the QT interval of the electrocardiogram, which may, under certain circumstances, lead to the polymorphic ventricular tachyarrhythmia, Torsade de Pointes (Keating and Sanguinetti, 2001). A wide range of pharmacological agents of different therapeutic classes is known to induce QT prolongation by blocking I_{hERG}, including type III antiarrhythmics

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doi:10.1016/j.ejphar.2003.11.003

(Sanguinetti and Jurkiewicz, 1990; Spector et al., 1996), antihistaminics (Roy et al., 1996; Zhou et al., 1999) and antipsychotics (Rampe et al., 1998; Drolet et al., 1999a,b; Kang et al., 2000; review of: Buckley and Sanders, 2000). Although there is no clear relationship between the potency of a compound to block I_{hERG} and the likelihood to prolong QT and to induce Torsade de Pointes, it is generally accepted to consider a high potency (low IC_{50}-value) for I_{hERG} inhibition as a risk factor for fatal arrhythmias related to QT interval prolongation, especially if the free therapeutic plasma concentration of a drug is near or even below the IC_{50}-value for I_{hERG} inhibition (Webster et al., 2002). Hence, to compare the proarrhythmic potential of drugs it is reasonable to calculate cardiac safety indices by dividing the hERG current inhibiting potency (e.g. IC_{50}-value) by the respective therapeutic free plasma concentration (Crump and Cavero, 1999; Cavero et al., 2000). Antimalarial drugs represent a chemically diverse group of compounds. Although these drugs are associated with a higher risk for QT prolongation, the literature is poor regarding their respective effects on cardiac ion currents in relation to their chemical structure and no clear structure activity relationships related to a risk of I_{hERG} inhibition have yet been elucidated.

The purpose of the present study was to assess the proarrhythmic potential and the structure–activity relationships with regard to I_{hERG} inhibition of the antimalarial drugs lumefantrine, desbutyl-lumefantrine in comparison with halofantrine, chloroquine and mefloquine. Electrophysiological experiments on HEK293 cells stably expressing the hERG channel were conducted using the patch-clamp technique.

2. Materials and methods

2.1. Cell culture

HERG.T.HEK (HEK293 cells stably transfected with HERG cDNA) were obtained from the University of Wisconsin (Zhou et al., 1998). The cells were continuously maintained in and passaged using Minimum Essential Medium (MEM; Gibco-BRL, UK) supplemented with 10% foetal bovine serum (Gibco-BRL, UK), 1% non-essential amino acids (lot no. 1085497, Gibco-BRL, UK) and 0.4 mg/ml geneticin (Gibco-BRL, UK). For studies, the cells were plated onto sterile glass coverslips in 35 mm^2 dishes (containing 3 ml geneticin-free medium) at a density of $1.1–1.5 \times 10^5$ cells per dish. That allowed isolated cells to be selected for patch clamping. The dishes were stored in a humidified and gassed (5% CO_2) incubator at 37 °C.

2.2. Drugs and solutions

The pipette solution was prepared in batches, aliquoted and stored at −20 °C until the day of use. The composition of the pipette solution was (mM): KCl 130; $MgCl_2$ 1.0; Ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) 5; MgATP 5; HEPES 10; pH 7.2. Bath solution was prepared at least every 2 days and stored at room temperature when in use and refrigerated when not in use. The composition of the bath solution was (mM): NaCl 137; KCl 4; $CaCl_2$ 1.8; $MgCl_2$ 1.0; D-glucose 10; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) 10; pH 7.4. Lumefantrine (MW: 528.9; Novartis Pharma, Switzerland), desbutyl-lumefantrine (MW: 472.8; Novartis Pharma) and halofantrine (MW: 500.4; IDIS World Medicines) were prepared as a 10 mg/ml stock solution in dimethylsulfoxide (DMSO) (Sigma, UK). Mefloquine (MW: 378.3; Roche, Switzerland) was prepared as a 5 mg/ml stock solution in DMSO. Chloroquine (MW: 319.9; Sigma) was prepared as a 15 mg/ml stock solution in reverse osmosis (RO) water. The highest test concentration represents the solubility limit for each compound. The corresponding vehicle concentration used for each perfusion concentration was 1% DMSO. Stock solutions of E-4031 (Wako, Germany) (100 μM) were prepared in RO water, aliquoted and stored at −20 °C until use.

