JACC: CLINICAL ELECTROPHYSIOLOGY
VOL. 2, NO. 7, 2016

ISSN 2405-500X/$36.00
PUBLISHED BY ELSEVIER
http://dx.doi.org/10.1016/j.jacep.2016.07.008

Potent Inhibition of hERG Channels by the Over-the-Counter Antidiarrheal Agent Loperamide

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ABSTRACT

OBJECTIVES The aim of this study was to determine the in vitro electrophysiological properties of loperamide. The authors' hypothesis was that loperamide is a potent blocker of the current carried by the human ether-à-go-go-related gene (hERG) potassium channel.

BACKGROUND Loperamide is a peripherally-acting μ-opioid agonist available worldwide as an over-the-counter treatment for diarrhea. Like most opioids, it is not currently known to be proarrhythmic. Recent cases of torsade de pointes in association with high-dose loperamide raise concern given its structural similarity to methadone, another synthetic opioid with an established arrhythmia risk.

METHODS Effects of loperamide on blockade of the hERG potassium channel ion current were assessed in Chinese Hamster Ovary (CHO) cells stably expressing hERG to elucidate current amplitude and kinetics. The concentration required to produce 50% inhibition of hERG current was assessed from the amplitude of tail currents and the impact on action potential duration was assessed in isolated swine ventricular cardiomyocytes.

RESULTS The 50% inhibitory concentration for loperamide inhibition of hERG ionic tail currents was approximately 40 nmol/l. In current-voltage measurements, loperamide reduced steady and tail currents and shifted the current activation to more negative potentials. Loperamide (10 nmol/l) also increased the action potential duration, assessed at 90% of repolarization, in ventricular myocytes by 16.4 ± 1.7% ($n = 6$; $p < 0.004$). The maximum rate of rise of phase 0 of the action potential, however, was not significantly altered at any tested concentration of loperamide.

CONCLUSIONS Loperamide is a potent hERG channel blocker. It significantly prolongs the action potential duration and suggests a causal association between loperamide and recent clinical cases of torsade de pointes.
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Loperamide is a peripherally acting μ-opioid receptor agonist available worldwide as an over-the-counter treatment for diarrhea. Like most opioids it is not currently known to be associated with delayed repolarization or arrhythmia, but we recently observed a case of torsade de pointes associated with high-dose ingestion (1). Methadone is a synthetic opioid structurally similar to loperamide and a potent blocker of the rapid component of the delayed rectifier potassium ion current, encoded by the human ether à-go-go-related gene (hERG) channel (2). In the current epidemic of prescription opioid abuse, recreational users are seeking inexpensive alternatives to achieve euphoria, and loperamide is a widely available option increasingly promulgated on the Internet (3). With rising opioid-associated

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Manuscript received May 31, 2016; revised manuscript received July 14, 2016, accepted July 21, 2016.

mortality, the use of nonprescription opioids is increasing and is expected to escalate further given recent cautionary guidelines from the Centers for Disease Control and Prevention (4). Given this backdrop, we evaluated the in vitro electrophysiological properties of loperamide, primarily its impact in blocking the hERG channel, a major determinant of cardiac repolarization and a principal mechanism for drug-induced torsade de pointes.

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METHODS

Effects of loperamide hydrochloride on the blockade of potassium ionic currents were assessed in Chinese Hamster Ovary (CHO) cells stably expressing hERG (KCNH2) channels (cell line courtesy of Professor Alfred George, Vanderbilt University). Chinese hamster ovary cells were grown and plated at 37°C and then incubated at 30°C for 24 h to increase hERG expression and current density (5). Ionic currents were assessed via whole-cell patch clamp. Extracellular solution contained 140 mmol/l NaCl, 5.4 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 10 mmol/l HEPES, and 5 mmol/l glucose (pH 7.4, temperature 30°C). Intracellular (pipette) solution contained 100 mmol/l K^+ aspartate, 30 mmol/l KCl, 10 mmol/l HEPES, 5 mmol/l Mg-ATP, 5 mmol/l Na_2 creatine phosphate, and 1 mmol/l EGTA (pH 7.2). In addition, $CaCl_2$ and $MgCl_2$ were added to obtain "free" levels of 30 nmol/l and 1 mmol/l, respectively, using MaxChelator software. Recordings were made using an Axopatch 200B amplifier and Digidata 1440 interface controlled by Clampex 10 software (Molecular Devices, Sunnyvale, California). Cell capacitance and series resistance (>80%) were compensated electronically. Patch pipettes had resistances of 1 to 3 MΩ when filled with intracellular solution. Ionic currents were acquired at a sampling rate of 10 kHz and filtered at 5 kHz. Loperamide hydrochloride (Sigma) was prepared daily as a 1 mmol/l stock solution in dimethyl sulfoxide and then diluted to the desired concentration in extracellular solution. The Chinese hamster ovary cell experiments were performed at 30°C to 32°C. The concentration of loperamide required to produce 50% inhibition (50% inhibitory concentration [IC_{50}]) of hERG channels was assessed from the amplitude of leakage-corrected tail currents following an activating pre-pulse while stimulating at 0.16 to 1 Hz. The voltage dependence of tail current amplitudes was fitted by a Boltzmann function ($I_{tail} = I_{Max}/\{1 + \exp[(V' - V)/k]\}$) to assess slope factor (k), for amplitude suppression (IMax), and for a shift in the midpoint voltage, V'.

