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Absence of clinically important HERG channel blockade by three compounds that inhibit phosphodiesterase 5—sildenafil, tadalafil, and vardenafil

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Abstract

Compounds that inhibit phosphodiesterase 5 (PDE5) have been developed for the treatment of erectile dysfunction. Because men with erectile dysfunction frequently have comorbid cardiovascular disease, they may have limited cardiac repolarization reserve and be at risk of arrhythmia if treated with medications that prolong ventricular repolarization. The human ether-a-go-go related gene (HERG) channel is important for repolarization in human myocardium and is a common target for drugs that prolong the QT interval. We studied the ability of three compounds that inhibit PDE5—sildenafil, tadalafil, and vardenafil—to block the HERG channel. Using a whole cell variant of the patch-clamp method, the HERG current was measured in a stably transfected human embryonic kidney cell line expressing the HERG channel. The compounds produced dose-dependent reductions in HERG current amplitude over a concentration range of 0.1 to 100 μM. The IC_{50} values were 12.8 μM for vardenafil and 33.3 μM for sildenafil. Because the maximum soluble concentration of tadalafil (100 μM) produced only a 50.9% inhibition of the HERG current amplitude, the IC_{50} value for tadalafil could not be determined with the Hill equation. Tadalafil had the weakest capacity to block the HERG channel, producing a 50.9% blockade at the maximum soluble concentration (100 μM), compared with 86.2% for vardenafil (100 μM) and 75.2% for sildenafil (100 μM). In conclusion, the concentrations of the PDE5 inhibitors required to evoke a 50% inhibition of the HERG current were well above reported therapeutic plasma concentrations of free and total compound. None of the three compounds was a potent blocker of the HERG channel.

Keywords: Phosphodiesterase 5; HERG channel; Potassium channel; Sildenafil; Tadalafil; Vardenafil

1. Introduction

The prevalence of cardiovascular disease is high among men with erectile dysfunction (Feldman et al., 1994; Kloner et al., 2003; Walczak et al., 2002). Patients with cardiovascular disease may have reduced repolarization reserve and be at increased risk for arrhythmia when taking medications that alter ventricular repolarization (Roden, 1998). Many different classes of drugs have been temporally associated with cardiac arrhythmia and sudden cardiac death (Anderson et al., 2002; Redfern et al., 2003). Some of these drugs have been shown to prolong the QT interval of the electrocardiogram (ECG) (Tan et al., 1995; Thomas, 1994). The lengthening of the QT interval usually reflects slowing of action potential repolarization in ventricular myocytes (Tan et al., 1995; Thomas, 1994). Although the mechanism is not certain, excessive prolongation of the QT interval can lead to the potentially life-threatening ventricular tachyarrhythmia, torsade de pointes. Several transmembrane ion currents contribute to the duration of the

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cardiac action potential, including inward depolarizing Na^+ and Ca^{2+} currents and outward repolarizing K^+ currents (mainly the different components of the delayed rectifier K^+ current) (Tamargo et al., 2004). A common mechanism by which drugs delay repolarization and prolong the QT interval is by blocking one or more outward K^+ currents (Barry et al., 1998; Jeron et al., 2000).

In humans, the most common channel linked to drug-induced QT interval prolongation is the rapid component of the delayed rectifier K^+ channel (I_{Kr}) (Mitcheson et al., 2000). The human ether-a-go-go-related gene, HERG, encodes the pore-forming unit of the channel responsible for the delayed rectifier K^+ current (Trudeau et al., 1995; Warmke and Ganetzky, 1994). Many drugs associated with QT interval prolongation have been found to block HERG channels (Rampe et al., 1998; Redfern et al., 2003; Splawski et al., 2000; Suessbrich et al., 1997).

