Antiarrhythmic Efficacy of Selective Blockade of the Cardiac Slowly Activating Delayed Rectifier Current, \( I_{KS} \), in Canine Models of Malignant Ischemic Ventricular Arrhythmia

Joseph J. Lynch, Jr, PhD; Melanie S. Houle, MS; Gary L. Stump, BS; Audrey A. Wallace, BS; David B. Gilberto, DVM; Hossain Jahansouz, PhD; Garry R. Smith, BS; Andrew J. Tebben, MS; Nigel J. Liverton, PhD; Harold G. Selnick, PhD; David A. Claremon, PhD; George E. Billman, PhD

Background—To date, the lack of potent and selective inhibitors has hampered the physiological assessment of modulation of the cardiac slowly activating delayed rectifier current, \( I_{KS} \). The present study, using the \( I_{KS} \) blocker L-768,673, represents the first in vivo assessment of the cardiac electrophysiological and antiarrhythmic effects of selective \( I_{KS} \) blockade.

Methods and Results—In an anesthetized canine model of recent (8.5 \( \pm \) 0.4 days) anterior myocardial infarction, 0.003 to 0.03 mg/kg L-768,673 IV significantly suppressed electrically induced ventricular tachyarrhythmias and reduced the incidence of lethal arrhythmias precipitated by acute, thrombotically induced posterolateral myocardial ischemia. Antiarrhythmic protection afforded by L-768,673 was accompanied by modest 7% to 10% increases in noninfarct zone ventricular effective refractory period, 3% to 5% increases in infarct zone ventricular effective refractory period, and 4% to 6% increases in QTc interval. In a conscious canine model of healed (3 to 4 weeks) anterior myocardial infarction, ventricular fibrillation was provoked by transient occlusion of the left circumflex coronary artery during submaximal exercise. Pretreatment with 0.03 mg/kg L-768,673 IV elicited a modest 7% increase in QTc, prevented ventricular fibrillation in 5 of 6 animals, and suppressed arrhythmias in 2 additional animals.

Conclusions—The present findings suggest that selective blockade of \( I_{KS} \) may be a potentially useful intervention for the prevention of malignant ischemic ventricular arrhythmias. (Circulation. 1999;100:1917-1923.)

Key Words: antiarrhythmia agents ■ arrhythmia ■ myocardial infarction ■ fibrillation

In several mammalian species, including humans, cardiac delayed rectifier potassium current has been dissected into 2 kinetically distinct components: a rapidly activating current (\( I_{Kr} \)) that inwardly rectifies at depolarized membrane potentials and a slowly activating current (\( I_{KS} \)) with a linear current-voltage relationship. Inhibition of \( I_{Kr} \) has been and continues to be explored as an antiarrhythmic mechanism. Selective inhibitors of \( I_{Kr} \) have been assessed clinically for the prevention of malignant ventricular arrhythmia as well as atrial arrhythmia. Although some clinical studies have demonstrated antiarrhythmic activity, proarrhythmic effects have also been reported. Specifically, torsade de pointes has been associated with \( I_{Kr} \) blockade, thereby limiting the therapeutic utility of these agents.

To date, the antiarrhythmic potential of \( I_{KS} \) inhibition has not been vigorously assessed because of the lack of potent and selective inhibitors. Recently, the chemical synthesis and preliminary cardiac electrophysiological properties of a potent and selective \( I_{KS} \) blocker, L-768,673 (Figure 1), were described. The purpose of the present study was to evaluate the cardiac electrophysiological and antiarrhythmic activities of L-768,673 in 2 canine models of malignant ischemic ventricular arrhythmia. An anesthetized canine model of recent anterior myocardial infarction was used to assess the ability of L-768,673 to suppress ventricular tachyarrhythmia induced by programmed ventricular stimulation (PVS) as well as ventricular fibrillation precipitated by thrombotically induced acute myocardial ischemia. A conscious canine model of healed myocardial infarction was used to investigate the effects of L-768,673 on ventricular fibrillation provoked by transient myocardial ischemia against a background of elevated sympathetic activity elicited by submaximal exercise.