2.3. Electrophysiology

Currents were recorded at room temperature (20–22 °C), using the whole-cell patch-clamp technique. The coverslips of cells were transferred to the recording chamber mounted on the stage of an inverted microscope (Nicon Diaphot, Nicon Corporation, UK) and continuously perfused (at approximately 1–2 ml/min) with bath solution at room temperature. The electrode off-set potential was set to zero before the seal formation. Gigaohm seals (resistance range: 1.3–160 GΩ) were formed between the patch electrodes (filled with pipette solution; resistance range: 1.5–5.0 MΩ) and individual cells. The membrane across the electrode tip was ruptured using suction and the whole-cell patch-clamp configuration was established. Cancellations of the fast and slow capacitive transients were performed after establishment of the cell attached and the whole cell configurations. The serial resistance (Rs) was compensated throughout the experiment (70%). Currents were amplified using an Axopatch 200A amplifier (Axon Instruments, UK). Once a stable patch had been achieved, recording commenced in voltage-clamp mode, with the cell initially clamped at a holding potential of −80 mV. To induce the hERG current, the following voltage protocol was used: +20 mV for 4.8 s, −50 mV for 5 s and −80 mV for 5.2 s, giving a total pulse length of 15 s. Voltage-dependence of block was investigated by application of depolarisation pulses ranging from −60 to +60 mV with 10 mV increments. The hERG peak tail currents were recorded upon repolarization to −50 mV. The voltage protocol was run for a minimum of 10 times, and then test substance or comparator compound was perfused through the bath; fluid exchange took approximately 60 s. The test substance was allowed to equilibrate for at least a further 5 min. Either single or multiple concentrations of the test substance were studied in each

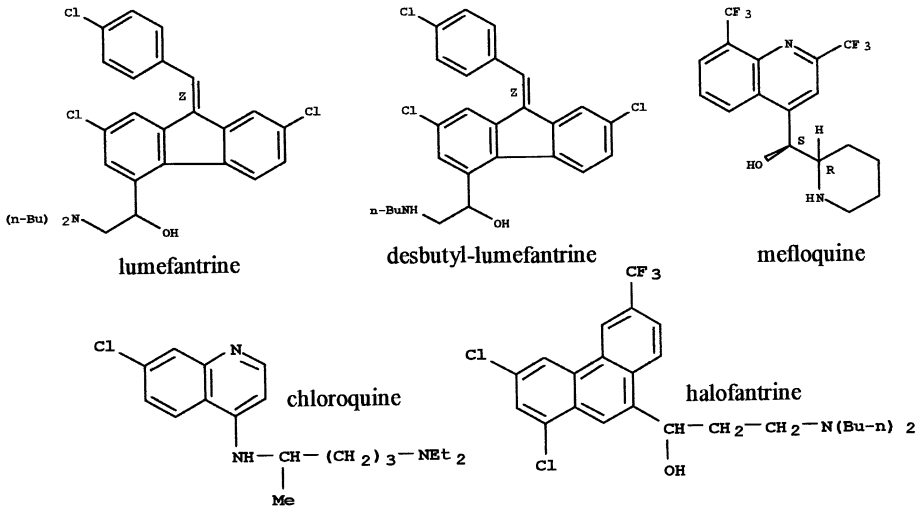

Fig. 1. Molecular structures of the investigated antimalarial drugs.

cell. The change in the magnitude of the currents in the vehicle treated cells was used to give an indication of any current rundown that was observed. Finally, the effect of E-4031was assessed by comparing the values for tail current after treatment with the vehicle and following 10 min perfusion with 100 nM E-4031. The data capture and analysis were undertaken using CED Signal v1.81 software.

2.4. Statistical analysis

The steady-state tail current was measured and entered into an Excel spreadsheet. Each value represents the mean current recorded over 4 voltage pulses. The residual current (% of control) for each test substance or comparator compound concentration was then calculated. Tail currents in the presence of test substance/comparator compound were first corrected for the mean vehicle rundown observed. The concentration–response curve was plotted and the IC_{25}-, IC_{50}- and IC_{75}-values were estimated from the sigmoid function fit to the data where appropriate. Concentrations of 0.1, 1, 3 and 10 μg/ml lumefantrine, 0.1, 1, 3 and 10 μg/ml desbutyl-lumefantrine, 0.001, 0.01, 0.03, 0.1 and 1 μg/ml halofantrine and 0.05, 0.5, 1 and 5 μg/ml mefloquine were compared to the 1% DMSO vehicle treatment group; and

