Electrophysiological Effects of LU111995 on Canine Hearts: In Vivo and In Vitro Studies

EUGENE A. SOSUNOV, RAVIL Z. GAINULLIN, PETER DANILO, Jr., EVGENY P. ANYUKHOVSKY, MICHAEL KIRCHENGAST, and MICHAEL R. ROSEN


Accepted for publication March 8, 1999

ABSTRACT

We studied the electrophysiological effects of LU111995 (1–15 mg/kg p.o.) in conscious dogs with chronic atrioventricular block and ventricular pacing at 50 to 130 beats/min. LU111995 had no effects on idioventricular rhythm, QRS duration, and ventricular conduction time. It significantly prolonged Q–T interval (by 5–8%) and effective refractory period (ERP) (by 5–12%) with the maximal effect at 4 h after a 10 mg/kg dose. At 10 and 15 mg/kg, it increased the ERP/Q–T ratio. In vitro, the effects of LU111995 (1 × 10⁻⁷ to 1 × 10⁻⁶ M) on action potentials of Purkinje fibers (PFs) and M cells were studied at cycle lengths (CL) of 300 to 2000 ms. It had no effects on maximum diastolic potential and action potential amplitude in either tissue. High concentrations induced a moderate, rate-independent decrease of Vₘₚₐₓ in M cells. In PFs and M cells, it produced reverse use-dependent lengthening of action potential duration (APD). In PFs at long CL, the drug exhibited a biphasic concentration-dependent effect on APD: maximum prolongation (by 26% at a CL of 2000 ms) was attained at 1 × 10⁻⁶ M, and a decrease of APD occurred at higher concentrations. In M cells, the maximum effect on APD occurred at 3 × 10⁻⁶ M. Early afterdepolarizations were seen in 50% of M cell preparations but only at CL of 2000 ms. Triggered activity did not occur. In summary, LU111995 prolongs the Q–T interval to a limited degree and is not arrhythmogenic over the physiological range of CLs.

In some instances, drug-induced long Q–T intervals are associated with torsade de pointes and sudden death (Tan et al., 1995). The mechanism generally assumed to be operative is reduction in the repolarizing K⁺ currents, I_Kr, and/or I_Ks; prolongation of the cardiac action potential duration (APD), and initiation of early afterdepolarizations (EADs) that lead to the classic torsade de pointes arrhythmia (Roden and Hoffman, 1985; Hondeghem and Snyders, 1990; Funkc-Brentano, 1993). These untoward events have led regulatory agencies to require the use of established intact animal and isolated tissue models (or the development of new models) to determine the likelihood of proarrhythmia occurring on administration of new drugs that prolong repolarization.

LU111995 [(+)-(1S,5R,6S)-exo-3-[2-[6-(4-fluorophenyl)-3-aza-bicyclo[3.2.0]heptan-3-yl]ethyl]-1H,3H-quinoxaline-2,4-dione fumarate; Fig. 1) is a recently identified antipsychotic agent with high 5-hydroxytryptamine_2 and dopamine D₄ receptor affinities as well as D₃ versus D₂ receptor selectivity (Steiner et al., 1998). Because of concern over the effects of some antipsychotic agents to excessively prolong repolarization and induce proarrhythmia, the purpose of the present study was to determine the effects of LU111995 on ventricular repolarization in vivo and in vitro. Therefore, we investigated the actions of LU111995 on the ECG and the ventricular effective refractory period (ERP) in conscious dogs in which chronic atrioventricular block had been induced to permit measurements over a range of heart rates that encompass the physiological range. In vitro, the electrophysiological properties of LU111995 were investigated in Purkinje fibers and mid myocardial cells (M cells) (Sicouri and Antzelevitch, 1991). M cells were studied because they constitute a significant fraction of ventricular myocardium (Antzelevitch et al., 1994; Sicouri and Antzelevitch, 1995; Anyukhovsky et al., 1996), they contribute importantly to T-wave configuration and the Q–T interval, and, due to their exceptional sensitivity to APD-prolonging agents, they develop EADs more readily than epicardial or endocardial cells (Antzelevitch and Sicouri, 1994; Antzelevitch et al., 1996; Shimizu and Antzelevitch, 1997).

