Gastrointestinal Prokinetic Drugs have Different Affinity for the Human Cardiac Human Ether-à-gogo K⁺ Channel

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ABSTRACT

Agonists of the serotonin 5-hydroxytryptamine 4 (5-HT4) receptor are widely used to activate motility in the gastrointestinal tract. Among these, cisapride was recently withdrawn from the U.S. market because of its proarrhythmic effects. Cisapride is a potent blocker of human ether-à-gogo (HERG) K⁺ channels and prolongs the cardiac action potential in a reverse use dependence manner. We compared the effects of four different 5-HT4 receptor agonists (cisapride, prucalopride, renzapride and mosapride) on cloned HERG channels with the objective to evaluate and compare their proarrhythmic potential. K⁺ currents from HERG-transfected COS-7 cells were recorded under physiological conditions using the whole cell configuration of the patch-clamp technique. Short (500 ms) depolarizing pulses were used and following deactivating HERG currents were measured. Cisapride inhibited the HERG channels in a concentration-dependent manner with an IC₅₀ of 2.4 10⁻⁷ M. The IC₅₀ value for prucalopride to block HERG (5.7 10⁻⁸ M) was 20-fold higher than that of cisapride. Renzapride was slightly more potent than prucalopride (IC₅₀ = 1.8 10⁻⁸ M). Mosapride produced no significant effects on the recombinant HERG current. The voltage dependence of HERG block was also investigated. The block mediated by cisapride or renzapride was voltage-dependent whereas that produced by prucalopride was not. We conclude that the rank order of potency of 5-HT4 agonists to block HERG is cisapride > renzapride > prucalopride > mosapride. We also conclude that 5-HT4 agonists devoid of side effects on the HERG current such as mosapride can be found as a safe alternative to cisapride.

Drugs that activate the serotonin receptor subtype 5-HT4 are widely used to facilitate or restore motility in the gastrointestinal tract (Tonini, 1996). Among these, cisapride, a benzamide derivative, was first marketed in the United States in 1993. As of December 31, 1999, the use of cisapride has been associated with 341 reports of heart rhythm disturbances including QT prolongation, syncope, serious ventricular arrhythmia such as torsades-de-pointes, and early after depolarization. Cisapride was subsequently withdrawn from the United States market (Food and Drug Administration, 2000). In isolated rabbit Purkinje fibers (Puisieux et al., 1996) and guinea pig isolated papillary muscle (Kii and Ito, 1997), cisapride causes prolongation of action potential in a reverse use dependence manner and early after depolarization.

Repolarization of cardiac myocytes is mainly triggered by activation of outward K⁺ currents including the delayed rectifier current I_K which has two components, I_Kr and I_Ks (Sanguinetti and Jurkiewicz, 1990). The human ether-à-gogo related gene, HERG, encodes the K⁺ channel that is responsible for the rapid component of the delayed rectifier current I_Kr found in both human ventricles and atria (Curran et al., 1995; Sanguinetti et al., 1995). A growing number of drugs associated with QT prolongation and its concomitant risks of arrhythmia has been shown to block I_Kr or cloned HERG (Witchel and Hancox, 2000). Therefore, the affinity of drugs for HERG channels appears as a risk factor for arrhythmias.

Cisapride is a potent inhibitor of HERG in mammalian heterologous expression systems as well as in native cardiac tissue (Mohammad et al., 1997; Rampe et al., 1997; Drolet et al., 1998). Other 5-HT4 receptor agonists are currently under investigation as an alternative to cisapride (Fig. 1). Renzapride, another benzamide derivative, is being considered for the treatment of irritable bowel syndrome. Prucalopride belongs to a new class of medications known as benzofurancarboxamides and is currently being developed for treatment of chronic constipation (Bouras et al., 2001). In clinical studies, the benzamide derivative mosapride alleviates gastroesophageal reflux disease (Kanaizumi et al., 1991) and is commercially available in Japan. Comparative studies of cisapride and mosapride activity on cardiac electrophysiological parameters in rabbits and guinea pigs indicate the absence of effect of mosapride in the context of QT prolongation (Carlsson et al., 1997; Kii and Ito, 1997). The investigations on the