Methods

Procedures related to the use of animals were approved by the Institutional Animal Care and Use Committees at Merck Research Laboratories, West Point, Pa, and the Department of Physiology, The Ohio State University, Columbus.
Conscious Canine Model of Healed Anterior Myocardial Infarction

The conscious canine model of healed anterior myocardial infarction in which ventricular fibrillation is precipitated by transient acute myocardial ischemia during exercise was described originally by Schwartz et al.11 Details of this preparation as used in this study have been described previously.12 In brief, studies were conducted with conscious mongrel dogs (19.8 ± 0.4 kg) at 3 to 4 weeks after anterior myocardial infarction. The animals were walked on a motor-driven treadmill and adapted to the laboratory during this period. Susceptibility to ventricular fibrillation was tested as previously described.11,12 Ten animals developed ventricular fibrillation (susceptible) during the exercise-plus-ischemia test, and 7 did not (resistant). Four susceptible animals were not successfully resuscitated. Thus, studies were conducted with 6 susceptible and 7 resistant animals. One week later, the exercise-plus-ischemia test was repeated after administration of L-768,673. L-768,673 formulated as described above was infused at a dose of 0.03 mg/kg IV over a period of 30 minutes. Thirty minutes after the end of the infusion, the exercise-plus-ischemia test was performed (n = 13; susceptible, n = 6; resistant, n = 7). One week after these studies, a second control exercise-plus-ischemia test was repeated after infusion with the microemulsion vehicle (susceptible animals, n = 5; resistant dogs that had arrhythmias, n = 2).

Statistical Analysis

Data are expressed as mean ± SEM. For the anesthetized canine model of recent infarction, pretreatment versus posttreatment comparisons were made by use of a 2-tailed paired Student’s t test. Comparisons among treatment groups were made with a single-factor ANOVA followed by Scheffe’s post hoc test or Fisher’s exact test, as appropriate. Data obtained from the conscious postinfarction animal studies were analyzed with a 2-factor ANOVA with repeated measures. When the F value exceeded a critical value (P < 0.05), post hoc comparisons were made with Scheffe’s test. The effects of the interventions on mortality were evaluated with Fisher’s exact test.

Results

Anesthetized Canine Model of Recent Anterior Myocardial Infarction

Table 1 summarizes the effects of 0.0003, 0.003, and 0.03 mg/kg L-768,673 IV on heart rate, mean arterial pressure,

**Table 1.** ECG and Cardiac Electrophysiological Effects of 0.0003, 0.003, and 0.03 mg/kg L-768,673 IV in Chloralose-Anesthetized Dogs With Anterior Myocardial Infarction