Materials and Methods

All experimental procedures conformed to the “Guiding Principles of the Care and Use of Animals” of the American Physiologic Society.
and were in accordance with the “Guide for the Care and Use of Laboratory Animals” (DHEW (DHHS) publication (NIH) 85-23, revised 1985).

**In Vivo Experiments.** Conditioned mongrel dogs of either sex weighing 20 to 25 kg were fasted overnight and anesthetized with propofol (6 mg/kg i.v.). After tracheal intubation, anesthesia was maintained with isoflurane (2%) inhalation. Under sterile conditions, a thoracotomy was performed in the right forth intercostal space, and the heart was positioned in a pericardial cradle. To achieve a low heart rate, complete heart block was produced by the injection of 0.1 to 0.3 ml of 40% formalin into the region of the AV node (Scherlag et al., 1967; Anyukhovsky et al., 1996). An epicardial screw-type electrode (model 6917; Medtronic) was placed in the left ventricle near the apex. The electrode lead was connected to a programmable Medtronic pacemaker (MINIX 8340) that was placed in a s.c. pouch. For studying ERP and for monitoring ventricular conduction time, three platinum bipolar electrodes were sewn to the epicardium of the right ventricle (electrodes were placed along a base-apex axis approximately 1–2 cm apart). The wires from the electrodes were secured to the ribs, tunneled s.c., and exteriorized through a small incision between the scapulae. The pericardium was left open, the chest wall was closed in layers, and the pneumothorax was evacuated by a chest tube. Each dog was fitted with a jacket to prevent damage to the pacemaker and wires. Animals were allowed to recover for 2 weeks. After recovery from surgery and during 1 week before the drug protocols were begun, each dog was trained to stand quietly in an animal sling during ECG monitoring. At this time, the ECG had completely stabilized. During the periods of recovery and training and between drug administration protocols, ventricular pacing was maintained at 60 beats/min.

ECC parameters were measured at heart rates of 50 (when idioventricular rhythm permitted), 60, 70, 80, 110, and 130 beats/min. The low heart rates were considered of particular importance because drugs that are reverse use dependent and induce torsade de pointes have a propensity to do so at long cycle lengths (CLs) (e.g., Funck-Brentano, 1993). Variability of the Q–T interval over the period of time of the study was minimal (i.e., at CL = 1000 ms, <5 ms; Fig. 2). To ensure a steady state, each rate was maintained for 3 min. To ensure a steady state, each rate was maintained for 3 min before data were collected (Anyukhovsky et al., 1996). ERP was determined at heart rates of 60, 80, and 130 beats/min. In these experiments, the heart was stimulated with 1-ms rectangular pulses of amplitude 2 × threshold (Pulsemaster A300, with isolation unit A360; WPI, Sarasota, FL) through the right ventricular epicardial electrode that was closest to the apex. After every 10 beats, an extrastimulus was delivered, the delay for which was gradually decreased by 10- and then 1-ms decrements. The earliest premature response that propagated to the most distant electrogram site was assumed to represent the end of the ERP. Conduction time was measured as the interval between the two bipolar right ventricular electrodes recorded when that located closest to the apex was stimulated. ECG and local electrogram signals were digitized with an analog-to-digital converter (D-210; DATAQ Instruments Inc., Akron, OH) and stored in a personal computer for further analysis.