Swine ventricular myocytes were isolated by serial enzymatic dissociation of explanted left ventricular free wall using procedures approved by the Uniformed Services University Institutional Animal Care and Use Committee. Whole-cell current-clamp methods were used to monitor action potentials using the aforementioned extracellular and pipette solutions, at a temperature range of 30°C to 34°C, then stimulated at a pacing frequency of 1 Hz. Loperamide was dissolved in extracellular solution as described, and action potential durations were assessed at 90% of repolarization (APD_{90}). All variables are expressed as mean ± SEM. Statistical significance were assessed using 2-tailed Student t tests, and a p value of <0.05 was considered to indicate statistical significance.

ABBREVIATIONS AND ACRONYMS

APD_{90} = action potential duration at 90% of repolarization

C_{max} = maximal plasma concentration

hERG = human ether-à-go-go-related gene

IC_{50} = 50% inhibitory concentration

CHO = Chinese Hamster Ovary

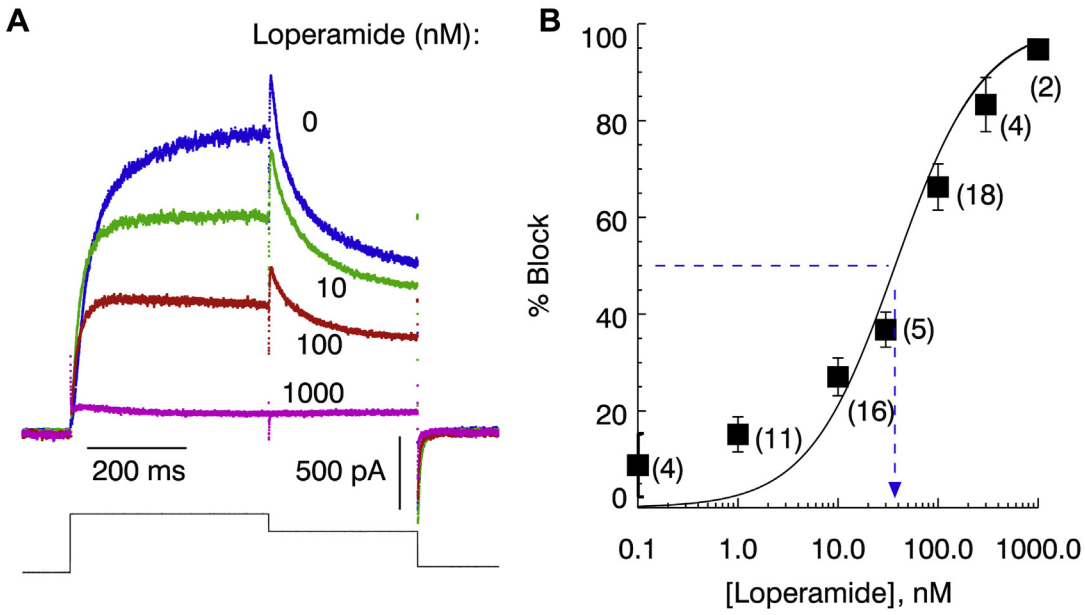
RESULTS

EFFECTS OF LOPERAMIDE ON POTASSIUM IONIC CURRENTS (hERG). Loperamide was a potent blocker of the hERG channel expressed in Chinese hamster ovary cells (Figure 1A). Loperamide suppressed steady currents during a depolarization (+10 mV) as well as tail currents at −50 mV (holding potential −80 mV) in a concentration-dependent manner. The IC_{50} for hERG channel blockade by loperamide was approximately 40 nmol/l for inhibition of tail currents (Figure 1B).

