PHARMACOKINETICS, PHARMACODYNAMICS AND DRUG METABOLISM

Lengthening of Cardiac Repolarization in Isolated Guinea Pigs Hearts by Sequential or Concomitant Administration of Two I_{Kr} Blockers

RAYMOND HREICHE,[1,2] ISABELLE PLANTE,[1,2] BENOIT DROLET,[3,4] PIERRE MORISSETTE,[1,2] JACQUES TURGEON[1,2]

[1]Faculty of Pharmacy, Université de Montréal, Montreal, Quebec, Canada, H2R2P6

[2]Research Centre of the University of Montreal Hospital Center, Montreal, Quebec, Canada, H2W 1T7

[3]Faculty of Pharmacy, Université Laval, Quebec City, Quebec, Canada, G1V 0A6

[4]Research Center, Institut universitaire de cardiologie et de pneumologie de Québec, Quebec City, Quebec, Canada. G1V 4G5

Received 24 September 2010; revised 15 November 2010; accepted 18 November 2010

Published online 23 December 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22437

ABSTRACT: Block of I_{Kr} is of major concern in drug safety. The objective of this study was to assess prolongation of cardiac repolarization during the combined use of two I_{Kr} blockers when administered concomitantly or sequentially. (1) When isolated hearts from male guinea pigs were perfused concomitantly with two I_{Kr} blockers, prolongation of monophasic action potential duration measured at 90% ($MAPD_{90}$) was less than the summation of effects observed for each drug perfused alone. (2) In sequential administration, when ketoconazole or erythromycin was perfused first, they antagonized $MAPD_{90}$-prolonging effects of domperidone. This effect was absent when domperidone or dofetilide was perfused first. Patch-clamp experiments confirmed that the order of sequential perfusion impacts the decrease in HERG tail amplitude. In conclusion, this study does not support the concept that potentiation of drug effects is observed during the combined administration of two I_{Kr} blockers. Furthermore, order of administration of two I_{Kr} blockers together may be an important factor in drug-induced long QT syndrome. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2469–2481, 2011

Keywords: drug targeting; drug interaction; In vitro/in vivo correlations (IVIVC); pharmacodynamics; physiological model

INTRODUCTION

Drug-induced long QT syndrome (LQTS) is a cardiac electrophysiological abnormality characterized by a prolonged QT interval on the surface ECG, which may predispose patients to the polymorphic ventricular arrhythmia termed torsades de pointes (TdP).[1,2] Clinicians have long been familiar with this severe adverse drug effect particularly in the context of antiarrhythmic drug therapy.[3,4] However, since the early 1990s, several noncardiovascular drugs with various primary pharmacological activities have been withdrawn from the market—or restrictly delivered—because of their "torsadogenic" effects.[5] These include, *inter alia*, terfenadine,[6] astemizole,[7] droperidol,[8] and cisapride[9] Today, more than 125 drugs currently available in the North American market have been associated with the drug-induced LQTS (http://www.Torsades.org/).

Except for a handful of them, it has been shown that most drugs known to prolong the QT interval are potent blockers of the delayed rectifier potassium current (I_K). I_K is the major outward current responsible for cardiac repolarization in humans. This current comprises rapidly (I_{Kr}) and slowly (I_{Ks}) activating components.[10–12] Class III antiarrhythmic agents, such as *N*-acetylprocainamide (NAPA), *d*-sotalol, E-4031, and dofetilide,[13,14] as well as many noncardiovascular drugs (antihistamines, macrolide

Correspondence to: Benoit Drolet (Telephone: (418)-656-8711 ext. 5755; Fax: (418)-656-4509; E-mail: benoit.drolet@pha.ulaval.ca)

Journal of Pharmaceutical Sciences, Vol. 100, 2469–2481 (2011)

antibiotics, etc.),[8,9,15–20] prolong action potential duration by selectively blocking I_{Kr}, whereas other drugs, such as indapamide or triamterene, block preferentially I_{Ks}.[21,22]

Drug-induced LQTS is often observed under conditions of impaired drug elimination as it was first described for the drug–drug interaction between the histamine H1-antagonist terfenadine and the macrolide antibiotic erythromycin.[23] Indeed, these events were explained mostly through an inhibition of terfenadine CYP3A-mediated metabolism by erythromycin (CYP3A inhibitor). Inhibition of CYP3As caused a major increase in terfenadine plasma concentrations leading to an increased block of I_{Kr} by terfenadine, prolongation of cardiac repolarization, and ultimately TdP.[24] It is noteworthy that macrolide antibiotics (including erythromycin) and imidazole antifungals that often cause the impaired metabolism status on CYP3As are also potent I_{Kr} blockers.[25–27] Yet, the question arises whether the true mechanism of drug-induced LQTS under conditions of drug–drug interactions is explained only by a pharmacokinetic interaction or whether potentiation of drug effects (pharmacodynamic interaction) is also part of the explanation.