Dogs were randomly assigned to receive 1, 3, 10, and 15 mg/kg LU111995 p.o. The highest dose used in early phase 2 studies was 200 mg/patient/day (Knoll, 1998), corresponding roughly to the 3 mg/kg dose in dogs. Cardiovascular assessments, which required about 20 min in toto, were performed at control (before drug administration) and 1, 2, 4, and 8 h after drug. Blood samples were drawn at appropriate intervals for measurements of serum levels of LU111995 and its major metabolites, LU195730 and LU273973, which are generated by glucuronidation (Steiner et al., 1998).

**Serum drug concentrations** were measured as follows: an HPLC method was used for the simultaneous determination of LU111995, LU195730, and LU273973 in dog plasma. LU81028 was the internal standard. After a liquid-liquid plasma extraction procedure, chromatographic separation was performed on a 4-μm Superspher 60, RPselect B analytical column (250 × 4.0 mm) using a mixture of 0.05 M KH₂PO₄ (pH 5) at a flow rate of 0.9 ml/min at 35°C. The chromatographic peaks were measured by UV detection at 240 nm. The calibration curves were linear with correlation coefficients of 0.999 or better from 5 ng/0.5 ml to 80 ng/0.5 ml for all three compounds in plasma. Other endogenous components did not interfere with this method. Testing of the method with 0.5 ml of plasma at a working range of 5 to 80 ng/ml yielded coefficients of variation of 3.8% to 17.8% for LU111995, 7.0% to 11.0% for LU195730, and 6.1% to 13.3% for LU273973. Throughout the working range, the method yielded accurate values from 93.4% to 107.0% for LU111995, 92.7% to 107.9% for LU195730, and 95.4% to 107.5% for LU273973.

**In vitro experiments.** Mongrel dogs of either sex weighing 20 to 22 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Their hearts were removed through a left lateral thoracotomy and immersed in cold Tyrode’s solution equilibrated with 95% O₂/5% CO₂ and containing 131 mM NaCl, 18 mM NaHCO₃, 4 mM KCl, 2.7 mM CaCl₂, 0.5 mM MgCl₂, 1.8 mM Na₂HPO₄, and 5.5 mM dextrose. Two types of preparations were used: free-running Purkinje fibers (PFs) obtained from either ventricle and myocardial slices (2 cm × 2 cm × 1 mm) taken from the right ventricle and immersed in cold Tyrode’s solution equilibrated with 95% O₂/5% CO₂ and containing 131 mM NaCl, 18 mM NaHCO₃, 4 mM KCl, 2.7 mM CaCl₂, 0.5 mM MgCl₂, 1.8 mM Na₂HPO₄, and 5.5 mM dextrose.
mm) filleted from the posterobasal left ventricular free wall parallel to the epicardial surface from the depth of 3 to 5 mm beneath it. The preparations were placed in a tissue bath and superfused with Tyrode’s solution warmed to 37°C (pH 7.35 ± 0.05). Solution was pumped through the tissue bath at a flow rate of 12 ml/min, changing chamber content three times per minute. The bath was connected to ground via a 3 M KCl/Ag/AgCl junction.

All preparations were impaled with 3 M KCl-filled glass capillary microelectrodes that had tip resistances of 10 to 20 MΩ. The maximum upstroke velocity of the action potential (V_max) was obtained through electronic differentiation with an operational amplifier. The electrodes were coupled by an Ag/AgCl junction to an amplifier with high input impedance and input capacity neutralization. Transmembrane action potentials and V_max were displayed on a digital storage oscilloscope (model 4074; Gould) and stored in digitized form for subsequent analysis. For stimulation of preparations, standard techniques were used to deliver square-wave pulses 1.0 ms in duration and 1.5× threshold through bipolar Teflon-coated silver electrodes. To investigate frequency dependence of drug effects, the preparations were driven at CLs of 2000, 1000, 500, and 300 ms in sequence.