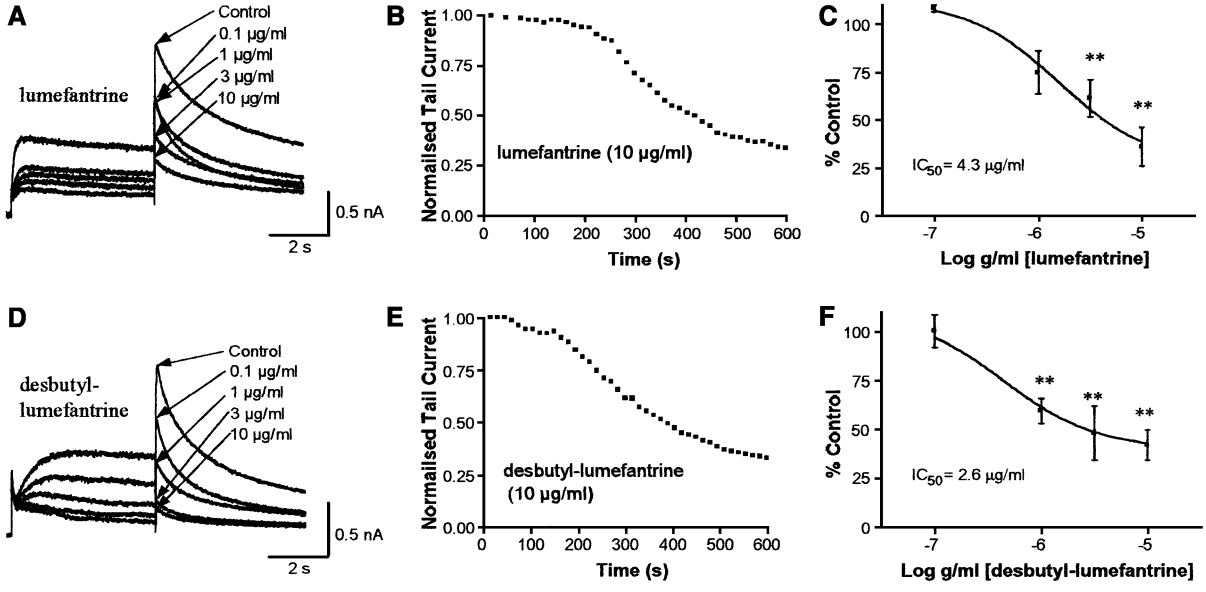

Fig. 2. Effects of lumefantrine (A–C) and desbutyl-lumefantrine (D–F) on hERG currents expressed in HEK293 cells. (A) and (D) show representative current traces recorded in one cell under control conditions and in the presence of different drug concentrations. (B) and (E) show a diary of the tail currents recorded in (A) and (D). (C) and (F) show the dose–response relationships for the hERG tail current block. Error bars denote S.E.M. ($n=4–6$); $^{**}P<0.01$ (ANOVA, followed by Dunnett's test).

0.005, 0.05, 0.5, 5 and 15 μg/ml chloroquine were compared to the 0.1% RO water treatment group using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. Statistical significance was assumed when $P < 0.05$. This analysis was performed in Excel 97 and GraphPad Prism v2.01 operating in Windows NT v4.0.

3. Results

Fig. 1 shows the chemical structures of five antimalarial drugs, which were electrophysiologically tested for their ability to inhibit I_{hERG}. In these experiments, a 4.8-s depolarization to +20 mV from a holding potential of −80 mV was followed by 5-s repolarization to −50 mV to produce large, slowly deactivating tail currents characteristic of hERG K^+ channels (Sanguinetti et al., 1995; Roy et al., 1996). The currents in the presence of each concentration of the respective drug were corrected for the mean vehicle rundown observed, and the concentration–response curves were plotted. Each drug concentration was applied to four or five cells.

3.1. Effects of lumefantrine and desbutyl-lumefantrine on hERG currents expressed in HEK293 cells

Fig. 2 describes the effects of lumefantrine (A–C) and desbutyl-lumefantrine (D–F) on hERG currents. The effects of both antimalarial drugs were studied at concentrations of 0.1, 1, 3 and 10 μg/ml. Lumefantrine inhibited the hERG tail current in a concentration-dependent manner, with a significant inhibition of the tail current observed at concentrations of 3 μg/ml and above. The estimated IC_{25}-value for lumefantrine block of the hERG tail current was 1.2 μg/ml (2.27 μM). The estimated IC_{50}-value was approximately four-fold higher at 4.3 μg/ml (8.13 μM). An IC_{75}-value could not be estimated for lumefantrine, as the maximum degree of inhibition was approximately 65% at the highest concentration tested (vehicle corrected data).