Our laboratory has previously shown that the coadministration of I_{Kr} and I_{Ks} blockers leads to a potentiation of drug effects on lengthening of cardiac repolarization.[28] We studied block of I_{Ks} by diuretic agents and subsequently demonstrated how they could potentiate QT-prolonging drug effects of dofetilide and NAPA (in isolated hearts) or sotalol (in whole animals).[6,29,30] This mechanism of potentiation of drug effects is easily understandable because I_{Kr} and I_{Ks} are separate currents with different binding sites on different proteins. However, to our knowledge, the effects of the concomitant administration of two I_{Kr} blockers on QT prolongation independently from their metabolic drug–drug interactions have hardly been assessed. Moreover, many clinical cases of drug-induced TdP have been described when treatment with an I_{Kr} blocker is being initiated while a long-term treatment with another I_{Kr} blocker was already in place,[30,31,32] Yet, the importance of the order of administration of drugs on lengthening of repolarization observed is still unknown.

With these concepts in mind, we investigated the modulation of cardiac repolarization lengthening during the administration of I_{Kr} blockers either alone or combined. Furthermore, we assessed the importance of the order of administration of two I_{Kr} blockers when administered sequentially. Our hypothesis was tested using buffer-perfused guinea pig hearts [monophasic action potential duration measured at 90% repolarization ($MAPD_{90}$)] and isolated cells using the whole-cell patch clamp technique; dofetilide, NAPA, erythromycin, ketoconazole, and domperidone were used as selective I_{Kr} blockers.

METHODS AND MATERIALS

All experiments were performed in accordance with our institutional committee (Université de Montréal) and guidelines on animal research. Animals were housed and maintained in compliance with the *Guide to the Care and Use of Experimental Animals* of the Canadian Council on Animal Care.

Drug Preparation

Dofetilide (generously supplied by Pfizer Inc., Groton, Connecticut) was dissolved in dimethylsulfoxide (DMSO) and then added to the buffer solution at a concentration of 20, 150, or 200 nM. Domperidone was dissolved in DMSO and added to the buffer solution at a final concentration of 5, 50, 150, and 300 nM. Ketoconazole was dissolved in methanol and then added to the buffer solution at a final concentration of 0.3, 1, 3, and 10 μM. NAPA- (100 μM or 1 mM) and erythromycin- (10, 60, or 120 μM) containing buffer solutions were prepared by adding the required amount of the drug to a freshly prepared Krebs-Heinseleit solution. NAPA, erythromycin, domperidone, and ketoconazole were purchased from Sigma–Aldrich (St. Louis, Missouri).

Experiments with Buffer-perfused Isolated Hearts

Heart Isolation and Perfusion Technique

Male Hartley guinea pigs (weight, 300–350 g; Charles River Laboratories, Montreal, Quebec, Canada) were anticoagulated by injection of heparin sodium (400 IU IP). After 20 min, animals were killed by cervical dislocation, and their hearts were rapidly extirpated and immersed in cold (4°C) Krebs-Henseleit buffer containing (mM) glucose 11.2, KCl 4.7, $CaCl_2$ 1.2, $NaHCO_3$ 25, NaCl 118.5, $MgSO_4$ 2.5, and KH_2PO_4 1.2. This solution was continuously gassed with 95% of oxygen plus 5% of carbon dioxide (pH 7.4, 37°C). Each heart was cannulated and retrogradely perfused via the aorta with the Krebs-Henseleit buffer at a constant pressure equivalent to 100 cm H_2O. To permit rapid exchange in perfusion solutions, a double warming coil heart perfusion system (Harvard Apparatus, Inc., South Natick, Massachusetts) and two parallel liquid columns were used.

Electrophysiological Measurements

Hearts were electrically stimulated (EP-2, clinical stimulator, and Digital Cardiovascular Instruments Inc.) at a basic cycle length of 250 ms (4 Hz) at three times threshold via two silver electrodes implanted in the epicardium of the right ventricle. A monophasic