Results

In Vivo Study. The idioventricular rhythm, QRS duration, conduction time, and Q–T duration all were stable and unchanging throughout the duration of the 3-week study period (Table 1). LU111995 had a consistent effect on the Q–T interval, as shown in Fig. 3. Q–T prolongation ranged from 5% to 8% with the maximum at 4 h after the 10 mg/kg dose (Fig. 3A). The magnitude of prolongation diminished at 15 mg/kg. The maximum effects of 10 mg/kg at 4 h after drug administration at all driving rates are presented in Fig. 3B. Prolongation reached significance at 60, 70, and 80 beats/min. The magnitude of the Q–T interval prolongation is explored across all doses in Fig. 1C.

The effects of LU111995 on the ERP at heart rates of 60 and 130 beats/min are shown in Fig. 4A. All doses prolonged the ERP significantly, with the maximal effect occurring at 2 to 4 h after drug administration. A similar picture was observed at 80 beats/min (data not shown). The maximal percent increases were 5% at 1 mg/kg, 7% at 3 mg/kg, 12% at 10 mg/kg, and 9% at 15 mg/kg. The changes in ERP at various pacing rates at 10 mg/kg and 4 h after drug administration are shown in Fig. 4B. Note that significant prolongation of ERP was seen across all rates tested. The magnitude of ERP prolongation across all doses at a rate of 60 beats/min is demonstrated in Fig. 4C. As in the case of the Q–T interval, a maximal effect was observed at 10 mg/kg, and the effect decreased at 15 mg/kg.

Figure 5 illustrates the effects of LU111995 on the ERP/Q–T ratio. ERP/Q–T increased at the 10 and 15 mg/kg doses,
2 and 4 h after drug administration (Fig. 5A). The increase was 7%. With an acceleration of pacing rate, the effect decreased; at 130 beats/min, no significant effect on ERP/Q–T ratio was seen. The changes in ERP/Q–T at various pacing rates at 10 mg/kg and 4 h after drug administration are shown in Fig. 5B. Significant prolongation of ERP/Q–T was seen only at 60 beats/min. The magnitude of effect across all doses at a stimulation rate of 60 beats/min is demonstrated in Fig. 5C. The drug induced a dose-dependent increase of ERP/Q–T ratio that reached significance at 10 and 15 mg/kg.

Figure 6 demonstrates serum concentrations of LU111995 and its metabolites achieved at each dosage schedule. A maximal level of parent compound was achieved at 4 h after administration and then slowly decreased. Maximal concentrations of the metabolites LU195730 and LU273973 were 25- and 70-fold less, respectively, than the parent compound. These levels were achieved at 2 h after LU111995 administration and then decayed faster than that of the parent compound.

**In Vitro Study.** Figure 7 illustrates representative PF and M cell transmembrane potentials recorded in control and in the presence of two LU111995 concentrations (10^{-6} and 10^{-5} M), at the longest and shortest CLs. Control maximum diastolic potential (MDP) at a CL of 1000 ms was −92 ± 1 mV in PFs and −91 ± 1 mV in M cells. There was no significant effect of LU111995 on MDP at LU111995 concentrations up to 3 × 10^{-6} M at any cycle length (e.g., at CL = 1000 ms, MDP = −91 ± 1 mV in PFs and −89 ± 1 mV in M cells at 3 × 10^{-6} M, P > .05 compared with control). Only in PFs at the fast rate of stimulation (300 ms) and at 10^{-5} M drug was a significant decrease in MDP seen (to −89 ± 1 mV, P < .05). Similar results were seen for action potential amplitude (A.P.D.) and V_{max} in both tissues; in PFs, amplitude and V_{max} were reduced significantly only at CL of 300 ms and 10^{-5} M drug from 130 to 126 ± 1 mV amplitude and from 543 ± 28 to 455 ± 31 V/s V_{max} (both P < .05). In M cells, V_{max}, but not amplitude, was reduced significantly at 3 × 10^{-6} M and 1 × 10^{-5} M drug at all cycle lengths. For example, at CL of 1000 ms, control V_{max} was 351 ± 42 V/s, and this was reduced to 290 ± 20 V/s at 3 × 10^{-6} M and 293 ± 23 V/s at 1 × 10^{-5} M (both P < .05).