Loperamide suppression of hERG current is voltage dependent, manifesting significant block during both steady and tail currents for large depolarizations (Figure 2) at 100 nmol/l loperamide. Steady depolarizations beyond approximately +10 mV also exhibited "sagging" of the current (Figures 2A and 2B), suggestive of preferential open- or inactivated-state block. At smaller depolarizations, the activation of hERG in the presence of loperamide is shifted toward more negative voltages. These features of loperamide suppression of hERG are depicted in current-voltage relationships (Figures 2C and 2D). The voltage dependence of tail current amplitudes, fitted by a Boltzmann function (see the Methods section), showed a significant suppression of the amplitude, I_{Max} (−62.7 ± 3.9%; $p < 0.002$, $n = 6$) and a shift of midpoint voltage, V' (−18.1 ± 1.9 mV; $p < 0.007$). The slope factor, k, was not significantly changed.

EFFECTS OF LOPERAMIDE ON ACTION POTENTIAL DURATION. Loperamide at 10 nM concentration increased the APD_{90} in swine ventricular myocytes

FIGURE 1 Loperamide Inhibition of hERG Potassium Ion Current



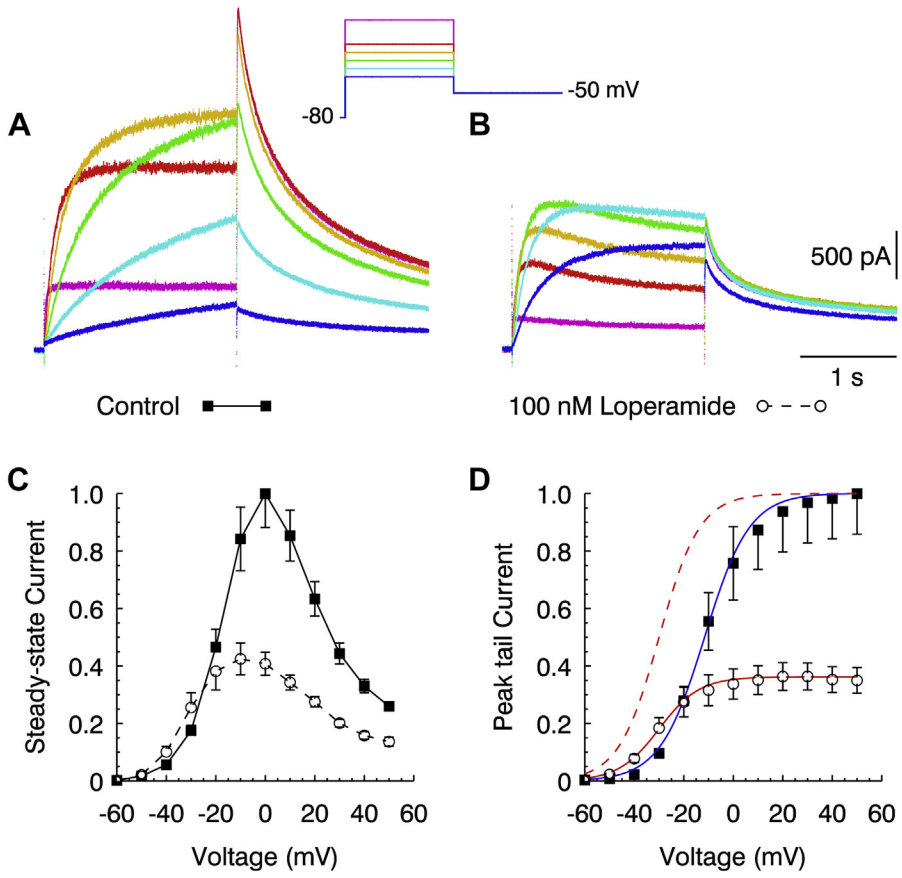
(A) Human ether-à-go-go-related gene (hERG) ionic currents in control (0 nmol/l) and during exposure to loperamide (10, 100, and 1,000 nmol/l). All records are from the same cell exposed to increasing concentrations of loperamide. The lower **(black)** record shows the timing of the voltage pulse protocol. **(B)** Concentration dependence of loperamide blockade of hERG currents. Data points represent mean ± SEM for the number of cells indicated. Inhibition was determined as the percentage block of tail currents at −50 mV following an activating pulse to +10 mV. The **solid line** is a single-site binding curve with K_D = 37.2 nmol/l. The **dashed lines** and **arrowhead** depict the loperamide concentration resulting in 50% block of hERG currents.

