Potentiation of Dofetilide LQT-Related Effects by Late Sodium Current Enhancement: A Simulation Study

K Cardona, J Saiz, L Romero, B Carbonell,
JM Ferrero, B Trenor

Universidad Politecnica de Valencia, Spain

Abstract

Torsades de pointes (TdP) is a life-threatening ventricular tachycardia associated to reduction of the rapid delayed rectifier potassium current (I_{Kr}) and prolongation of ventricular action potential duration (APD) and long QT (LQT) syndrome. An abnormal increase of the late sodium current (I_{NaL}) is also associated to susceptibility to TdP. In this work, a mathematical model of I_{NaL} has been proposed and incorporated to the Shannon model of the rabbit ventricular AP. We analyzed the effects of I_{NaL} enhancement alone and in combination with dofetilide, a blocker of I_{Kr}, on the AP. This study demonstrates that an increase of I_{NaL} prolongs APD in a rate dependent manner. Also, triangulation is increased by I_{NaL}, especially at higher BCLs. Finally, the effect of dofetilide was included in order to assess the mixed action of the drug and I_{NaL} enhancement. Under these conditions, EADs arose only when I_{NaL} was increased 10-fold.

1. Introduction

Torsades de pointes is a serious ventricular tachycardia caused by a reduction in repolarizing currents or an increase in depolarizing currents, resulting in prolonged APD [1]. Therefore, TdP arises usually in case of LQT syndrome [2,3].

Early after depolarizations generation is thought to be a major cause of TdP in humans [5]. Early afterdepolarizations (EADs) are membrane potential oscillations that occur at AP plateau voltages; they may trigger ventricular arrhythmias and can be induced by drugs that delay AP repolarization, such as clofilium and dofetilide [4]. EADs are induced by intracellular calcium (Ca^{2+}) oscillations that arise from an imbalance between sarcolemmal Ca^{2+} entry and release from sarcoplasmic reticulum stores.

There are several abnormalities in ionic currents leading to the prolongation of the duration of the ventricular AP. One of them is the inhibition of the rapid delayed rectifier potassium current, I_{Kr}, caused by drugs, such as dofetilide or by some pathology [6].

Additionally, recent studies have found that the increase of I_{NaL} seems to be an important factor in APD prolongation, variability and EADs generation [7].

The late I_{Na} persists throughout the plateau of the AP, and circulates through a few sodium (Na^+) channels that fail to inactivate completely, an enter into either a long-opening or a bursting mode. I_{NaL} of normal myocytes is generally a small current (30 pA at plateau potentials), but it may be enhanced by more than 3-fold in various pathological conditions (LQT syndrome, heart failure (HF), and mutations in the gene SCN5A) [8, 9, 10, 11].

There are at least two mechanisms by which enhanced I_{NaL} can trigger arrhythmias. The first mechanism is related to the interruption of repolarization, leading to EADs and initiation of TdP [12, 13]. The second one is more gradual and is attributed to intracellular Na^+ loading, leading to Ca^{2+} overload, oscillatory calcium realease and the initiation of late afterdepolarizations. Therefore, several authors have hypothesized that a small increase of I_{NaL} would potentiate and unmask the proarrhythmic effects of QT prolonging drugs, including drugs that have a very low risk of causing ventricular trachycardia (VT) [14, 15].

The main goal of this work is to introduce the model of the late sodium current in the ventricular action potential in order to evaluate the effects of the enhancement of I_{NaL} on different biomarkers for arrhythmic risk. Additionally, we have combined the effect of dofetilide, an I_{Kr} blocker, and the increase of I_{NaL} in rabbit ventricular cells to evaluate the impact on arrhythmic risk.

