Blockage of the HERG human cardiac K⁺ channel by the gastrointestinal prokinetic agent cisapride

SAEED MOHAMMAD, ZHENGFENG ZHOU, QIUMING GONG, AND CRAIG T. JANUARY
Section of Cardiology, Department of Medicine, University of Wisconsin, Madison, Wisconsin 53792

Mohammad, Saeed, Zhengfeng Zhou, Qiuming Gong, and Craig T. January. Blockage of the HERG human cardiac K⁺ channel by the gastrointestinal prokinetic agent cisapride. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2534–H2538, 1997.—Cisapride, a gastrointestinal prokinetic agent, is known to cause long Q-T syndrome and ventricular arrhythmias. The cellular mechanism is not known. The human ether-á-go-related gene (HERG), which encodes the rapidly activating delayed rectifier K⁺ current and is important in cardiac repolarization, may serve as a target for the action of cisapride. We tested the hypothesis that cisapride blocks HERG. The whole cell patch-clamp recording technique was used to study HERG channels stably expressed heterologously in HEK293 cells. Under voltage-clamp conditions, cisapride block of HERG is dose dependent with a half-maximal inhibitory concentration of 6.5 nM at 22°C (n = 25 cells). Currents rapidly recovered with drug washout. The onset of block by cisapride required channel activation indicative of open or inactivated state blockage. Block of HERG with cisapride after channel activation was voltage dependent. At −20 mV, 10 nM cisapride reduced HERG tail-current amplitude by 5%, whereas, at +20 mV, the tail-current amplitude was reduced by 45% (n = 4 cells). At −20 and +20 mV, 100 nM cisapride reduced tail-current amplitude by 66 and 90%, respectively. We conclude that cisapride is a potent blocker of HERG and cause LQT by inhibiting IKr or HERG. We used HERG stably transfected HEK293 cells to test the hypothesis that cisapride is a potent blocker of HERG channel current.

METHODS

Stable transfection. HERG cDNA (22, 23, 28) was subcloned into BamHI and EcoRI sites of the pCDNA3 vector (Invitrogen, San Diego, CA). This vector contains a CMV promoter and an SV40 promoter, which drive the expression of the inserted cDNA (HERG) and neomycin-resistant gene, respectively. The HEK293 cells were transfected with this construct using the lipofectamine method (GIBCO, Grand Island, NY). After selection in 800 μg/ml Geneticin (G-418, Gibco) for 15–20 days, single colonies were picked with cloning cylinders and tested for HERG current. The stably transfected cells were cultured in minimal essential medium (MEM) supplemented with 10% fetal bovine serum and 400 μg/ml G-418. All cells were from a single cell line producing high current levels and were stable for >6 mo. For electrophysiological study, the cells were harvested from the culture dish by trypsinization, washed twice with standard MEM medium, and stored in this medium at room temperature for later use. Cells were studied within 8 h of harvest.

Patch-clamp recording technique. Cells used for electrophysiological study were transferred to a small cell bath mounted on the stage of an inverted microscope (Diaphot, Nikon) and were superfused with N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered Tyrode solution containing (in mM) 137 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES (pH 7.4 with NaOH). Solution exchanges were completed within 2 min. Membrane currents were recorded in a whole cell configuration using suction.
pipettes (7, 24). The pipettes had inner diameters of 1–1.5 µm and, when filled with the internal pipette solution, had resistances of 2–4 MΩ. The internal pipette solution contained (in mM) 130 KCl, 1 MgCl₂, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N',N',N''-tetraacetic acid, 5 MgATP, and 10 HEPES (pH 7.2 with KOH). An Axopatch 1D patch-clamp amplifier was used to record membrane currents. Computer software (pCLAMP; Axon Instruments, Foster City, CA) was used to generate voltage-clamp protocols, acquire data, and analyze voltage and current signals. All experiments were performed at room temperature (22–23°C).

Drugs and chemicals. Cisapride was obtained from Research Diagnostics (Flanders, NJ; drug purity >99.8%). Cisapride was dissolved in 100% ethanol to give a stock concentration of 5 mM. Final drug concentrations were made within 48 h of experiments by diluting stock solution with the extracellular HEPES-buffered Tyrode solution. Ethanol control (n = 3 cells), at a concentration (0.01%) equivalent to the highest cisapride dilution studied, had no effect on HERG current. Other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Statistical methods. Data are given as means ± SE. Curve fitting was done using a nonlinear least-squares regression analysis (Sigmaplot; Jandel Scientific, Corte Madera, CA). Statistical significance was analyzed using Student's t-test and permutation analysis.