Desbutyl-lumefantrine also inhibited the hERG tail current in a concentration-dependent manner, with a significant inhibition of the tail current observed at concentrations of ≥1 μg/ml. The estimated IC_{25}-value for desbutyl-lumefantrine block of the hERG tail current was 0.4 μg/ml (0.84 μM). The estimated IC_{50}-value was approximately seven-fold higher at 2.6 μg/ml (5.49 μM). An IC_{75}-value

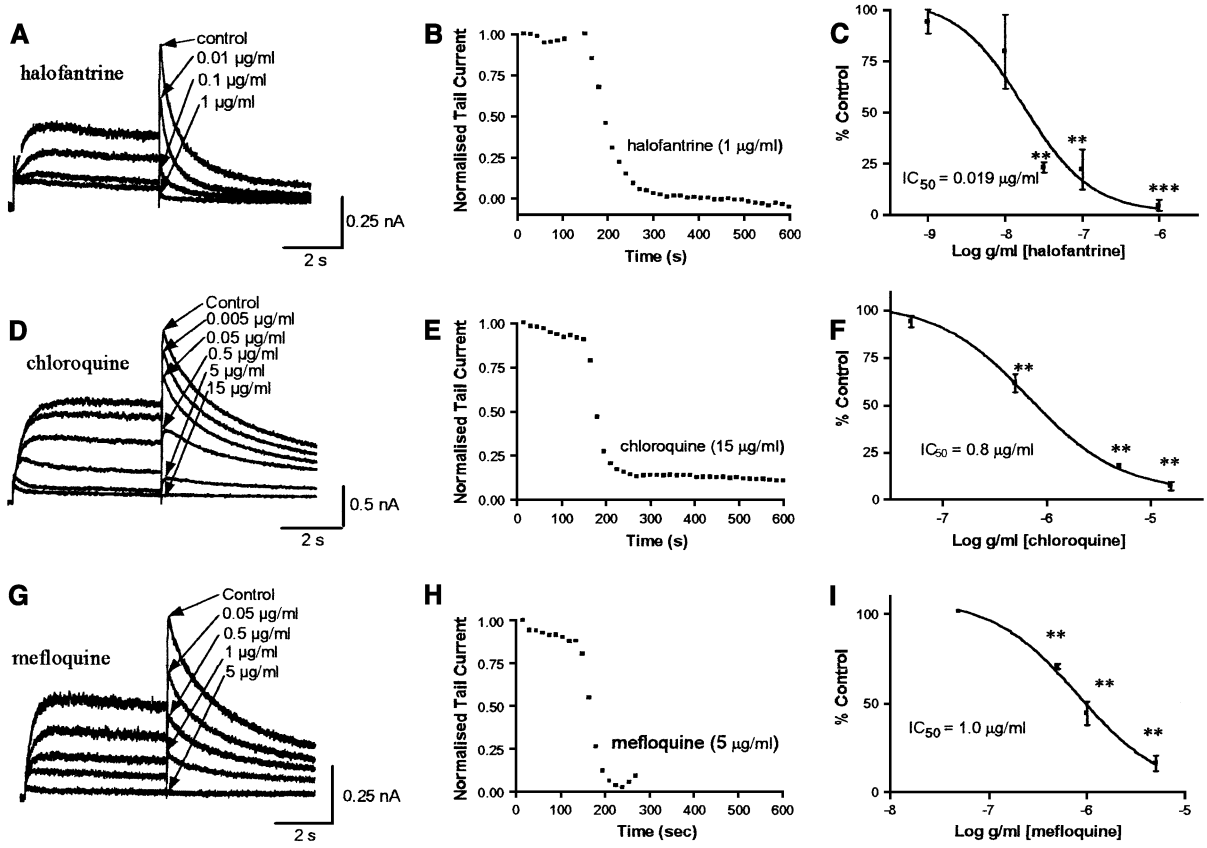

Fig. 3. Effects of halofantrine (A–C), chloroquine (D–F) and mefloquine (G–I) on hERG currents expressed in HEK293 cells. (A), (D) and (G) show representative current traces recorded in one cell under control conditions and in the presence of different drug concentrations. (B), (E) and (H) show a diary of the tail currents recorded in (A) and (D). (C), (F) and (I) show the dose–response relationships for the hERG tail current block measured after 600 s of incubation. Error bars denote S.E.M. ($n = 4–6$); $^{**}P < 0.01$, $^{***}P < 0.001$, (ANOVA, followed by Dunnett's test).