2. Methods

I_{NaL} in dog was modeled previously by Hund and coworkers [16]. Their model was based on Luo and Rudy Model of guinea pig using Hodgkin Huxley formalism, and formulated in equations (1).

$$I_{NaL} = \bar{g}_{NaL} \cdot m_L^{\,3} \cdot h_L \cdot (V - E_{NaL})$$
$$\alpha_{m,L} = \frac{0.32 \cdot (V_m + 47.13)}{1 - e^{(-0.1 \cdot (V_m + 47.13))}} \qquad (1)$$
$$\beta_{m,L} = 0.08 e^{\left(-V_m / 11\right)}$$

Inactivation gate equation (2) and time constant of inactivation changed with respect to Luo and Rudy model and were based on Maltsev et al. data [9, 19]:

$$h_{L,\infty} = \frac{1}{1 - e^{\left((V_m + 91) / 6.1\right)}} \qquad (2)$$
$$\tau_h = 600 \text{ ms}$$

We adapted this model to the rabbit ventricular behavior based on experimental data obtained by Persson [17], where I_{NaL} yielded 0.2 pA/pF. Next, we introduced the model of I_{NaL} into the rabbit action potential model formulated by Shannon et a. [18].

The effect of dofetilide on I_{Kr} was modeled with a formulation proposed previously by our group [19].

To measure the I_{NaL}, APD, rate dependence and triangulation, we paced continuously with a basic train of pulses with a pulse duration of 2 ms and an amplitude of 1.5 times diastolic threshold. The steady-state was always reached. The basic cycle length (BCL) was modified in order to observe the rate-dependency of I_{NaL} enhancement.

Triangulation of the AP was defined as the difference between the APD at 90% repolarization and the APD at of 40% of repolarization (APD_{90}-APD_{40}, in ms). This parameter represents an accurate indicator for changes in AP morphology during phases two and three of the AP and is a strong predictor for proarrhythmic risk.

3. Results and discussion

Firstly, we have applied a voltage clamp protocol to compare our formulation of I_{NaL} against experimental data. We simulated I_{NaL} applying a holding potential of -80 mV during 300 ms and the voltage test was – 30 mV. Under control conditions, simulated I_{NaL} yielded 0.2 pA/pF, which was a similar value to the recordings obtained by Persson et al. [17]. When we increased I_{NaL} by 5 and 10-fold, the current was enhanced to -1.2 pA/pF and -2.3 pA/pF respectively, as shown in figure 1.

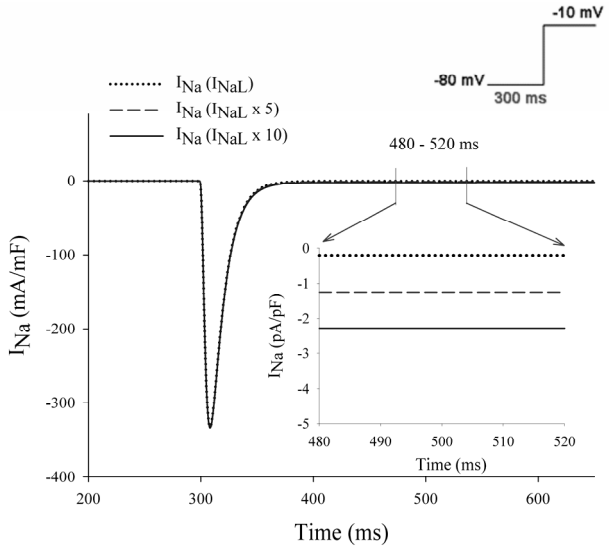

Figure 1. Simulated sodium current obtained after the voltage clamp protocol shown in the inset. I_{NaL} was measured between 480 and 520 ms after despolarization. The magnification of this area shows the value of I_{NaL}.

Analyzing the effects of this current on AP, we observed that the increase in the maximum conductance of I_{NaL} prolonged APD, which is in agreement with experimental observations obtained by So *et al* [3]. When the maximum conductance was increased 5 and 10-fold, APD_{90} underwent an increase of 15 % and 31 % respectively, for a BCL of 1000 ms. Figure 2 clearly represents this effect.