RESULTS

The effect of cisapride on HERG current was studied in a stably transfected HEK293 cell line. Figure 1, A and B, shows voltage-clamp data obtained with 100 nM cisapride. Families of current traces from one cell are shown for control conditions (Fig. 1A) and after exposure to cisapride (Fig. 1B), with the voltage-clamp protocol shown in Fig. 1A, inset. Cells were clamped at a holding potential of −80 mV. Depolarizing steps were applied for 4 s to voltages between −60 and +50 mV in 10-mV increments. For control conditions, depolarizing steps activated a time-dependent outward current that increased in amplitude with more positive voltage steps to reach a maximum at +10 mV. Depolarizing steps to more positive voltages resulted in inward rectification. After the repolarizing step to −50 mV, an outward tail current was recorded. Tail-current amplitude, measured as the difference between the peak current and leak-corrected baseline current at −50 mV, increased with depolarizing steps from −60 to +20 mV and was then superimposed on further depolarizing steps to +50 mV. Cisapride (100 nM) suppressed both the outward and tail currents, as shown in Fig. 1B. In other experiments, Zhou et al. (28) showed that HERG current in these cells is blocked nearly completely by E-4031 (300 nM). Finally, there is minimal contamination of HERG current in these cells by endogenous current, as shown in Fig. 1C in the current records obtained from a nontransfected HEK293 cell.

Current-voltage plots of steady-state current present at the end of the depolarizing step and peak tail current are depicted in Fig. 1, D and E, respectively. For control conditions, the threshold for activating HERG current was close to −40 mV and full activation was obtained at voltages near +20 mV. In the presence of 100 nM cisapride, steady-state current and peak tail-current amplitude were reduced compared with control conditions.

Figure 2 shows drug block of HERG current by cisapride in a different transfected cell. In this experiment, HERG current was rapidly activated by a 100-ms-
long depolarizing step to +60 mV (see Ref. 18) from a holding potential of −80 mV, and the cell was then clamped at +10 mV for 10 s before tail currents were obtained by repolarization to −50 mV. Control current, current activated by first depolarizing step after a 10-min-long exposure to 100 nM cisapride, and the current obtained after 10 min of drug washout are shown. The cell was held at −80 mV during the washin and washout periods. After drug washin, the initial outward current amplitude with the first depolarizing step was only slightly decreased compared with control current, indicating little closed-state block. The outward current amplitude subsequently decreased during the depolarizing step to reach maximal block. Current was recovered nearly completely after 10 min of drug washout.

Cisapride block of HERG current was concentration dependent. Steady-state block was obtained by applying depolarizing steps from −80 to +10 mV for a 20-s period every 30 s, and peak tail current was measured after repolarizing steps to −50 mV for 5 s at different drug concentrations. Analysis of the data obtained from a total of 25 cells with the Hill equation gave a half-maximal inhibitory concentration (IC50) value of 6.5 nM. The decrease in peak tail current as a function of drug concentration is shown in Fig. 3.

Block of HERG current by cisapride varied with voltage, exhibiting a higher degree of block at more positive voltages, as shown in Fig. 4. In four transfected cells, 10 nM cisapride at −20 mV reduced peak tail-current amplitude by 5% from 282 ± 91 to 268 ± 77 pA (P > 0.05), whereas at +20 mV it reduced peak tail-current amplitude by 45% from 944 ± 89 to 511 ± 97 pA (P < 0.05). At 100 nM cisapride, the voltage dependence of drug block was again evident, with HERG current reduced from 232 ± 95 to 80 ± 17 pA (66% reduction) and from 1,010 ± 48 to 96 ± 55 pA (90% reduction), at −20 and +20 mV, respectively (see Fig. 1; P < 0.05 compared with control). The amount of block at each drug concentration was greater at +20 mV than at −20 mV (P < 0.05). Thus cisapride block of HERG current exhibits voltage dependence.

DISCUSSION

Several reports of cardiac arrhythmias with the use of cisapride have appeared in the literature over the last decade (1, 2, 13). Recently, the Food and Drug Administration reported 57 cases of torsades de pointes...
and/or Q-T prolongation in patients that were adminis-
tered cisapride (27). The development of Q-T prolonga-
tion and torsades de pointes in cisapride users fre-
quently appeared to be associated with the concomitant
use of medications that compete for drug metabolism
by the cytochrome P-450 3A4 isozyme, the presence of
renal insufficiency, and with administration of high
doses of cisapride.

After oral administration, total cisapride peak serum
values of 60–80 µg/l (120–170 nM) are achieved, of
which >95% is plasma protein bound (11, 25a). The
drug is extensively metabolized by the cytochrome
P-450 enzyme system in the liver with an elimination
half-life of ~10 h. The half-life of cisapride may be
prolonged in patients with hepatic disease and in the
elderly (11). Coadministration of drugs metabolized
through the cytochrome P-450 enzyme system can also
result in higher drug levels.