<table>
<thead>
<tr>
<th>Parameter/Site</th>
<th>Base</th>
<th>L-768,673 0.0003 mg/kg IV</th>
<th>Base</th>
<th>L-768,673 0.003 mg/kg IV</th>
<th>Base</th>
<th>L-768,673 0.03 mg/kg IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus heart rate, bpm</td>
<td>122 ± 3</td>
<td>114 ± 4</td>
<td>123 ± 5</td>
<td>120 ± 6</td>
<td>129 ± 2</td>
<td>125 ± 3</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>55 ± 2</td>
<td>56 ± 5</td>
<td>62 ± 7</td>
<td>64 ± 7</td>
<td>62 ± 4</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>P-R interval, ms</td>
<td>111 ± 4</td>
<td>110 ± 4</td>
<td>116 ± 6</td>
<td>114 ± 5</td>
<td>110 ± 3</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>QRS interval, ms</td>
<td>42 ± 3</td>
<td>44 ± 3</td>
<td>46 ± 3</td>
<td>46 ± 3</td>
<td>44 ± 3</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>QTc interval, ms</td>
<td>353 ± 15</td>
<td>365 ± 14*</td>
<td>352 ± 12</td>
<td>372 ± 12†</td>
<td>370 ± 8</td>
<td>385 ± 9†</td>
</tr>
<tr>
<td>Paced (2.5 Hz) QT interval, ms</td>
<td>233 ± 7</td>
<td>238 ± 7</td>
<td>229 ± 9</td>
<td>239 ± 8*</td>
<td>240 ± 6</td>
<td>250 ± 6†</td>
</tr>
<tr>
<td>Noninfarct zone Excitation threshold, mA</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Effective refractory period, ms</td>
<td>161 ± 3</td>
<td>176 ± 3†</td>
<td>166 ± 4</td>
<td>180 ± 4†</td>
<td>165 ± 3</td>
<td>177 ± 5†</td>
</tr>
<tr>
<td>Infarct zone Excitation threshold, mA</td>
<td>0.10 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.20 ± 0.05</td>
<td>0.20 ± 0.05</td>
<td>0.13 ± 0.02</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Effective refractory period, ms</td>
<td>159 ± 4</td>
<td>165 ± 3</td>
<td>160 ± 8</td>
<td>168 ± 8*</td>
<td>156 ± 5</td>
<td>161 ± 7</td>
</tr>
</tbody>
</table>

Data are mean ± SEM; n = 9 or 10.

*P < 0.05, †P < 0.01 vs matching baseline value by a 2-tailed Student’s t test.

L-768,673

Figure 1. Chemical structure of (–)-2-[2,4-bis(trifluoromethyl)phenyl]-N-[3R-2,3-dihydro-2-oxo-5-phenyl-1-(2,2,2-trifluoroethyl)-1H-benzo[e][1,4]diazepin-3-yl]acetamide (L-768,673).
ischemia. Thrombotically induced acute posterolateral myocardial infarction expressed as a function of time after onset of recent anterior myocardial infarction also are summarized in Table 2. In the microemulsion vehicle group, the incidence of lethal ischemic arrhythmias was reduced slightly but nonsignificantly by low-dose 0.0003-mg/kg L-768,673 (6 of 10, 60%). However, the higher 0.003- and 0.03-mg/kg IV doses of L-768,673 significantly reduced the incidence of lethal arrhythmias (1 of 10, 10%, and 2 of 10, 20%, respectively, all ventricular fibrillation). Left circumflex coronary artery thrombus mass generally mirrored survival rate; treatment groups with the lowest arrhythmic mortality and hence longer survival times after the onset of ischemia possessed the largest thrombus masses on postmortem analysis. Times to onset of thrombotically induced posterolateral ischemia and underlying anterior infarct size did not vary significantly among treatment groups.

**Conscious Canine Model of Healed Anterior Myocardial Infarction**

As in previous studies, ventricular flutter degenerating into ventricular fibrillation was reproducibly induced with each presentation of the control exercise-plus-ischemia test in susceptible animals. The average time to ventricular fibrillation onset was 64.1 ± 7.9 seconds (range, 37 to 94 seconds) for the first control occlusion and 63.7 ± 9.5 seconds (range, 45.2 to 100 seconds) for the second control occlusion. The control exercise-plus-ischemia tests also elicited similar changes in heart rate (first occlusion: control, 202.0 ± 9.3; occlusion, 210.8 ± 12.8 bpm; second occlusion: control, 208.8 ± 10.2; occlusion, 226.0 ± 11.2 bpm).