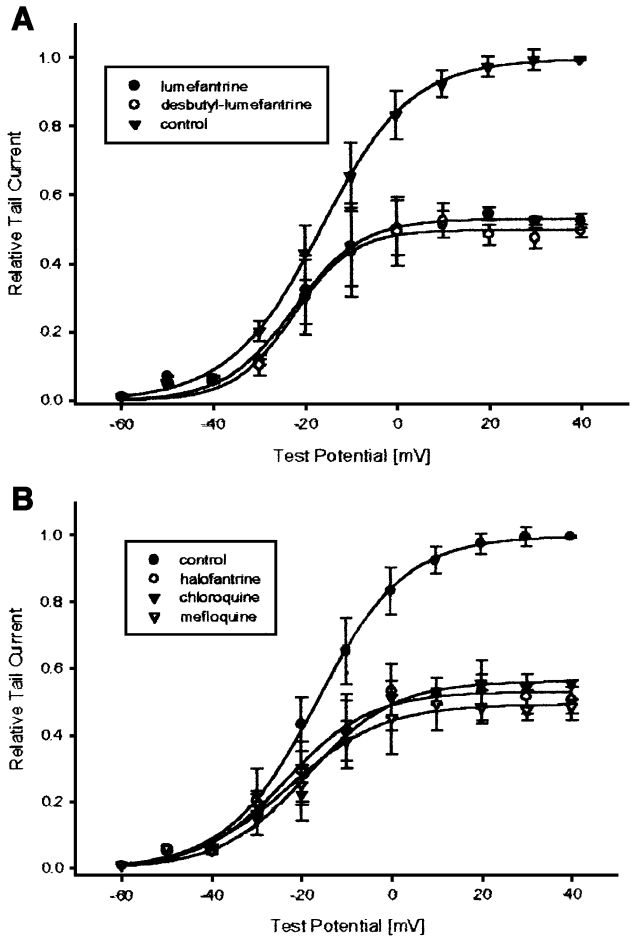

Fig. 4. Effect of antimalarial drugs on the hERG peak tail current at different test potentials. A shows $I-V$ relationships for tail currents under control conditions and in the presence of 8.1 μM lumefantrine and 5.5 μM desbutyl-lumefantrine, respectively. B shows $I-V$ relationships for tail currents under control conditions and in the presence of 0.04 μM halofantrine, 2.5 μM chloroquine and 2.6 μM mefloquine. Tail currents were normalized to the amplitude of the maximal control current and fitted with Boltzmann functions ($n=4$ cells per antimalarial drug).

could also not be estimated for desbutyl-lumefantrine, as the maximum degree of inhibition was approximately 60% at the highest concentration tested (vehicle corrected data). The onset of the I_{hERG} inhibition was comparable for lumefantrine and its major metabolite desbutyl-lumefantrine (Fig. 2B,E). I_{hERG} started to decrease about 200 s after the drugs had entered the bath chamber. Inhibition of I_{hERG} reached a steady state approximately 400 s later. Complete fluid exchange in the bath and reaching nominal drug concentration took approximately 60 s.

The $I-V$ relationships for tail currents under control conditions and after application of 8.1 μM lumefantrine and 5.5 μM desbutyl-lumefantrine are shown in Fig. 4A. The drug concentrations were chosen based on the determined IC_{50}-values. The tail currents increased with voltage at − 50 mV and plateaued at test potentials positive to + 10 mV. The half maximal activation voltage ($V_{1/2}$) shifted non-significantly from -17.1 ± 0.9 mV ($n=4$) in controls to -22.1 ± 2.6 mV ($n=4$) after lumefantrine and to -20.8 ± 1.2 mV ($n=4$) after desbutyl-lumefantrine treatment, respectively.