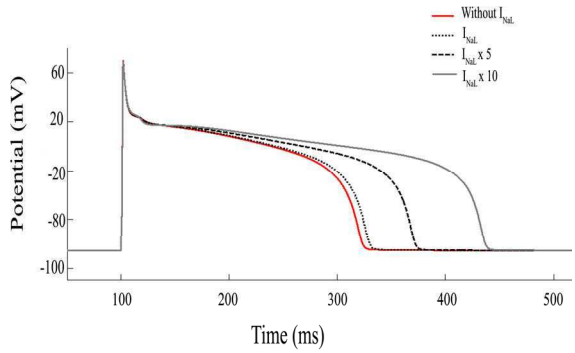

Figure 2. Effect of I_{NaL} on the action potential duration. I_{NaL} was enhanced 5 (discontinuous line) and 10 fold (continuous line). AP in the absence of I_{NaL} is represented with red line and AP in the presence of I_{NaL} with dotted line.

Additionally, we stimulated the cell with different BCLs (300, 400, 600, 800 and 2000 ms), and observed an APD rate-dependency in all conditions studied (without and with I_{NaL} multiplied by 5 and 10 times). For a BCL of

600 ms, the increase of APD_{90} was 9 % and 18 %, when I_{NaL} was enhanced 5 and 10-fold respectively. The prolongation of APD_{90} became higher (22 and 53 %, respectively) for a BCL of 2000 ms, as depicted in figure 3. Experimentally, Wu *et al.* also observed the rate-dependency, as the increase of APD was significantly higher at longer BCLs [6]. Milberg and coworkers observed how veratridine, an activator of I_{NaL}, had more important effects at higher BCLs [5]. Thus, our results reproduce the experimental behavior.

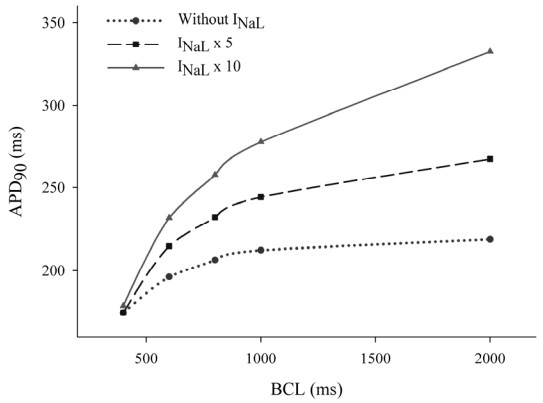

Figure 3. Rate dependence of APD_{90} under the effects of enhanced I_{NaL} (I_{NaL} was raised 5 and 10-fold).

Triangulation was also increased in a rate–dependent manner when I_{NaL} was increased 5 and 10-fold. However, the most significant change in triangulation was observed when I_{NaL} was 10 times higher and for a higher BCL, as shown in table 1. In fact, when I_{NaL} was increased 10-fold, triangulation was 34% and 61 % higher for BCL 1000 and 2000 ms, respectively. Lu *et al.* studied the action of anthopleurin, an activator of I_{NaL}, on APD and on triangulation. They observed a higher increase of APD and triangulation when the frequency was lower (higher BCL) [2]. So, our simulations are in agreement with the experimental data found by different authors.

	APD_{90} – APD_{40} (ms)		
BCL (ms)	Without I_{NaL}	I_{NaL} x 5	I_{NaL} x 10
400	75,5	75,5	75,8
1000	99,7	99,7	134,4
2000	117,4	117,4	189,6

Table 1. Effect of the I_{NaL} on triangulation (APD_{90} – APD_{40}).