**ABBREVIATIONS:** 5-HT4, 5-hydroxytryptamine 4; HERG, human ether-à-gogo related gene; PEI, polyethylenimine; APD, action potential duration; I_Kr, rapidly activating delayed rectifier K⁺ current.
action potential of other structurally related 5-HT4 receptor agonists such as renzapride and prucalopride has not been conducted yet. The purpose of the present study was to compare the effects of four prokinetic agents on cloned HERG channel activity recorded in physiological conditions with the objective to evaluate their potential proarrhythmic effects. We show that 5-HT4 agonists devoted of side effects on the HERG current can be identified.

Materials and Methods

Cell Culture and Transfection. African green monkey kidney cells transformed with SV40 (COS-7) obtained from the American Type Culture Collection (Manassas, VA) were cultured in Dulbecco's modified Eagle's medium, which contained L-glutamine (2 mM), 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere with 5% CO2 (Invitrogen, Paisley, Scotland). Cells cultured in Petri dishes were transfected using polyethylenimine (PEI) vector as described elsewhere (Pollard et al., 1998) 24 h prior to the experiments. HERG cDNA (a kind gift from S. Kupershmidt, D. Snyder, and D. Roden, Nashville, TN) was cloned into the mammalian expression vector pSI (Promega, Madison, WI) under the control of a simplex herpes virus enhance-promoter (SV40). Briefly, 0.4 μg of pSI-HERG and 1.6 μg of pTR-green fluorescent protein plasmids per milliliter of culture medium were complexed with PEI using a PEI/cDNA ratio of 5 equivalents free-Mg2+, 2 mM K2ATP, pH 7.2 with KOH, whereas the extracellular medium used to record K+ currents contained 145 mM sodium-glucuronate, 4 mM potassium-glucuronate, 7 mM hemi calcium-gluconate (1 mM free-Ca2+), 2 mM EGTA, 2 mM free-Mg2+, 5 mM HEPES, 5 mM glucose, pH 7.4 with NaOH. The intracellular medium contained 145 mM potassium-glucuronate, 5 mM HEPES, 2 mM EGTA, 2 mM hemi magnesium-glucuronate (0.1 mM free-Mg2+), 2 mM K2ATP, pH 7.2 with KOH, whereas the extracellular medium used to record K+ currents contained 145 mM sodium-glucuronate, 4 mM potassium-glucuronate, 7 mM hemi calcium-gluconate (1 mM free-Ca2+), 4 mM hemi magnesium-glucuronate (1 mM free-Mg2+), 5 mM HEPES, 5 mM glucose, pH 7.4 with NaOH (all from Sigma-Aldrich Chimie SARL, St. Quentin Fallavier, France). Free activities were calculated using software designed by G. L. Smith (University of Glasgow, UK).

Electrophysiology. Ionic currents from HERG-transfected COS-7 cells maintained at 35°C were recorded using the whole cell configuration of the patch-clamp technique. Cells were placed on the stage of an inverted microscope and continuously superfused with the standard extracellular solution. Patch pipettes with a tip resistance of 2.5 to 5 MΩ were electrically connected to a patch-clamp amplifier (Axopatch 200A, Axon Instruments, Foster City, CA). Stimulation, data recording, and analysis were performed through an A/D converter (Tecmar TM100 Labmaster, Scientific Solutions, Solon, OH) and Acquis1 software (Bio-Logic, Claix, France). A microperfusion system allowed local application and rapid change of the different experimental solutions warmed at 35°C. The cell membrane capacitance was determined by analyzing the kinetics of the capacitive current recorded during a step to −70 mV applied for 50 ms from a holding potential of −60 mV. The membrane potential was clamped at a holding potential of −80 mV. A voltage step was applied every 3 s to +10 mV for 500 ms and then to −60 mV for 500 ms when tail currents were measured. Current values were normalized using cell capacitance. Patch-clamp measurements are presented as the mean ± S.E.M. Statistical significance of the observed effects was assessed by means of the one-way or two-way analysis of variance when needed. A value of p < 0.05 was considered significant.