3.2. Effects of halofantrine, chloroquine and mefloquine on the hERG current

Fig. 3 describes the effect of halofantrine on hERG tail current was studied at concentrations of 0.001, 0.01, 0.03, 0.1 and 1 μg/ml. halofantrine inhibited I_{hERG} in a concentration-dependent manner, with a significant inhibition of tail current observed at concentrations of ≥ 0.03 μg/ml. The estimated IC_{25}-, IC_{50}- and IC_{75}-values for halofantrine block of hERG tail currents were 6.9 ng/ml (0.01 μM), 19 ng/ml (0.04 μM) and 59 ng/ml (0.12 μM), respectively. Chloroquine inhibited the hERG tail current also in a concentration-dependent manner, with a significant inhibition of tail current observed at concentrations of ≥ 0.5 μg/ml. The estimated IC_{25}-value for chloroquine block of the hERG tail current was 0.3 μg/ml (0.94 μM), the estimated IC_{50}-value was 0.8 μg/ml (2.5 μM) and the IC_{75}-value for chloroquine block of the hERG tail current was determined to be 2.6 μg/ml (8.11 μM). The effect of mefloquine was studied at concentrations of 0.05, 0.5, 1 and 5 μg/ml. Mefloquine inhibited I_{hERG} in a concentration-dependent manner, with a significant inhibition of the tail current observed at concentrations of 0.5 μg/ml. The estimated IC_{25}-, IC_{50}-and IC_{75}-values for mefloquine block of the hERG tail current were 0.4 μg/ml (1.06 μM), 1.0 μg/ml (2.64 μM) and 2.8 μg/ml (7.39 μM), respectively.

The onset of the I_{hERG} inhibition was comparable for these three antimalarial drugs. The hERG current decreased about 150 s after the drugs had entered the bath chamber and reached a steady state very rapidly within 100 s compared to approximately 400 s for lumefantrine and desbutyl-lumefantrine (Fig. 3B,E,H).

The $I-V$ relationships for tail currents under control conditions and after application of 0.04 μM halofantrine, 2.5 μM chloroquine and 2.6 μM mefloquine are shown in Fig. 4B. The drug concentrations were chosen based on the determined IC_{50}-values. $V_{1/2}$ shifted significantly from -17.1 ± 0.9 mV ($n=4$) in controls to -23.2 ± 2.9 mV ($n=4$, $P<0.05$) after halofantrine treatment. Application of chloroquine and mefloquine

Table 1
Table comparison

Drug	IC_{50} (μM)	FTPC[a] (μM)	CSI
Lumefantrine	8.13	0.17	48
Desbutyl-lumefantrine	5.49	ND	ND
Halofantrine	0.04	0.57	0.07
Chloroquine	2.50	0.41	6.1
Mefloquine	2.64	0.05	53

Overview of the estimated IC_{50}-values for the block of hERG current by the tested antimalarial drugs, their free therapeutic plasma concentration (FTPC) and the respective calculated caridac safety indices (CSI).

[a] Morant and Ruppaner, 2001.

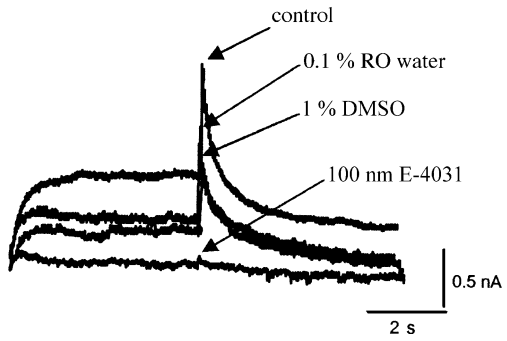

Fig. 5. Original recordings of the effect of 0.1% reverse osmosis water, 1% DMSO and 100 nM E-4031 on the hERG tail current after 10 min of incubation.

shifted $V_{1/2}$ not significantly to -17.9 ± 1.1 mV ($n=4$) and to -21.9 ± 2.5 mV ($n=4$), respectively.

All estimated IC_{50}-values for the I_{hERG} inhibition of the tested antimalarial drugs are summarized in Table 1 together with their free therapeutic plasma concentrations and the calculated cardiac safety indices. Cardiac safety indices were calculated by division of the IC_{50}-values of I_{hERG} inhibition by the free therapeutic plasma levels (Crump and Cavero, 1999; Cavero et al., 2000; Webster et al., 2002).

3.3. Effect of E-4031 and vehicles

After 10 min of exposure to 0.1% RO water, a residual hERG tail current of $78.8 \pm 2.4\%$ (Fig. 5) was produced. This equates to a decrease in the tail current of 21.2% ($n=4$). Treatment with 1% DMSO produced a residual tail current of $68.1 \pm 2.4\%$ after 10 min of exposure. This equates to a decrease in the tail current of 31.9% ($n=7$). E-4031 (100 nM) inhibited the hERG tail current by $96.3 \pm 4.1\%$ ($n=12$, $P<0.001$) when compared with the values recorded under control conditions (Fig. 5).

