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Short communication

Effects of fluoroquinolones on HERG currents

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Abstract

We have investigated the effects of four fluoroquinolones on the human ether-à-go-go-related gene (HERG) mediated K^+ currents to evaluate their potential to induce QT-prolongation. HERG currents were measured from stably transfected Chinese hamster ovary (CHO) cells by means of the patch-clamp technique. Bath application of sparfloxacin, moxifloxacin and grepafloxacin produced an inhibition of HERG outward currents at -40 mV with EC_{50} of 13.5 ± 0.8, 41.2 ± 2.0 and 37.5 ± 3.3 μg/ml, respectively. Current inhibitions were reversible after washout of the compounds. By contrast, ciprofloxacin at concentrations of up to 100 μg/ml did not effect HERG outward currents.

Keywords: HERG; QT-prolongation; Fluoroquinolones; Patch-clamp technique

1. Introduction

Because of an increasing number of pathogens, which have become resistant to commonly used antibiotics (e.g. penicillins and macrolides), it is an important challenge in present drug development to find new antibiotics. The interest in treating infectious diseases (e.g. pneumonia, chronic bronchitis) with a new class of antibiotics, the fluoroquinolones, is rising (review: Appelbaum, 1999; Blondeau, 1999). According to recent studies, a quinolone-induced QT-prolongation with a potential for associated ventricular tachyarrhythmias is discussed (Jaillon et al., 1996; Ball, 2000). Frequently, QT-prolongations are correlated with changes in cardiac ion channel activity. In particular, a blockade of the human ether-à-go-go-related gene (HERG) K^+ channel, which plays an important role for the repolarization of ventricular action potentials, can induce QT-prolongation (Curran et al., 1995; Sanguinetti, 1999). Therefore, we investigated the effects of the fluoroquinolones ciprofloxacin, sparfloxacin, moxifloxacin, and grepafloxacin on the HERG K^+ channel.

2. Material and methods

Investigations were performed with stably transfected Chinese hamster ovary (CHO) cells expressing the HERG channel. The HERG protein carried a flag-tag at the C-terminus. This allowed us to continuously control the cellular expression of HERG protein by immunostaining. Cells were grown in 50-ml flasks (Nunc, Roskilde, Denmark) in 6-ml minimum essential medium (MEM)-ALPHA (Gibco 22571-020) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 1% (v/v) penicillin/streptomycin/L-glutamin solution (Gibco 10378-016) and GENETICIN-418 (Gibco 10131-019) at 37°C in a 5% CO_2-atmosphere. The cells were plated at a density of 1.25×10^4 cells/ml on plastic dishes. [For the experimental cells were superfused with the following solution (in mM): NaCl 130, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1, glucose 5, and HEPES 10. The pH was adjusted to 7.4 with NaOH. Micropipettes were filled with the following solution (in mM): KCl 130, HEPES 10, EGTA 1, ATP-Mg 2, and glucose 5. The pH was adjusted to 7.2 with KOH]. Whole-cell patch-clamp experiments were performed in the voltage-clamp mode (Hamill et al., 1981) using borosilicate glass tubes (GC 150, Clark Electromedical Instruments, Pangbourne, UK). Current signals were amplified and digitized by an EPC-9 patch-clamp amplifier (HEKA-Electronics), stored and analyzed on a personal

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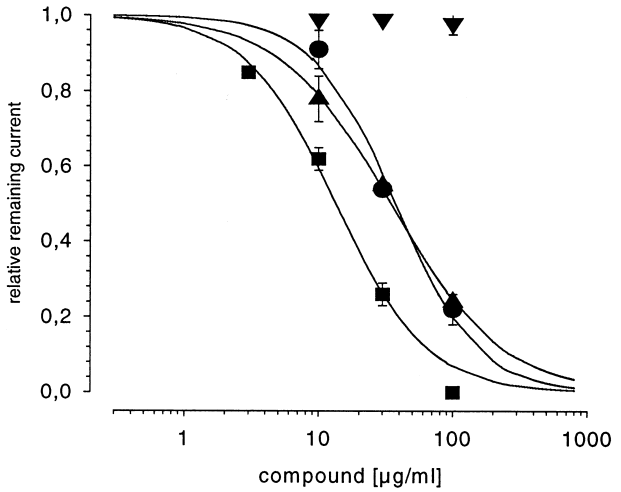

Fig. 1. Concentration response relationships of four fluoroquinolones. Comparison of the effect of the fluoroquinolones ciprofloxacin, grepafloxacin, moxifloxacin and sparfloxacin on HERG K^+ currents. Concentration/response relations were calculated by a non-linear least squares fit of equation $f = 1/(1+(x/EC_{50})^{n_H})$ to the data. Hill coefficient (n_H) and the half-maximum inhibiting concentration (EC_{50}) were calculated. Symbols explained in Table 1.

computer using the Pulse/Pulsefit software (HEKA, Lambrecht, Germany). Resistance of the pipettes ranged between 2 and 3 MΩ. Experiments were conducted at room temperature (23 ± 1°C).