Sildenafil, tadalafil, and vardenafil, are used to treat erectile dysfunction based on their capacity to amplify nitric-oxide-stimulated vasodilation by inhibiting the breakdown of cGMP (Ignarro et al., 1990; Lue, 2000). Because cardiovascular disease is common in men with erectile dysfunction, it is important to determine the potential for medications used to treat erectile dysfunction to prolong ventricular repolarization. Many factors influence the QT interval and its measurement (Roden, 1998). Although changes of up to 5 ms may occur in the heart rate corrected QT interval with placebo treatment alone, drugs that increase the risk of sudden cardiac death may produce only slightly greater increases (i.e., 5–10 ms) (Malik, 2001; Malik and Camm, 2001). In a study examining the effects of therapeutic and supratherapeutic doses of vardenafil and sildenafil compared with placebo on the human QT interval, the Fridericia-corrected QT interval increased by 8 ms and 10 ms after vardenafil 10 mg and 80 mg, respectively, and by 6 ms and 9 ms after sildenafil 50 mg and 400 mg, respectively (Ilson et al., 2004). In a separate study of the effects of supratherapeutic doses of tadalafil on the QT interval in human subjects, the Fridericia-corrected QT interval increased by 3.5 ms after tadalafil 100 mg compared with placebo (Cialis® Package Insert, 2003). To evaluate further the effects of these compounds on ventricular repolarization, we measured the capacity of sildenafil, tadalafil, and vardenafil to block the HERG channel using whole-cell patch clamp methodology.

2. Materials and methods

2.1. Transfection and cell culture

Human embryonic kidney (HEK 293) cells were stably transfected through the lipofectamine method (Saldeen et al., 1996) with the HERG clone. Cells were maintained in minimum essential medium with Earle's salts supplemented with nonessential amino acids, sodium pyruvate, penicillin, streptomycin, geneticin, and fetal bovine serum.

2.2. Solutions

Drugs were dissolved in dimethyl sulfoxide (DMSO) to make 10 mM stock solutions on the day of experimentation. Dilution of stock solutions was made immediately before the experiments to create the desired concentrations. The external solution (solution bathing the cell) used for recording the HERG potassium currents had an ionic composition of (in mM): 137 NaCl, 4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 11 dextrose, 10 HEPES, adjusted to a pH of 7.4 with NaOH. The internal (pipette) potassium solution had an ionic composition of (in mM): 130 KCl, 1 $MgCl_2$, 5 NaATP, 5 EGTA, 5 HEPES, adjusted to a pH of 7.2 with KOH. Experiments were performed at 37 ± 1 °C.

2.3. Data acquisition and analysis

Currents were measured using the whole-cell variant of the patch-clamp method (Hamill et al., 1981). Glass pipettes were pulled from borosilicate glass by a horizontal puller (Sutter Instruments, USA), then fire polished to produce tip openings of 1–2 μm. Pipette tip resistance was approximately 1.0–2.0 MΩ when filled with internal potassium solution. Bath temperature was measured by a thermistor placed near the cell under study and was maintained by a thermoelectric device (model no. 806-7243-01, Cambion/Midland Ross, Cambridge, MA). An Axopatch 1-B amplifier (Axon Instruments, Foster City, CA) was used for whole-cell voltage clamping. Creation of voltage clamp pulses and data acquisition was controlled with an IBM PC utilizing pClamp software (version 6.0, Axon Instruments).

After rupture of the cell membrane (entering whole-cell mode), current kinetics, and amplitudes were allowed to stabilize as the cell was dialyzed with internal solution and paced at 0.1 Hz (typically 5–7 min). If the HERG current did not stabilize over this time, the cell was discarded. Currents were considered stable if 2–3 currents elicited by a series of voltage pulses given at 0.1 Hz were superimposable. Rate-dependent effects were determined by a train of 20 depolarizing voltage steps at 1, 2, and 3 Hz from a holding potential of −75 mV. Peak HERG current was measured for the first and last pulse of the train under control conditions and in the presence of the drug. Because current amplitude may be reduced with rapid pacing in the absence of drug (control), to avoid overestimating the degree of current reduction in the presence of drug, rate-dependent drug effects were calculated by subtracting the current reduction in control from that observed in the presence of drug in the same cell at the same pacing rate. Peak HERG current was measured as the maximum outward deflection of the tail current elicited upon return to −40 mV (Fig. 1A,B).