Solutions and Drugs. The standard extracellular medium contained 145 mM NaCl, 4 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 5 mM HEPES, 5 mM glucose, pH adjusted to 7.4 with NaOH. The intracellular medium contained 145 mM potassium-glucuronate, 5 mM HEPES, 2 mM EGTA, 2 mM hemi magnesium-glucuronate (0.1 mM free-Mg2+), 2 mM K2ATP, pH 7.2 with KOH, whereas the extracellular medium used to record K+ currents contained 145 mM sodium-glucuronate, 4 mM potassium-glucuronate, 7 mM hemi calcium-gluconate (1 mM free-Ca2+), 4 mM hemi magnesium-glucuronate (1 mM free-Mg2+), 5 mM HEPES, 5 mM glucose, pH 7.4 with NaOH (all from Sigma-Aldrich Chimie SARL, St. Quentin Fallavier, France). Free activities were calculated using software designed by G. L. Smith (University of Glasgow, UK).

Cisapride was diluted in dimethyl sulfoxide to reach a concentration of 3 · 10−3 M. Mosapride and renzapride were dissolved in distilled water. Prucalopride was diluted in acetic acid and then distilled water to obtain a 3 · 10−2 M stock solution (10% acetic acid). The stock solutions were then further diluted with the Cl−-free extracellular medium to the final concentration. Dimethyl sulfoxide or acetic acid was added in experimental solutions when needed to reach the same final concentration (1% or 0.1%, respectively). The pH value of each experimental solution was adjusted to 7.4.

Results

Effects of Cisapride on Recombinant HERG K+ Currents. Upon transfection with HERG cDNA, COS-7 cells expressed a large K+ current with biophysical properties that were reminiscent of those from the rapid component of the cardiac delayed rectifier K+ current recorded in human cardiac cells (Fig. 2A, top; Wang et al., 1994). When repolarized to −60 mV, expressing COS-7 cells exhibited a large
deactivating current (I tail). Cisapride (3 \times 10^{-6} M) induced a slow decay of the activating current recorded at +10 mV with a lesser effect at the initial phase of activation. This pattern of inhibition was observed during the drug application at steady state until cisapride was washed out. In the presence of cisapride, the deactivating current density decreased in a dose-dependent manner from 10.4 pA/pF in control conditions (control), after 1 min (1), or 2 min (2) exposure to 3 \times 10^{-6} M cisapride and after washout. The cell maintained at −80 mV was depolarized for 500 ms to +10 mV and then repolarized to −60 mV for 500 ms when deactivating current was measured (I tail: vertical bar, 500 pA; horizontal bar, 200 ms); bottom, tail current amplitude versus time. Horizontal bars denote increasing cisapride concentrations (3 \times 10^{-7} M, 3 \times 10^{-6} M, 3 \times 10^{-5} M, and 3 \times 10^{-4} M). Same voltage protocol as in top (frequency, 0.33 Hz; time scale, 2 min). B, current-voltage relationship for activating current density in control (Ipp/Cm, □) and in the presence of 3 \times 10^{-6} M cisapride (■, n = 5). Voltage protocol consisted of 500 ms depolarizing and hyperpolarizing prepulses to various potentials between −100 and +60 mV followed by a test pulse to −60 mV for 500 ms. Holding potential, −80 mV; frequency, 0.33 Hz. C, current-voltage relationship for the deactivating tail current density under control conditions (I tail/Cm; ○) and with 3 \times 10^{-6} M cisapride (●, n = 5). Statistical significance versus I tail/Cm in control conditions: *, p < 0.05; **, p < 0.01. Inset, inhibition ratio (I tail control − I tail late) versus prepulse potential. D, dose-response curve for HERG K^+ tail current (n = 6). Tail current was normalized to the control value and expressed as a function of the drug concentration. Solid line, fit of experimental data to the Hill equation: \( y = a + (d/(1 + (x/c)^n)) \) where x is the drug concentration, and b the Hill coefficient. IC_{50} value was calculated as the drug concentration for which y = 50%. Statistical significance versus I tail/Cm in control conditions: *, p < 0.05; ***, p < 0.001.