The results of our study demonstrate that cisapride
is a potent blocker of HERG channels expressed hetero-
dalogously in HEK293 cells. The drug concentrations
we studied were similar to the therapeutic levels achieved
in the clinical use of this drug. We conclude that
blockage of HERG current may underlie the proarrhyth-
ic effect of cisapride. Thus this report shows HERG to
be a molecular target for the action of cisapride and
may help to explain the mechanism of Q-T prolongation
and occurrence of torsades de pointes with this drug.

Block of HERG by cisapride was voltage dependent.
Drug block does not occur via closed-state block, be-
cause nearly normal peak outward current was re-
corded on the first depolarizing step after drug washin,
as shown in Fig. 2. Rather, the onset of HERG-current
block by cisapride required channel activation, sugges-
tive of open- or inactivated-state drug block. Our proto-
cols, however, do not easily distinguish between open-
and inactivated-state block. In the study of cisapride
block of HERG, it is important to recognize that the
IC50 of drug block is voltage dependent.

Cisapride is a widely used gastrointestinal prokinetic
agent in humans and animals. Cisapride augments
motility throughout the gastrointestinal tract and is
used in the treatment of gastroesophageal reflux dis-
ease and gastroparesis (3, 25a). The mechanism of
action is not fully elucidated but is thought to be
through the release of acetylcholine mediated by post-
ganglionic nerve endings in the myenteric plexus of the
gut (9). Cisapride is an agonist of serotonin [5-
hydroxytryptamine (5-HT)] at the 5-HT1 receptor as
well as an antagonist at the 5-HT3 receptor (4). Cisapride
seems to enhance gastrointestinal motility in humans
by enhancing cholinergic transmission through stimula-
tion of 5-HT1 receptors on the enteric nerve endings
(17, 21, see also Ref. 20). Cisapride also has a direct
stimulating effect on gastrointestinal smooth muscle
tissue without the involvement of 5-HT receptors. In
experiments with guinea pig stomach circular smooth
muscle, cisapride was found to cause depolarization of
the muscle membrane (6), and, in taenia coli prepara-
tions from guinea pig colon, cisapride was shown to
cause depolarization, enhancement of spike activity,
increases in muscle tone, and potentiation of contrac-
tion (12).

Our findings raise the possibility that the prokinetic
effect of cisapride may be explained, in part, by its
blockage of gastrointestinal K+ channels located either
on the smooth muscle cells of the gut or on the
presynaptic nerve terminals of the myenteric plexus.
Recent reports showed widespread distribution of
HERG mRNA in different tissues, including small
intestine (26). It is possible that cisapride causes
membrane depolarization of smooth muscle cells or
causes release of excitatory neurotransmitters from
terne endings by blocking HERG channels present in
the gastrointestinal tract. Further studies are needed
to define the cellular distribution of HERG in the
gastrointestinal tract and the possible interaction of
HERG and cisapride in the gut.

The findings of this study have several important
implications. Our study shows that cisapride in low
concentrations blocks HERG; therefore, this may be the
mechanism responsible for the Q-T prolongation and
torsades de pointes associated with cisapride use in
humans. Moreover, the concentration of drug associ-
ated with 50% block in vitro (IC50 = 6.5 nM) is within
the therapeutic range achieved with oral dosing in
humans. The reason that arrhythmic effects are not
observed more frequently may be due to the fact that
>95% of the drug is bound to plasma proteins. Only
when high free-drug levels are achieved, such as from
liver disease, drugs competing for metabolism, or over-
dosage, do Q-T prolongation and torsades de pointes
occur. In addition, these findings may prove helpful in
developing newer and safer gastrointestinal prokinetic
drugs devoid of the undesirable side effects of Q-T
prolongation.

Because we only studied HERG-transfected cells,
possible effects of cisapride on other membrane cur-
rents cannot be excluded. In addition, we did not study
the effects of metabolites, such as norcisapride, on
HERG current. Therefore, in vivo contributions by the
metabolites to the drug effects cannot be excluded.
Finally, cisapride has been demonstrated to be a partial
agonist of 5-HT4 receptors in human right atrium. This
mechanism is unlikely to be important in the genesis of
ventricular arrhythmias because of the absence of
functional 5-HT4 receptors in the human ventricle (8).

In conclusion, cisapride blocks HERG-encoded chan-
nels expressed in HEK293 cells. This effect may account
for the clinical occurrence of Q-T prolongation and ventricu-
lar arrhythmias observed with the use of cisapride. This
is a new class of drugs shown to block HERG, further
supporting the evidence that HERG is an important target
for many cardiovascular and noncardiovascular drugs.

The authors thank Drs. Jonathan Makielski and Chandar Singa-
ram for reviewing the manuscript.
Address for reprint requests: C. T. January, Section of Cardiology,
Rm. H6/352 CSC, Univ. of Wisconsin Hospitals and Clinics, 600
Highland Ave., Madison, WI 53792.
Received 21 May 1997; accepted in final form 12 July 1997.
REFERENCES