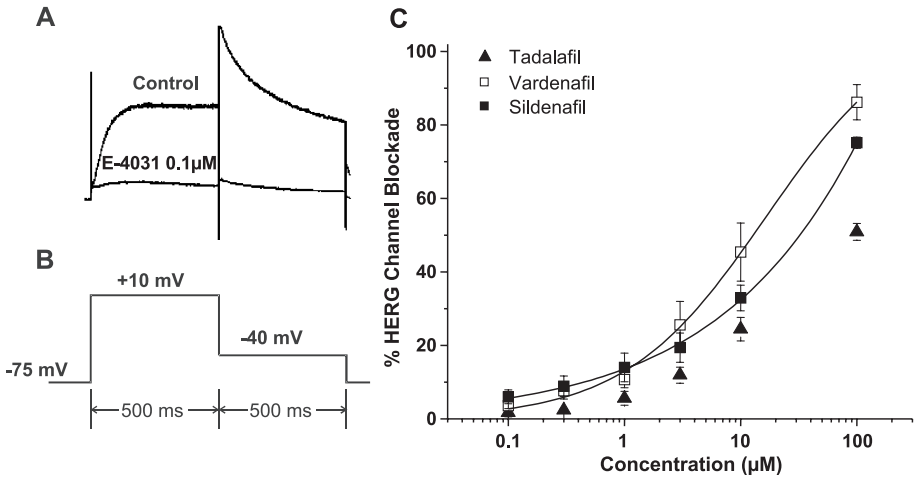

Fig. 1. (A) Current recorded from a HERG-transfected HEK-293 cell indicating that the current is E-4031 sensitive. (B) Pulse protocol used to elicit HERG currents at 37 °C. (C) Concentration–response relationship for block of HERG channel by sildenafil (n=5), tadalafil (n=5–6), and vardenafil (n=5). Data for sildenafil and vardenafil were fit with the equation given in Materials and methods. Data are mean±S.E.M.

2.4. Statistical analysis

Raw data and mean±S.E.M. are given. Data are given as percentage reduction of current amplitude. This was measured as current reduction after a steady state drug effect had been reached in the presence of drug relative to current amplitude before drug was introduced (control). Each cell served as its own control. Drug effects were compared by a paired Student's t-test for significance (P<0.05) using MicroCal Origin version 6.0 software (Microcal Software, Northampton, MA).

Log-linear plots were created of the mean percent blockade±S.E.M. at the concentrations that were tested. A nonlinear curve fitting routine was utilized to fit a three-parameter Hill equation to the results for vardenafil and sildenafil using MicroCal Origin, version 6.0 software. The equation is of the form:

$$y = V_{\max}\frac{x^n}{k^n + x^n}$$

where $V_{\max}$, k, and n are unconstrained variables (except $V_{\max}$>0). Because the $V_{\max}$ parameter is not constrained to 100%, the parameter k does not represent an IC_{50} (or IC_{20}) for ion channel blockade. Thus, the IC_{50} and IC_{20} values were calculated from the inverse of the previous equation by solving for x with y=50 and y=20, respectively. A curve was not fit to the tadalafil results because the maximum inhibition that could be achieved with tadalafil at the maximum achievable concentration in solution was 50.9%. IC_{50} and IC_{20} values for tadalafil were estimated from visual inspection of the data.

3. Results

Fig. 1 (panel C) shows that sildenafil, tadalafil, and vardenafil dose-dependently reduced the HERG current amplitude. Using nonlinear curve fitting and the Hill equation, the mean dose–response relationships for sildenafil (n=5) and vardenafil (n=5) yielded IC_{50} and IC_{20} values in the low micromolar range (Table 1). Tadalafil could not be accurately fit with the nonlinear equation, but the estimated IC_{50} was 100 μM (50.9%±2.3% reduction in HERG current amplitude at 100 μM, n=5). To show that HERG channels continued to be sensitive to blockade in the presence of 100 μM tadalafil, the HERG channel blocker, E-4031, was added to the bath (Herzberg et al., 1998; Imamura et al., 1998; Shinmura et al., 1998). After the addition of 0.1 μM E-4031 in the presence of 100 μM tadalafil, the HERG current was blocked by 87.8%. It is unlikely that the reduction in current amplitude was the result of vehicle effects or current rundown, because over the course of 8–10

Table 1
Mean C_{max}, IC_{20}, IC_{50} and ratios of IC_{20} and IC_{50} to mean C_{max} for tadalafil, vardenafil, and sildenafil

Drug	Molecular weight	C_{max}-total (ng/ml)	C_{max}-total (μM)	Protein binding (%)	C_{max}-free (μM)	IC_{20} (μM)	IC_{50} (μM)	IC_{20}/ C_{max}-free	IC_{50}/ C_{max}-free	IC_{50}/ C_{max}-total
Tadalafil	389.4	351	0.901	94	0.054	7.0	100	130	1852	111
Vardenafil	488.6	31.8	0.065	95	0.003	1.9	12	633	4000	185
Sildenafil	474.6	400	0.843	96	0.034	2.9	33	85	971	39