**Effects of Prucalopride on HERG K^+ Currents Expressed in COS-7 Cells.** The effects of prucalopride were also evaluated on recombinant HERG current. At 3 \times 10^{-6} M, prucalopride significantly blocked both the activating and deactivating HERG K^+ current (p < 0.001; Fig. 3A). Unlike cisapride, the block of the activating current produced by prucalopride reached a steady state within the 500 ms duration of the test pulse (compare Fig. 2A and Fig. 3A). Furthermore, prucalopride block did not show voltage dependence (Fig. 3B, inset) and the inhibition level was conserved during the repolarizing pulse after depolarization to +40 mV (42 ±
16% versus 44 ± 16%; inhibition percentage of I tail init and I tail late, respectively; n = 4; N.S.). The IC\textsubscript{50} value for prucalopride to block recombinant HERG current (5.7 \times 10\textsuperscript{-6} M, n = 8; Fig. 3C) was more than 20-fold higher than that for cisapride. The effects of prucalopride were partially reversed upon washout of the drug (not illustrated).

**Effects of Renzapride on Recombinant HERG.** We also evaluated the effects of renzapride on HERG K\textsuperscript{+} current. As illustrated in Fig. 4, renzapride inhibited activating and deactivating current in a voltage- and dose-dependent manner. The renzapride-induced block of the tail current was significantly more effective at depolarized potentials (Fig. 4B, inset). In that sense, the block of the HERG current produced by renzapride more closely resembles that produced by cisapride than the block produced by prucalopride, which was voltage-independent. The recovery from the block could also be observed when the membrane potential was repolarized. After depolarization to +40 mV, I tail init was significantly more inhibited than I tail late (86 ± 3% versus 69 ± 7%; inhibition percentage of I tail init and I tail late, respectively; n = 5; p < 0.01). Renzapride (IC\textsubscript{50} = 1.8 \times 10\textsuperscript{-6} M) was less potent than cisapride but slightly more potent than prucalopride to block the HERG current. The effects of renzapride were also partially reversible.

**Effects of Mosapride.** As illustrated in Fig. 5A, 3 \times 10\textsuperscript{-6} M mosapride did not significantly alter the deactivating current amplitude (11.9 ± 1.5 pA/pF versus 13.4 ± 1.8 pA/pF in control; n = 6). The tail current was not affected by applying from 3 \times 10\textsuperscript{-8} M to 3 \times 10\textsuperscript{-5} M mosapride at the cell surface, irrespective of the potential at which the membrane was

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**Fig. 3.** Effects of prucalopride on recombinant HERG K\textsuperscript{+} currents. A, representative current traces recorded in a cell under control conditions and in the presence of 3 \times 10\textsuperscript{-6} M prucalopride. Same protocol as in Fig. 2A (vertical bar, 100 pA; horizontal bar, 200 ms). B, current-voltage relationship of the I tail density in control conditions (○) and in the presence of 3 \times 10\textsuperscript{-7} M prucalopride (●, n = 6). Same protocol as Fig. 2C. Inset, inhibition ratio versus prepulse potential. C, dose-response curve for HERG K\textsuperscript{+} tail current after 500 ms of depolarization to +10 mV (n = 8).

**Fig. 4.** Effects of renzapride on HERG K\textsuperscript{+} currents expressed in COS-7 cells. A, representative current traces recorded in a cell under control conditions and in the presence of 3 \times 10\textsuperscript{-6} M renzapride. Same protocol as Fig. 2A (vertical bar, 250 pA; horizontal bar, 200 ms). B, current-voltage relationships of the current tail density in control conditions (○) and in the presence of 3 \times 10\textsuperscript{-6} M renzapride (●, n = 5). Same protocol as Fig. 2C. Inset, inhibition ratio versus prepulse potential (n = 5). C, dose-response curve for HERG K\textsuperscript{+} tail current after 500 ms of depolarization to +10 mV (n = 5).
driven during the prepulse (Fig. 5B). As shown in Fig. 5C, the
dose-response curve for mosapride perfectly superimposed the
spontaneous run-down of the tail current amplitude determined in
distinct experiments in cells superfused with the vehicle alone (n = 8).