The most prominent effect of LU111995 was a lengthening of the APD in both types of tissues. In PFs, at low stimulation
rates, the compound exhibited a concentration-dependent bi-phasic effect on APD to 90% repolarization (APD 90): the maximum prolongation was attained at 1 \times 10^{-2} \text{ M}, and a decrease in APD 90 was then seen at higher concentrations (Fig. 8A). This decrease was accompanied by significant shortening of APD to 50% repolarization (APD 50) (Fig. 8B). The APD 90 prolongation manifested reverse use dependence: no prolongation took place at high stimulation rates.

As in PFs, LU111995 induced reverse use-dependent prolongation of APD in M cells (Fig. 9). At low stimulation rates, about the same lengthening of APD 90 and APD 50 was observed. The maximum effect was attained at 3 \times 10^{-2} \text{ M}. In contrast to PFs, no APD shortening was seen at high LU111995 concentrations. In three of six experiments with M cells, LU111995 of 3 \times 10^{-2} \text{ M} or more induced EADs that developed at the end of the plateau (Fig. 7). These depolarizations were observed only at the longest CL (2000 ms) and had low amplitude. Triggered activity was never seen.

Discussion

LU111995 had no effect on the variables measured in vivo except for the Q–T interval and the ERP. The comparison of time courses of Q–T interval changes (Fig. 3) and of LU111995 and its serum metabolite levels (Fig. 9) suggests that the parent compound rather than its metabolites was mainly responsible for Q–T prolongation. The extent of Q–T increase was small, never reaching 10% even at its greatest magnitude. Moreover, in clinical studies, at a maximum dosage of 200 mg/patient/day, the peak plasma level of parent compound attained was approximately 500 ng/ml, with steady-state levels in the range of 50 to 100 ng/ml (Knoll, 1998). This suggests that the 3 mg/kg dose in dogs is the likely clinical surrogate. This dose had minimal effects on the Q–T interval.

The fact that the largest increase in Q–T interval occurred at the 10 mg/kg dose and that the Q–T interval decreased at the higher dose suggests that in addition to inducing K^+ channel blockade, which would prolong the APD, the drug may reduce inward plateau currents, thereby limiting the extent of prolongation of the Q–T interval. In vitro results with PFs and M cells are consistent with this suggestion. The drug significantly decreased APD 50, a result consistent with inhibition of Na^+ and/or Ca^{2+} plateau currents. Although no APD shortening was observed in M cells, a decrease of the magnitude of APD lengthening occurred at the highest drug concentration. Also consistent with an effect on inward Na^+ current was the decrease in V_{\text{max}} of phase 0.

PFs are vulnerable to the effects of APD-prolonging agents. Their long APD and steep APD-rate relationship make them a likely site for the occurrence of EADs and triggered activity as seen especially at slow stimulation rates (Brachman et al., 1983). In light of the above, the action potential prolongation and reverse use dependence of LU111995 effects seen in PFs are a potential drawback. However, there was a limit of

Fig. 6. Time courses of serum levels of LU111995 (A) and its metabolites LU195730 (B) and LU273973 (C). All doses of LU111995 are shown. Horizontal axis, time in hours after LU111995 administration. Values are mean ± S.E.M. (n = 7 for 1 mg/kg, n = 10 for 3, 10, and 15 mg/kg). * P < .05 compared with 0 h (before LU111995 administration).