Molecular weights were calculated as the free base. C_{max}-total represents the mean maximum total plasma concentration (free plus protein-bound drug) measured after the administration of a single maximum therapeutic oral dose of each drug. C_{max}-free=mean maximum free (unbound) plasma concentration. The IC_{20} and IC_{50} values for tadalafil were estimated by visual inspection of the data in Fig. 1.

min the addition of a 100:1 dilution of DMSO produced a 1.4±1.5% (n=11) reduction in the HERG current amplitude. The lowest dilution of DMSO used in this study was 100:1.

To test for rate-dependent effects that may occur at different heart rates, cells were paced over the range of 1–3 Hz. Over this pacing range, there was no substantial rate-dependent block of HERG (less than 2%) when cells were exposed to 100 μM of sildenafil (n=5), tadalafil (n=5), or vardenafil (n=5).

4. Discussion

Sildenafil, tadalafil, and vardenafil were not potent HERG channel blockers. The IC_{50} values for vardenafil and sildenafil were in the micromolar range and blockade by tadalafil, at the maximum soluble concentration (100 micromolar), was 51%. Our findings with sildenafil (IC_{50}=33 μM) are comparable to those reported by Geelen et al. (2000) where the IC_{50} for HERG channel blockade by sildenafil was reported as 100 μM.

Drugs that affect the QT interval commonly block the HERG channel in the nanomolar range (Rampe et al., 1998; Redfern et al., 2003), whereas the IC_{50} values for sildenafil, tadalafil, and vardenafil were in the micromolar range (Table 1). However, the most appropriate consideration for these results is in the context of (1) the degree of HERG channel blockade necessary to evoke a clinically important delay in ventricular repolarization and thereby increase the risk of ventricular tachydysrhythmia; (2) the plasma concentration of drug (free and/or total) necessary to produce a clinically important degree of HERG channel blockade; and (3) the plasma concentration of drug expected after administering a therapeutic dose either alone or under conditions likely to increase plasma concentrations (e.g., conditions of inhibited drug metabolism or overdose).

There is no consensus regarding a confirmed threshold for the predication of clinically important HERG channel blockade based on an in vitro model. However, there are several hypotheses regarding this threshold. Some authors believe that drugs that fail to produce a 20% blockade at the highest achievable free plasma concentration will be free of liability for the ventricular arrhythmia torsade de pointes (Webster et al., 2002). Yet other researchers have suggested that drugs for which the IC_{50} is at least 30-fold greater than the highest achievable free or total plasma concentration will be free of liability for torsade de pointes (Redfern et al., 2003; Webster et al., 2002).

Limited data are available with regard to expected maximum plasma concentrations after oral administration of sildenafil, tadalafil, or vardenafil. However, comparable data for the arithmetic mean C_{max} value for single administrations of maximum therapeutic doses (i.e., tadalafil [20 mg], vardenafil [20 mg], sildenafil [100 mg]) in classical clinical pharmacology studies are available: tadalafil 351 ng/ml (data on file, Lilly Research Laboratories); vardenafil 31.8 ng/ml (Klotz et al., 2002); sildenafil 400 ng/ml (Viagra® Package Insert, 2001). Although maximum C_{max} values would be useful in estimating a worst-case clinical relevance of HERG channel blockade, the mean C_{max} values do allow relative comparisons of the three PDE5 inhibitors. Table 1 provides mean C_{max} values at the highest intended therapeutic doses, IC_{20} and IC_{50} values for HERG channel blockade, and the ratios of the IC values to the mean C_{max} concentrations. For all three compounds, the C_{max}-free concentrations are less than 2% of the IC_{20} values, and the IC_{50} values are well over 30-fold greater than the C_{max}-free concentrations. Table 1 also shows that none of the compounds block the HERG channel, on routine administration, to an extent that would predict an increased risk of arrhythmia based on this potential mechanism of delaying ventricular repolarization.

Changes in drug metabolism, protein binding, or overdose would affect the values in Table 1 such that if the metabolism of sildenafil, tadalafil, or vardenafil were significantly inhibited, then their plasma concentrations might approach a threshold where a given compound might have a relevant effect on the HERG channel (Muirhead et al., 2000). However, the degree of metabolic inhibition required for such a scenario would be considerable.

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