Representative recordings obtained from the same animal before and after pretreatment with L-768,673 are displayed in Figure 3. In contrast to the control occlusion, L-768,673 significantly reduced the incidence of ventricular fibrillation, protecting 5 of 6 animals (83% reduction, \( P = 0.008 \)). The arrhythmias were completely suppressed in 2 animals, whereas the remaining 3 animals still had a few single or coupled premature ventricular beats (6, 7, and 27 beats, respectively). In addition, L-768,673 completely suppressed arrhythmias in 2 resistant animals in which multiple ventricular complexes were induced during the control exercise-plus-ischemia test. Finally, the microemulsion vehicle (n = 5) failed to protect any animal from malignant arrhythmias, nor did this treatment prevent arrhythmias in resistant animals (n = 2).
The effects of L-768,673 on resting heart rate and ECG parameters are displayed in Figure 4. L-768,673 significantly increased QTc interval (control, 258.6±6.4 ms; L-768,673, 275.5±5.4 ms; change, 16.9±5.8 ms, 7.0±2.3%) but did not alter resting heart rate (control, 109.7±3.0 bpm; L-768,673, 105.5±3.4 bpm). Likewise, L-768,673 did not alter the heart rate response to exercise (Figure 5). Exercise significantly increased QTc interval (control, 258.6±6.4 ms; exercise, 282.9±7.9 ms). The QTc interval was not increased further with exercise in L-768,673–treated animals (control, 275.5±5.4 ms; exercise, 284.9±8.9 ms). Finally, L-768,673 did not alter the heart rate response to the coronary artery occlusion (control, 202±9.3; occlusion, 210.8±12.8 bpm; L-768,673, 211.7±9.8; occlusion, 214±15 bpm).

Discussion

Two distinct components of cardiac delayed rectifier current, \( I_{Kr} \) and \( I_{Ks} \), have been identified in multiple mammalian species, including humans.\(^1\)–\(^5\) \( I_{Kr} \) is a rapidly activating current that exhibits prominent inward rectification at depolarized membrane potentials, whereas \( I_{Ks} \) is a more slowly activating and deactivating current with a linear current-voltage relationship.\(^14\) It is noteworthy, however, that the biophysical characteristics of \( I_{Ks} \) may vary among species, with the rate of deactivation of \( I_{Ks} \) reported to be more rapid in dog than in guinea pig myocytes.\(^5\) \( I_{Kr} \) and \( I_{Ks} \) are differentially modulated in varied experimental conditions that mimic physiological or pathological states. During rapid heart rate, the contribution of \( I_{Kr} \) to repolarization is functionally diminished because of the enhanced contribution of other currents, including \( I_{Ks} \), which accumulates at faster rates as a result of incomplete deactivation.\(^13\) This differential rate-dependence of the delayed rectifier subtypes has been proposed to underlie the diminished activities of \( I_{Kr} \) blockers at faster heart rates and exaggerated activity at slower heart rates (ie, reverse frequency–dependence).\(^13\),\(^16\) \( I_{Ks} \) is unaffected per se by \( \beta \)-adrenergic receptor stimulation, whereas \( I_{Ks} \) is enhanced; however, the functional contribution of \( I_{Ks} \) to repo-

Figure 3. Representative recordings from same conscious dog with healed anterior myocardial infarction before and after treatment with \( I_{Ks} \) antagonist L-768,673 (0.03 mg/kg IV). Note that L-768,673 prevented ventricular flutter despite a similar heart rate (HR) response to occlusion and ischemic ECG changes. Bar represents 1 second.

Figure 4. Effects of L-768,673 (n=13, 0.03 mg/kg IV) on heart rate and ECG parameters before onset of exercise. *P<0.01 control vs treated.