Fig. 7. Examples of the effects of LU111995 on PF and M cell action potentials at CL of 2000 and 300 ms. Top, transmembrane action potential. Bottom, V_{\text{max}}. Vertical calibration is for the action potential and V_{\text{max}}; horizontal calibration is for the action potential. Numbers represent molar concentrations of the compound. C, control.
LU111995-induced APD prolongation in PFs: maximal prolongation was attained at $1 \times 10^{-6}$ M, and a shortening of APD occurred at higher concentrations. In this setting of high LU111995 concentrations, EADs did not occur in PFs. In contrast, EADs were seen in three of six M cell preparations but only at the highest drug concentrations and at a cycle length (2000 ms) outside the usual physiological range, although within the range of long sinus pauses.

In vivo, the effect of LU111995 to prolong ERP was greater than its effect on Q–T. The results of the experiments in vitro help explain this action. In M cells, the drug induced a moderate, concentration-dependent decrease of $V_{\text{max}}$ that was independent of simulation rate. Although not reaching a statistically significant level, a qualitatively similar effect was observed in PFs. This type of local anesthetic action may prolong refractoriness and increase the ERP/APD ratio. Although not reaching a statistically significant level, a qualitatively similar effect was observed in PFs. This type of local anesthetic action may prolong refractoriness and increase the ERP/APD ratio (Bigger and Mandel, 1970; Gintant, 1990). Thus, even if there were some arrhythmogenic potential of the Q–T prolongation, this would tend to be counteracted by the greater prolongation of the ERP. The inhibition of $V_{\text{max}}$ implies a decrease of conduction velocity that, however, was not seen in vivo. This can be explained as follows: The LU111995-induced decrease of $V_{\text{max}}$ did not exceed 20%. The speed of impulse propagation is directly related to the square root of $V_{\text{max}}$ (Buchman et al., 1985); hence the $V_{\text{max}}$ inhibition in our study implies a less than 10% slowing of conduction velocity. This is why no significant changes in CT and QRS duration was seen in the experiments in vivo.

The results of the present study relate to an important additional question: What type of isolated ventricular tissue adequately reflects the effects of APD-prolonging agents on ventricular repolarization in vivo? Free running PFs are widely used in vitro as a multicellular preparation. Because PFs have long APD and are sensitive to action potential prolonging effects of antiarrhythmic compounds (Varro et al., 1986; Anyukhovsky et al., 1997), they are suitable for the estimation of proarrhythmic propensity. However, to estimate in vitro the effects of drugs on ventricular repolarization in vivo (on Q–T interval), ventricular myocardial tissue is a more appropriate model. The use of M cells here is reasonable because they constitute a significant fraction of ventricular myocardium (Antzelevitch et al., 1994; Sicouri and Antzelevitch, 1995; Anyukhovsky et al., 1996) and can contribute importantly to T-wave configuration and the Q–T interval. In addition, APD-prolonging agents produce a marked APD lengthening, EADs, and triggered activity in M more than in
endocardial or epicardial cells (Antzelevitch and Sicouri, 1994; Antzelevitch et al., 1996; Shimizu and Antzelevitch, 1997).

In summary, the results of the present study showed that in a model in which heart rate was carefully controlled, LU111995 prolonged the Q–T interval to an extent that is not considered of clinical importance. Hence, in the absence of any contradictory information, there should be minimal concern regarding the proarrhythmic potential of this Q–T-prolonging drug when administered to an individual with a healthy heart.

Acknowledgments

We express our gratitude to Dietmar Schoebel for performing the HPLC determinations, to Dr. Natalia Egorova for assistance with certain of the experiments, and to Ms. Eileen Franey for her careful attention to preparation of the manuscript.

References


Hondeghem LM and Snyder DJ (1990) Class III antiarrhythmic drugs have a lot of potential but a long way to go: Reduced effectiveness and dangers of reverse use-dependence. Circulation 81:686–690.


Send reprint requests to: Michael R. Rosen, M.D., Gustavus A. Pfeiffer Professor of Pharmacology, Professor of Pediatrics, College of Physicians and Surgeons of Columbia University. Department of Pharmacology, 630 West 168 St., PH7 West-321, New York, NY 10032. E-mail: emf3@Columbia.edu