(Figure 3A) by 16.4 ± 1.7% (n = 6; p < 0.004) (Figure 3B). In 2 myocytes, 100 nmol/l loperamide increased APD_{90} by 31.3 ± 16.2%, whereas 1 μmol/l loperamide caused a small elevation of APD_{90} (1.4 ± 10.3%; n = 2), as shown in Figure 3B. Recovery of APD_{90} was incomplete 15 min after washout of the drug. The maximum rate of rise of phase 0 of the action potential, taken as an index of the magnitude of Na^+ conductance, was not significantly changed at any concentration of loperamide investigated, though there appears to be a trend toward a reduction in maximum rate of rise of phase 0 of the action potential at the micromolar level, Therefore, a small effect on sodium channels cannot be excluded.

DISCUSSION

Although not well established in the peer-reviewed scientific literature, loperamide addiction and misuse appears to be a growing international concern. To our knowledge, the present study is the first to demonstrate that this synthetic opioid is a highly potent hERG channel blocker and prolongs the action potential duration in isolated cardiac myocytes. Loperamide is chemically similar to methadone, a long-acting prescription opioid used to treat heroin addiction and known to be associated with torsade de pointes (5). The present study demonstrated concentration-dependent blockade of hERG current that was more potent than with methadone (2), which exhibits an IC_{50} of 9.8 μmol/l, a maximal plasma concentration (C_{max}) of 3.6 μmol/l, and an IC_{50}/C_{max} ratio of 2.7 without accounting for plasma protein binding. Given an expected C_{max} of 4.5 nmol/l for loperamide after normal dosing for the treatment of diarrhea (6), the IC_{50}/C_{max} ratio is 8.9, where ratios <30 reflect a diminished cardiac safety margin (7). Loperamide is significantly (95% to 97%) bound, yielding a hERG IC_{50}/C_{max} value of about 300 for standard dosing. However, a recent case study demonstrated that serum concentrations as high as 83.2 ng/ml (162 nmol/l) may be achieved among individuals abusing loperamide (8). In this scenario, assuming 3% of the drug is not protein bound, results in a free plasma loperamide concentration of 4.9 nmol/l and an IC_{50}/C_{max} (free) ratio of 8.2, well within a potential range for drug-associated QT-prolongation liability.

FIGURE 2 **Voltage Dependence of hERG Current Blockade by Loperamide**

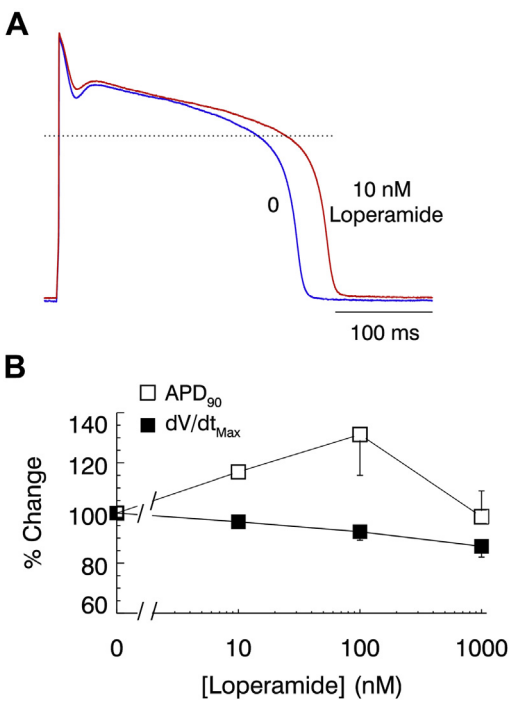


Human ether-à-go-go-related gene (hERG) currents in control **(A)** and 100 nmol/l loperamide (**B**, same cell) at increasing test depolarizations. The voltage pulse protocol is shown in the inset. Current-voltage relationships for the steady current (end of test depolarization, **C**) and the peak tail current **(D)** in control and 100 nmol/l loperamide. Points are mean ± SEM for 6 cells, normalized to the maximum of the control. In **(D)**, the points are fitted to a Boltzmann function with the following parameters: control **(blue line)**, $V' = -11.6$ mV, $k = 9.1$ mV; loperamide **(red line)**, $I_{Max} = 0.36$, $V' = -29.9$, $k = 8.2$ mV. The **dashed red line** is the same as the **solid red line** but scaled to 1.0 to show the relative changes of V' and k compared with control.