Figure 5. Effect of L-768,673 (n=13, 0.03 mg/kg IV) on heart rate response to exercise. Note that L-768,673 did not alter exercise heart rate response. Exercise levels: 1=0 kph/0% grade, 2=4.0 kph/0% grade, 3=6.4 kph/0% grade, 4=6.4 kph/4% grade, 5=6.4 kph/8% grade, 6=6.4 kph/12% grade, and 7=6.4 kph/16% grade.
larization is diminished during β-adrenergic receptor stimulation. The differential modulation of $I_{Kr}$ and $I_{Ks}$, particularly the maintained or enhanced activity of $I_{Ks}$ relative to $I_{Kr}$ with faster heart rate and enhanced sympathetic tone, has led to the speculation that $I_{Ks}$ may represent the more effective target current for therapeutic modulation of cardiac action potential duration (APD). The in vivo assessment of the antiarrhythmic potential of $I_{Ks}$ blockade has been hampered by the lack of potent and selective inhibitors. Chromanol 293B has been reported to block $I_{Kr}$ selectively with $IC_{50}=2.0$ to 9.9 $\mu$mol/L in guinea pig myocytes, *Xenopus* oocytes, and COS-7 cells. Chromanol 293B produces frequency-independent prolongations of cardiac APD in guinea pig and human ventricular myocytes, in contrast to the strong reverse frequency-dependent profile displayed by the $I_{Kr}$ blocker E-4031. Chromanol 293B has been used to evaluate the contribution of $I_{Ks}$ to action potential morphology and arrhythmogenesis in isolated canine cardiac preparations. The exposure of canine epicardial, midmyocardial, and endocardial ventricular myocytes to chromanol 293B resulted in no afterdepolarization-type activity, whereas combined exposures to either 293B and the $I_{Kr}$ blocker E-4031 or 293B and isoproterenol resulted in early and delayed afterdepolarizations, respectively, suggesting arrhythmogenic risk with simultaneous $I_{Kr}$ and $I_{Ks}$ blockade as well as with $I_{Ks}$ blockade in the setting of high sympathetic tone. In an isolated canine ventricular wedge preparation, perfusion with chromanol 293B homogeneously prolonged QT interval and APD of epicardial, midmyocardial, and endocardial myocytes but did not widen the T wave, increase transmural dispersion of repolarization, or induce torsade de pointes. However, combined perfusion with chromanol 293B and isoproterenol widened the T wave, accentuated transmural dispersion of repolarization, and elicited torsade de pointes, again suggesting arrhythmogenic risk with $I_{Ks}$ blockade in the setting of high sympathetic tone.

L-768,673 is a recently described benzodiazepine blocker of $I_{Kr}$. In guinea pig ventricular myocytes, L-768,673 blocks $I_{Kr}$ selectively and potently ($IC_{50}=6$ nmol/L) and elicits a self-limiting, maximal 30% prolongation of APD. No early afterdepolarizations were noted with exposure of guinea pig ventricular myocytes to L-768,673, whereas comparable lengthening of APD with $I_{Kr}$ blockade resulted in the development of early afterdepolarizations. L-768,673 was used in the present studies to assess the antiarrhythmic efficacy of selective $I_{Kr}$ blockade in 2 canine models of ischémically induced malignant ventricular arrhythmias. In anesthetized dogs with recent anterior myocardial infarction, L-768,673 suppressed both electrically induced ventricular tachyarrhythmia and ventricular fibrillation precipitated by thrombically induced posterolateral myocardial ischemia. Efficacy in this preparation was associated with modest 3% to 10% increases in ventricular refractory periods and 4% to 6% increases in QT interval measured before the acute ischemic triggering event. Significant antiarrhythmic efficacy with only modest increases in ventricular refractoriness and QTc interval with L-768,673 contrasts with the profiles observed in previous studies with the selective $I_{Kr}$ blockers d-sotalol, dofetilide, E-4031, and MK-499. These $I_{Kr}$ blockers required 12% to 27% increases in ventricular refactoriness and 12% to 17% increases in QTc interval to achieve >50% reductions in the incidence of malignant ventricular arrhythmias in previous studies using conscious and anesthetized versions of this canine model. The greater increase in ventricular refactoriness and QTc interval required for selective $I_{Kr}$ blockers for significant suppression of ischemic ventricular arrhythmias may be necessary to offset reduced $I_{Ks}$ in the settings of elevated heart rate and high sympathetic tone such as occur during acute myocardial ischemia. Conversely, the modest effects on ventricular refactoriness and QTc interval required for antiarrhythmic activity with L-768,673 are consistent with a maintenance, if not enhancement, of $I_{Ks}$ activity during acute myocardial ischemia.