HERG K^+ currents were elicited by 1-s depolarizing voltage steps to +20 mV from a holding potential of −80 mV followed by a 1-s repolarization back to −40 mV. Outward tail-currents were analysed. Cells responded with averaged tail current amplitudes of about 977.34 ± 528.39 ($n = 41$) at −40 mV. After six control stimuli, the extracellular solution was changed to a solution containing the test compound at concentrations of 10, 30 and 100 μg/ml. In the case of sparfloxacin, a concentration of 3 μg/ml was also applied. Forty-four additional stimuli were given. Stimulation frequency was 0.1 Hz. Concentrations are given in microgram per milliliter for a better comparison with anti-microbial active concentrations and pharmacokinetic data.

3. Results

Ciprofloxacin did not reduce the HERG current amplitude. The comparison of potencies from grepafloxacin, moxifloxacin, and sparfloxacin to block the HERG channel shows the latter to have the greatest inhibition effect on the HERG current amplitude. The effects occurred immediately after exposure to the cells and reached a steady state within 2 min of superfusion. The compounds can be washed-out fast and almost completely. At highest applied concentrations of the fluoroquinolones, current amplitudes recovered on the average of 85–90% of initial current amplitude. HERG current reductions at −40 mV were calculated by dividing the current amplitude in the presence of a fluoroquinolone (44th stimulus) through the initial current amplitude under control conditions (6th stimulus). Grepafloxacin, moxifloxacin, and sparfloxacin, each displayed a concentration-dependent inhibition of the HERG current amplitude. Concentration/response relations were calculated by a non-linear least squares fit of equation: $f = 1/(1+(x/EC_{50})^{n_H})$ to the data (Fig. 1). Grepafloxacin and moxifloxacin suppressed the HERG current with EC_{50} of 37.5 ± 3.3 μg/ml and 41.2 ± 2.0 μg/ml and $n_H = 1.4 \pm 0.2$ and 1.1 ± 0.1, respectively. Sparfloxacin inhibited HERG currents with an EC_{50} of 13.5 ± 0.8 μg/ml and n_H of 1.3 ± 0.1 (Table 1).

The results indicated that ciprofloxacin does not interact with HERG channels at tested concentrations. Grepafloxacin and moxifloxacine showed an equal potency for the reduction of HERG K^+ current, in comparison to sparfloxacin that displayed the highest potency of the four fluoroquinolones tested. In order to correlate the electrophysiologically determined EC_{50} concentrations with the potential potency of compounds to induce QT-prolongation, we calculated the free plasma concentration for each of the fluoroquinolones after single 400-mg oral dose

Table 1
Comparison between EC_{50} of different fluoroquinolones calculated from electrophysiological experiments in CHO cells stably expressing the HERG channel and between the mean free plasma concentrations measured in humans

Compound	Symbol	EC_{50} ± S.E.M. (μg/ml)	n_H ± S.E.M.	Mean free plasma concentration (μg/ml)	EC_{50}/mean free plasma concentration (μg/ml)
Ciprofloxacin	▼	–	–	2.03[a]	–
Grepafloxacin	●	37.5 ± 3.3	1.4 ± 0.2	0.46[a,b]	82
Moxifloxacin	▲	41.2 ± 2.0	1.1 ± 0.1	1.68[a,c]	25
Sparfloxacin	■	13.5 ± 0.8	1.3 ± 0.1	0.72[a,d]	19

Values for the EC_{50} and the Hill coefficient. Data are presented as means ± standard error of the mean (S.E.M.; $n = 3$–4). Mean free plasma concentrations were calculated from mean maximum concentrations observed in plasma after single 400-mg oral dose application of each of the fluoroquinolones and the plasma protein binding according to current literature.

[a] Data from web site: http://cp.gsm.com.

[b] Data from Geddes, 1999.

[c] Data from Wise et al., 1999.

[d] Data from Morgenroth et al., 1999.

application in humans (Table 1). For grepafloxacin, the free plasma concentration is estimated to 0.46 μg/ml, which is approximately 80-fold less than the calculated EC_{50} of 37.5 μg/ml. In cases of moxifloxacin and sparfloxacin, the free plasma concentrations are about 25- and 20-fold less than the estimated EC_{50} values (Table 1). The present experiments allowed us to estimate the percentage of HERG current reduction after single 400-mg dose application of each compound. Thus, calculated free concentrations for sparfloxacin, moxifloxacin, and grepafloxacin corresponded to a current reduction of 2.4%, 4.2%, and 0.2%, respectively.

4. Discussion

The results indicate that the investigated compounds only induce minor reductions of HERG currents at the calculated free plasma levels. However, it has to be taken into account that secondary factors (e.g. hereditary QT-syndrome or hypokaliaemia), or elevated plasma levels of compounds caused by alterations in their pharmacokinetics, may generate a situation where some of these compounds could induce QT-prolongation in patients.

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