4. Discussion

This is the first report of a direct blockade of the hERG channel activity by desbutyl-lumefantrine and of the comparison of its hERG inhibitory potency with four other antimalarials, among them its parent compound lumefantrine (also known as benflumetol).

All agents tested (lumefantrine, desbutyl-lumefantrine, halofantrine, chloroquine and mefloquine) inhibited I_{hERG} of stably transfected HEK293 cells in a concentration-and time-dependent manner. Our results reveal halofantrine to be the most potent hERG channel inhibitor and lumefantrine to be the weakest one. The ranking of these antimalarial agents with regard to their I_{hERG} blocking potency, based on their IC_{50}-values, was: halofantrine $\ll$ chloroquine < mefloquin < desbutyl-lumefantrine < lumefantrine. Halofantrine is known to induce a prolongation of QT/QTc (>30 ms) in man at therapeutic doses, most likely via an inhibition of I_{Kr} (Nosten et al., 1993; Wesche et al., 2000; Mbai et al., 2002; Touze et al., 2002). The results of our study support these findings and confirm that halofantrine is a very potent hERG channel inhibitor with an IC_{50}-value of 0.04 μM, a value similar to that previously described in the literature (Mbai et al., 2002).

A striking difference in the time course of the hERG current inhibition between lumefantrine, desbutyl-lumefantrine and halofantrine, chloroquine and mefloquine was detected. The onset of inhibition was similar for all five compounds at their highest tested concentration but lumefantrine and desbutyl-lumefantrine showed a much slower development of I_{hERG} inhibition. This could be explained by lumefantrine's and desbutyl-lumefantrine's higher IC_{50}-value, suggesting a slightly weaker affinity for the hERG channel protein or by a different mode of channel inhibition.

Desbutyl-lumefantrine is a metabolite and, as a 2,3-benzindene, is closely related in structure to lumefantrine. Lumefantrine carries a dibutylamino-ethanol side chain in the 4-position, whereas desbutyl-lumefantrine has a monobutylamino-ethanol group at this position. Orally administered lumefantrine is *N*-debutylated in the liver by P-450A cytochromes to its major metabolite desbutyl-lumefantrine. This contributes to the antimalarial activity of the parent drug, as shown by its four times higher potency in the in vitro response of *Plasmodium falciparum* (Noedl et al., 2001).

Interestingly, the *N*-debutylated isoform of halofantrine showed a 3.3 fold higher IC_{50}-value compared with its parent compound (Mbai et al., 2002), whereas in our study the *N*-debutylation of lumefantrine led to 1.5-fold lower IC_{50}-value. This suggests that the *N*-butylgroup does not play a major role in modifying the interaction of these antimalarial compounds with the hERG channel. At present there are only a few structure–activity approaches which try to find structural similarities between hERG blocking agents (Wempe, 2001; Ekins et al., 2002; Cavalli et al., 2002; Roche et al., 2002). Compounds with a tri-alkylated nitrogen like halofantrine and lumefantrine, which undergo protonation in aqueous solution or cellular environment, display inhibitory effects on hERG and other voltage gated K^+ channels. One of the hypotheses suggests that quaternary ammonium ions block the channel pore by interacting with the four tyrosine residues at the extracellular entryway (Wempe, 2001). Additonally, inhibitory effects on the hERG channel may also be influenced by the molecular mass/charge ratio and by the adjacent moieties of the tri-alkylated nitrogen (Wempe, 2001).

An aromatic ring in proximity to the alkylated nitrogen showing a strong field effect could effectively shield the quaternary ammonium ion and therefore decrease the affinity for hERG K^+ channels. Increased potency in blocking I_{hERG} has been suggested when the length of the alkyl side chain attached to the nitrogen is increased (Cavalli et al., 2002). Moreover, our data suggest that the longer the distance is between the trialkylated nitrogen and the aro-

matic system (created by alkyl residues), the more potent the compound is with regard to I_{hERG} inhibition. This could explain the strong inhibition of I_{hERG} by halofantrine. However, secondary amines such as desbutyl-lumefantrine, mefloquine or chloroquine also showed an inhibition of I_{hERG}. Further quantitative structure activity studies have to be done to create a valid pharmacophore that discriminates between the very diverse classes of drugs inhibiting the hERG K^+ channel.