Thus, when loperamide is used in strict accordance to the prescription label, it likely has a reasonable cardiac safety margin, yet it appears to have substantial arrhythmic liability when misused. Very recently, this risk appraisal was mirrored in an international dynamic registry that stratifies the QT-prolonging and proarrhythmic properties of both over-the-counter and prescription drugs (9). Within this registry, loperamide was designating as possessing "conditional risk" for the development of torsade de pointes, particularly in very high doses among individuals addicted to narcotics (10). This characterization is consistent with the previous patient we cared for, who developed torsade de pointes after ingesting a large quantity of loperamide to achieve a euphoric effect (1).

STUDY LIMITATIONS. We investigated a single potassium ion current; another study observed loperamide-induced sodium-channel blockade with an IC_{50} of 2.9 μmol/l, roughly 100-fold less potent than the hERG blockade demonstrated in our study. Presently, we cannot rule out clinically relevant effects of loperamide on other cardiac ionic currents. Nonetheless, the present investigation demonstrates that loperamide potently blocks hERG current and prolongs APD_{90} in cardiomyocytes. Because loperamide is not classified as a drug associated with QRS complex prolongation (11), we suspect that the predominant mechanism of conduction/repolarization alteration associated with loperamide is related to delayed rectifier potassium ion current blockade. However, recent case reports indicate that loperamide

FIGURE 3 Effects of Loperamide on Action Potential Waveform



Loperamide (10 nmol/l) causes an increase of the action potential duration **(red trace)** compared with control **(blue trace)** in an isolated swine ventricular myocyte **(A)**. The **dashed line** denotes 0 mV. Mean ± SEM changes of action potential duration at 90% of repolarization (APD_{90}) **(open squares)** and maximum rate of rise of action potentials (dV/dt_{Max}, **solid squares**) at different concentrations of loperamide compared with paired controls **(B)**. Data for n = 6 (10 nmol/l) and n = 2 (100 and 1,000 nmol/l loperamide) ventricular myocytes.

intoxication can significantly prolong the QRS complex in a manner similar to tricyclic antidepressant overdose (8,12). It may be that the subjects reported in these cases had unsuspected sodium channel abnormalities that increased their susceptibility, or perhaps that loperamide alters cardiac conduction through an unrecognized mechanism. Inhibition of gap junctions has recently been described as an off-target effect of P-glycoprotein inhibitors, including loperamide (13). Such an effect, unfortunately, cannot be evaluated in a single cell, yet it might be an important contributor to the cardiotoxicity associated with supratherapeutic levels of loperamide.

Given the molecular and electrophysiological similarity of loperamide to methadone, and the primacy of hERG block among causes of drug-induced QT prolongation and torsade de pointes, a causal association likely exists between supratherapeutic doses of loperamide and recent cases of malignant ventricular arrhythmia. Loperamide abusers often consume up to 200 standard 2-mg pills to achieve euphoria or avoid withdrawal symptoms, leading to loperamide's being referenced colloquially as "poor man's methadone" (14). Given the fact that loperamide costs 1 to 3 cents per pill and is widely available for purchase via both retail and Internet sources (15), we are concerned that arrhythmia incidence associated with misuse of this drug in the current opioid landscape may continue to rise.

CONCLUSIONS

Although generally considered a safe, over-the-counter preparation, the synthetic opioid loperamide appears to have important effects on cardiac repolarization and conduction properties. Further characterization of the cardiac safety profile of loperamide seems warranted. In the interim, cardiologists should regard loperamide as a putative culprit agent in patients presenting with unexplained QT-interval prolongation, syncope, ventricular arrhythmia, or cardiac arrest prior to consideration of implantable defibrillator therapy.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The present study suggests that the synthetic opioid loperamide blocks the delayed rectifier potassium ion current, which is the primary mechanism for drug-induced QT prolongation and torsade de pointes. Clinicians should consider the potential role of loperamide in opioid-dependent patients misusing loperamide and presenting with torsade de pointes.

TRANSLATIONAL OUTLOOK: Experimental studies are an important complement to clinical observations. With increasing misuse of loperamide and its electrophysiologic properties, examination of optimal risk mitigation strategies and the regulatory approach for this drug seems warranted.

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KEY WORDS action potential, hERG, loperamide, QT, torsade de pointes