The prolongation of APD is an essential prerequisite for induction of EADs. In this way, pure I_{Kr} inhibition preferentially prolongs repolarization and facilitates EADs at slow pacing rates. There is also experimental evidence that the combined action of different drugs can potentiate proarrhythmic effects. In order to analyze these proarrhythmic effects, specifically those provoked by I_{Kr} inhibition concomitant with I_{NaL} activators, we tested the action of several degrees of I_{NaL} enhancement (5-fold and 10-fold) combined with dofetilide (30, 100 and 300 nM), which is a I_{Kr} blocker. In our simulations, we obtained that EADs arose for concentrations of 100 and 300 nM of dofetilide and only when I_{NaL} was increased 10-fold and for a BCL of 2000 ms, as depicted in figure 4. Moreover, we observed a dramatical rate-dependency AP prolongation.

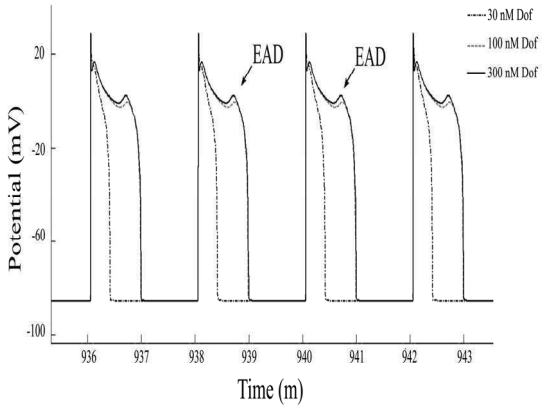

Figure 4. Concomitant effect of I_{NaL} enhancement (10–fold) and different concentrations of dofetilide (30, 200 and 300 nM) on AP.

Wu *et al* suggested that the increased late I_{Na} and reductions in repolarizing K^+ currents, are the major factor risk for TdP in humans [1]. These results were also supported by experimental data obtained by Fedida and coworkers [4]. Therefore, our results are consistent with experimental observations.

4. Conclusions

In this study, we proved the importance of I_{NaL} enhancement and its proarrhythmic action in the virtual rabbit ventricular cell. In particular, I_{NaL} has a determinant role in the prolongation of APD. Additionally, drugs like dofetilide can initiate EADs and extra beats under conditions of enhanced I_{NaL}, especially at a low pacing rate. These actions can lead to the development of serious ventricular tachycardias. From our study, it can be induced that targeting I_{NaL} block would have antiarrhythmic effects.

Acknowledgements

This work was partially supported by the European Commission preDiCT grant (DG-INFSO-224381) and by

the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica del Ministerio de Ciencia e Innovación of Spain (TEC2008-0290).

References

[1] Wu L, Shryock JC, Song Y, Belardinelli L. An increase in late sodium current potentiates the proarrhythmic activities of low-risk QT-prolonging drugs in female rabbit hearts. J Pharmacol Exp Ther 2006 Feb; 316[2]:718-26.

[2] Lu HR, Vlaminckx E, Van Ammel K, De Clerck F. Drug-induced long QT in isolated rabbit Purkinje fibers: importance of action potential duration, triangulation and early afterdepolarizations. Eur J Pharmacol 2002 Oct 4; 452[2]:183-92.

[3] So PP, Backx PH, Dorian P. Slow delayed rectifier K+ current block by HMR 1556 increases dispersion of repolarization and promotes Torsades de Pointes in rabbit ventricles. Br J Pharmacol 2008 Dec; 155[8]:1185-94.

[4] Fedida D, Orth PM, Hesketh JC, Ezrin AM. The role of late I and antiarrhythmic drugs in EAD formation and termination in Purkinje fibers. J Cardiovasc Electrophysiol 2006 May; 17 Suppl 1:S71-S78.

[5] Milberg P, Pott C, Fink M, Frommeyer G, Matsuda T, Baba A, et al. Inhibition of the Na+/Ca2+ exchanger suppresses torsades de pointes in an intact heart model of long QT syndrome-2 and long QT syndrome-3. Heart Rhythm 2008 Oct; 5[10]:1444-52.