The antiarrhythmic potential of $I_{Ks}$ blockade with L-768,673 was further evaluated in the conscious canine model of healed anterior myocardial infarction. In this model, ventricular fibrillation was induced by the reversible occlusion of the left circumflex coronary artery during submaximal exercise to increase cardiac sympathetic activity. This model differs in several respects from the preceding preparation, including study in the conscious versus anesthetized state, healed versus recent anterior infarction, production of secondary ischemic insult by mechanical versus thrombotic coronary occlusion, and most notably the occurrence of the secondary ischemic insult in the setting of high sympathetic tone and elevated heart rate elicited by exercise. Pharmacologically, $I_{Ks}$ blockers, including d-sotalol, have demonstrated efficacy in suppressing lethal ventricular arrhythmias provoked by thrombotic coronary artery occlusion in the setting of recent myocardial infarction studied in either the anesthetized or conscious state. In contrast, d-sotalol was ineffective in preventing the provocation of malignant ventricular arrhythmias by transient coronary artery occlusion during exercise in the setting of healed myocardial infarction, presumably because of an attenuation of the effects of $I_{Ks}$ blockade in the setting of high heart rate and high sympathetic tone. In this conscious model of healed anterior myocardial infarction, pretreatment with L-768,673 prevented malignant arrhythmias provoked by coronary artery occlusion in the setting of exercise-induced high sympathetic tone in 5 of 6 animals previously susceptible to ventricular fibrillation. Efficacy in this preparation was accompanied by a modest 7% increase in preexercise QTc interval, whereas QTc was not further increased during exercise. The lack of overt proarrhythmia with $I_{Kr}$ blockade with L-768,673 in the present in vivo study apparently contrasts with the arrhythmogenic risk of combined exposure to isoproterenol and chromanol 293B in canine ventricular myocytes and in the canine ventricular wedge preparation. The demonstration of proarrhythmia in preclinical models is highly preparation-dependent, however; therefore, further studies are required to determine whether the observed differences are compound- or preparation-specific.

The question remains whether or not selective $I_{Ks}$ blockade may have proarrhythmic activity in humans. This is a particularly important question, given the discouraging results from large clinical studies using $I_{Kr}$ blockers (ie, SWORD and DIAMOND). Recent genetic linkage and molecular biolog-
ichological studies of patients with congenital long-QT syndrome (a rare cardiac disorder characterized by prolonged ventricular repolarization, long QT interval, and a high risk for sudden death) have identified multiple mutations of several genes encoding cardiac ion channels, including KvLQT1, encoding the I\(K_a\) o-subunit, and KCNE1, encoding IsK, thought to be an I\(K_s\) \(\beta\)-subunit. The incidence of cardiac arrhythmia and sudden death are reportedly more likely to be associated with adrenergic factors (eg, physical or emotional stress) in long-QT syndrome patients with KvLQT1 mutations compared with other mutations. Whether these associations between specific genetic defects in KvLQT1 and IsK proteins and congenital long-QT syndrome identify a small high-risk population for which class III therapy would be absolutely contraindicated or, conversely, constitute genetic evidence that I\(K_s\) blockade representing an inherently unacceptable risk for the general population is at present unknown. Therefore, although the present studies with the I\(K_s\) blocker L-768,673 indicate antiarrhythmic activity in preclinical models, it is probable that under appropriate conditions, I\(K_s\) blockade could be arrhythmogenic. Further preclinical and clinical studies are required to better assess the overall antiarrhythmic potential and arrhythmogenic risk of selective I\(K_s\) blockade.

References
Antiarrhythmic Efficacy of Selective Blockade of the Cardiac Slowly Activating Delayed Rectifier Current, I_{Ks}, in Canine Models of Malignant Ischemic Ventricular Arrhythmia


Circulation. 1999;100:1917-1922
doi: 10.1161/01.CIR.100.18.1917

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/100/18/1917

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/