Discussion

Our data show that three different 5-HT4 receptor agonists
(cisapride, prucalopride, and renzapride) block recombinant
HERG channels recorded under physiological conditions (de-
polarizing pulse duration close to the duration of the human
ventricular action potential and 37°C). They also demonstrate that the 5-HT4 receptor agonist mosapride, which shares structural similarities with the other drugs tested, is

essentially neutral vis-à-vis cardiac HERG K⁺ current. We therefore conclude that 5-HT4 receptor agonists devoid of side effects on cardiac repolarization can be a safe alternative to cisapride. Our results also show that prucalopride and renzapride are intermediary between cisapride and mosapride. These drugs need further investigation in in vivo models to evaluate their innocuousness in the context of the induced long QT syndrome. Both prucalopride and renzapride are active on HERG in the same range of concentration although prucalopride, unlike cisapride or renzapride, did not show voltage dependence of the block, which may make it relatively more efficient to prolong the action potential duration. However, it remains inconclusive whether the time and voltage dependence of HERG inhibition has some informative value when the potential proarrhythmic efficiency of a drug is evaluated.

In our study, the experimental IC₅₀ for cisapride (240 · 10⁻⁹ M) was significantly higher than that previously reported for native or recombinant HERG channels (6.5 · 10⁻⁹ M, Mohammad et al., 1997; 44.5 · 10⁻⁹ M, Rampe et al., 1997; 15 · 10⁻⁹ M, Drolet et al., 1998). As shown here and in previous work performed in CHO-K1 HERG-transfected cells (Walker et al., 1999), the inhibition produced by cisapride increased during the depolarizing pulse and was partially removed during repolarization. In CHO-K1 HERG-transfected cells the inhibition kinetics were evaluated and a time constant of 383 ms was calculated. In the present work, the cells were depolarized for 500 ms only, a duration close to the human action potential duration but much shorter than the duration used by previous investigators (2–20-s duration pre-pulses). Since cisapride produces a time-dependent open-channel block, we believe that the affinity of cisapride for HERG is overestimated when long depolarization duration protocols are used. In accordance with this assumption, cisapride is less effective on the action potential duration (APD) of rabbit Purkinje fiber than dofetilide, a class III antiarrhythmic agent. Dofetilide is a potent blocker of the HERG current with an affinity within the nanomolar or tens of nanomolar concentration range. Applied at a concentration of 10⁻⁶ M, it produces an 83% prolongation in the APD₉₀ (Lu et al., 2001) whereas at the same concentration, cisapride failed to prolong the APD significantly (Puisieux et al., 1996; Carlsson et al., 1997). This is in agreement with an overestimation of cisapride affinity when tested on HERG current in non-physiological conditions.

Mosapride, a 5-HT4 agonist developed as an alternative to cisapride, produces no deleterious effect on HERG current. In accordance, mosapride at concentrations 1000 times higher than the free plasma concentration in humans does not affect repolarization of rabbit Purkinje fibers (Carlsson et al., 1997) or guinea pig isolated papillary muscle (Kii and Ito, 1997). Also, mosapride does not affect significantly the QTU interval in a rabbit model of the acquired long QT syndrome (Carlsson et al. 1997). In spite of this safe profile on HERG and cardiac repolarization, mosapride has most recently been reported to trigger torsades-de-points in a patient (Ohki et al., 2001). However, in that case report, mosapride was coprescribed with flecaïnide, a potent class IC agent. In addition, this patient had hypokalemia and a permanent pacemaker because of sick sinus syndrome.
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References


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