[6] Wu L, Shryock JC, Song Y, Li Y, Antzelevitch C, Belardinelli L. Antiarrhythmic effects of ranolazine in a guinea pig in vitro model of long-QT syndrome. J Pharmacol Exp Ther 2004 Aug; 310[2]:599-605.

[7] Milberg P, Reinsch N, Osada N, Wasmer K, Monnig G, Stypmann J, et al. Verapamil prevents torsade de pointes by reduction of transmural dispersion of repolarization and suppression of early afterdepolarizations in an intact heart model of LQT3. Basic Res Cardiol 2005 Jul; 100[4]:365-71.

[8] Zilberter Y, Starmer CF, Starobin J, Grant AO. Late Na channels in cardiac cells: the physiological role of background Na channels. Biophys J 1994 Jul; 67[1]:153-60.

[9] Maltsev VA, Sabbah HN, Higgins RS, Silverman N, Lesch M, Undrovinas AI. Novel, ultraslow inactivating sodium current in human ventricular cardiomyocytes. Circulation 1998 Dec 8; 98[23]:2545-52.

[10] Maltsev VA, Kyle JW, Mishra S, Undrovinas A. Molecular identity of the late sodium current in adult dog cardiomyocytes identified by Nav1.5 antisense inhibition. Am J Physiol Heart Circ Physiol 2008 Aug; 295[2]:H667-H676.

[11] Zaza A, Belardinelli L, Shryock JC. Pathophysiology and pharmacology of the cardiac "late sodium current." Pharmacol Ther 2008 Sep; 119[3]:326-39.

[12] Zygmunt AC, Eddlestone GT, Thomas GP, Nesterenko VV, Antzelevitch C. Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. Am J Physiol Heart Circ Physiol 2001 Aug; 281[2]:H689-H697.

[13] Song Y, Shryock JC, Wu L, Belardinelli L. Antagonism by ranolazine of the pro-arrhythmic effects of increasing late INa in guinea pig ventricular myocytes. J Cardiovasc Pharmacol 2004 Aug; 44[2]:192-9.

[14] Noble D, Noble PJ. Late sodium current in the pathophysiology of cardiovascular disease: consequences of sodium-calcium overload. Heart 2006 Jul;92 Suppl 4:iv1-iv5.

[15] Orth PM, Hesketh JC, Mak CK, Yang Y, Lin S, Beatch GN, et al. RSD1235 blocks late INa and suppresses early afterdepolarizations and torsades de pointes induced by class III agents. Cardiovasc Res 2006 Jun 1; 70[3]:486-96.

[16] Hund TJ, Rudy Y. Rate dependence and regulation of action potential and calcium transient in a canine cardiac ventricular cell model. Circulation 2004 Nov 16; 110[20]:3168-74.

[17] Persson F, Andersson B, Duker G, Jacobson I, Carlsson L. Functional effects of the late sodium current inhibition by AZD7009 and lidocaine in rabbit isolated atrial and ventricular tissue and Purkinje fibre. Eur J Pharmacol 2007 Mar 8; 558[1-3]:133-43.

[18] Shannon TR, Wang F, Puglisi J, Weber C, Bers DM. A mathematical treatment of integrated Ca dynamics within the ventricular myocyte. Biophys J 2004 Nov; 87[5]:3351-71.

[19] J Saiz, M Monserrat, JM Ferrero (Jr), J Gomis-Tena, K Cardona, J Chorro, JM Alonso. Modulation of the regional dispersion of repolarization by the action of class III antiarrhythmic drug dofetilide. Computers in Cardiology 2005. Lyon-France, 2005:885-9

Karen Cardona Urrego
Instituto Interuniversitario de Investigación en Bioingeniería y Tecnología Orientada al Ser Humano
Universidad Politécnica de Valencia
Valencia- Spain

E-mail: kcardona@gbio.i3bh.es