The HEK293 cells used in this study expressed only the hERG α-subunit because it is still under debate if minK-related protein (MiRP1) could act as an essential constituent of the hERG channel complex carrying I_{Kr} and if the pharmacological and biophysical properties of the hERG α-subunit are influenced by a potential in vivo association with MiRP1. Recently it has been suggested that coexpression of the hERG α-subunit with MiRP1 in *Xenopus laevis* oocytes reconstitutes native I_{Kr} (Abbott et al., 1999). In the meantime several laboratories have shown that the pharmacological sensitivity and biophysical properties of the hERG α-subunit are only weakly modified or even unaffected by the coexpression of MiRP1 (Kamiya et al., 2001; Scherer et al., 2002). Additionally, a recent study demonstrated that the inhibitory activity of quinidine, E-4031 and dofetilide on hERG currents expressed in Chinese hamster ovary cells and in *X. laevis* oocytes was not modulated by the presence of MiRP1 and that the sensitivity of the α-subunit hERG tail current to these three drugs was indistinguishable from that of native I_{Kr} (Weerapura et al., 2002).

We focussed mainly on the IC_{50}-determination of the antimalarial drugs since it is not clearly established whether voltage-or time-dependent inhibition of hERG K^+ channels under in vitro conditions is predictive enough for the proarrhythmic potential of a drug. However, according to our experiments only halofantrine was able to to inhibit hERG tail current voltage-dependently by inducing a hyperpolarizing shift of 6.1 mV which is comparable to the observations of Tie et al. (2000). Voltage-dependent inhibition of hERG currents has been reported as a common characteristic of open-channel blocking compounds such as dofetilide (Snyders and Chaudhary, 1996).

Several authors suggested a calculation of cardiac safety indices for compounds as a useful tool to assess the likelihood of inducing cardiac dysrhythmias, i.e. IC_{50}-values of I_{hERG} inhibition divided by the free therapeutic plasma concentration (Crump and Cavero, 1999; Food and Drug Administration, 2003; Cavero et al., 2000; Webster et al., 2002). The order of the safety margin for the antimalarial compounds based on their cardiac safety indices (see Table 1) was: halofantrine (0.07) ≪ chloroquine (6.2) < lumefantrine (48) < mefloquine (50). The free therapeutic plasma concentration for desbutyl-lumefantrine has not been determined yet. Clinical trials have shown that only halofantrine (Wesche et al., 2000) and chloroquine (Bustos et al., 1994) are able to produce a significant QT/QTc prolongation, whereas no QT prolongation was observed for lumefantrine, desbutyl-lumefantrine and mefloquine (van Vugt et al., 1999; Lefevre et al., 2001; Bindschedler et al., 2002). These findings demonstrate that the IC_{50}-value for I_{hERG} inhibition alone is not sufficient to predict the proarrhythmic potentials of a drug because all of the tested antimalarials showed a significant inhibition of I_{hERG} but only the two with the lowest cardiac safety index were positive in man. Chloroquine and mefloquine have nearly identical hERG IC_{50}-values but only mefloquine with a 10 × higher cardiac safety index shows no adverse cardiac side effects in the clinic. Additionally, this study provides additional evidence consistent with an interpretation that lower cardiac safety indices are associated with a higher risk for QT prolongation. However, the potential QT prolongation could theoretically be counterbalanced by impacts on other types of cardiac ion channels. Other in vivo factors, like an accumulation in the myocardium, could lead to discrepancies compared with in vitro results. This could be another reason why lumefantrine and desbutyl-lumefantrine may have no torsadogenic effect. The blockade of the hERG current is one risk factor for compounds under development, which could translate into cardiotoxic events in patients when combined with other factors known to prolong QT (e.g. hypokalemia, female gender, bradicardia, etc.) or when the compound will be co-prescribed with other cardioactive drugs (e.g. negative chronotropic effect, increase of sodium and/or calcium inward currents, etc.).

We showed that lumefantrine and desbutyl-lumefantrine inhibited the hERG tail current with similar IC_{50}-values. The proarrhythmic risk of lumefantrine, desbutyl-lumefantrine and mefloquine may be reduced, compared with halofantrine and chloroquine, because of the considerably higher cardiac safety index.

Acknowledgements

The authors wish to thank the staff of Quintiles Limited (Edinburgh, UK), especially Kirsty McGullogh for major experimental contributions.

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