

# Alfuzosin Delays Cardiac Repolarization by a Novel Mechanism

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Received July 10, 2007; accepted November 5, 2007

## ABSTRACT

The United States Food and Drug Administration (FDA) uses alfuzosin as an example of a drug having QT risk in humans that was not detected in nonclinical studies. FDA approval required a thorough clinical QT study (TCQS) that was weakly positive at high doses. The FDA has used the clinical/nonclinical discordance as a basis for mandatory TCQS, and this requirement has serious consequences for drug development. For this reason, we re-examined whether nonclinical signals of QT risk for alfuzosin were truly absent. Alfuzosin significantly prolonged action potential duration (APD)<sub>60</sub> in rabbit Purkinje fibers ( $p < 0.05$ ) and QT in isolated rabbit hearts ( $p < 0.05$ ) at the clinically relevant concentration of 300 nM. In man, the QT interval corrected with Fridericia's formula increased 7.7 ms, which exceeds the 5.0-ms threshold for a positive TCQS. Effects on hK<sub>v</sub>11.1, hK<sub>v</sub>4.3, and hK<sub>v</sub>7.1/hKCNE1 potassium currents and

calcium current were not involved. At 300 nM,  $\sim 30 \times C_{\max}$ , alfuzosin significantly increased whole-cell peak sodium (hNa<sub>v</sub>1.5) current ( $p < 0.05$ ), increased the probability of late hNa<sub>v</sub>1.5 single-channel openings, and significantly shortened the slow time constant for recovery from inactivation. Alfuzosin also increased hNa<sub>v</sub>1.5 burst duration and number of openings per burst between 2- and 3-fold. Alfuzosin is a rare example of a non-antiarrhythmic drug that delays cardiac repolarization not by blocking hK<sub>v</sub>11.1 potassium current, but by increasing sodium current. Nonclinical studies clearly show that alfuzosin increases plateau potential and prolongs APD and QT, consistent with QT prolongation in man. The results challenge the FDA grounds for the absolute primacy of TCQS based on the claim of a false-negative, nonclinical study on alfuzosin.

Alfuzosin hydrochloride is marketed in the United States (Uroxatral, alfuzosin HCl-extended release) as a treatment for benign prostatic hyperplasia. Alfuzosin is a selective antagonist of postsynaptic  $\alpha_1$ -adrenergic receptors located in the prostate, bladder base, bladder neck, prostate capsule, and prostate urethra. Outside the United States, the immediate-release formulation (2.5 mg three times a day) was marketed since 1987, the sustained-release formulation (5 mg two times a day) was marketed since 1993, and the extended-release formulation (10 mg once a day) was marketed since 1999 without reports of adverse cardiac events clearly attributable to alfuzosin (<http://www.fda.gov/cder/foi/>

[nda/2003/021287\\_uroxatral\\_toc.htm](http://www.fda.gov/cder/foi/nda/2003/021287_uroxatral_toc.htm)). A United States Food and Drug Administration (FDA) search of the World Health Organization adverse event database included data from 22,912 patients in 194 clinical trials and found that the most frequent adverse events were hypotension (57), syncope (53), postural hypotension (42), palpitations (28), angina (14), myocardial infarction (14), tachycardia (13), and atrial fibrillation (13) ([http://www.fda.gov/cder/foi/nda/2003/21-287\\_Uroxatral\\_Admindocs\\_P2.pdf](http://www.fda.gov/cder/foi/nda/2003/21-287_Uroxatral_Admindocs_P2.pdf)). The World Health Organization data did not include the specific terms of QT prolongation and Torsade de Pointes; however, seven cases of arrhythmia and three cases of sudden death were listed. In a recent evaluation of cardiovascular tolerability of the extended-release formulation (10 mg once a day), age, cardiovascular comorbidity, and antihypertensive co-medication had no impact on the safety profile of alfuzosin (Hartung et al., 2006).

During a review of the New Drug Application (NDA), the FDA commented that alfuzosin might increase the rate-corrected QT interval (QTc) and that the clinical pharmacology

This work was supported in part by Grant HL082060 from the NHLBI, National Institutes of Health and ChanTest Corporation.

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Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.

doi:10.1124/jpet.107.128405.

**ABBREVIATIONS:** FDA, United States Food and Drug Administration; NDA, New Drug Application; QTc, rate-corrected QT interval; TCQS, thorough clinical QT study; AP, action potential; I<sub>Ca,L</sub>, cardiac myocyte L-type calcium channel current; DMSO, dimethyl sulfoxide; HB-PS, HEPES-buffered physiological solution; PFT, Purkinje Fiber Tyrode; BCL, basic cycle length; APD, action potential duration; KH, Krebs-Henseleit; LQT3, long QT syndrome 3; PF, Purkinje fiber; HEK, human embryonic kidney.

data submitted in the NDA were insufficient to evaluate the QTc risk ([http://www.fda.gov/cder/foi/nda/2003/21-287\\_Uroxatral\\_Admindocs\\_P3.pdf](http://www.fda.gov/cder/foi/nda/2003/21-287_Uroxatral_Admindocs_P3.pdf)). To better evaluate the cardiac risk, the FDA required a thorough clinical QT study (TCQS). In discussion summaries, the FDA stated that in vitro data would not be helpful in determining the QT risk and that postmarketing surveillance (phase IV studies) would be inadequate ([http://www.fda.gov/cder/foi/nda/2003/21-287\\_Uroxatral\\_Admindocs\\_P3.pdf](http://www.fda.gov/cder/foi/nda/2003/21-287_Uroxatral_Admindocs_P3.pdf)). Sanofi-Synthelabo (now known as sanofi-aventis, Bridgewater, NJ) performed the TCQS that resulted in the inclusion of summary QTc data in the prescribing information for alfuzosin (<http://products.sanofi-aventis.us/uroxatral/uroxatral.html>). The outcome of the TCQS was that, at four times the therapeutic dose (40 mg; chosen to mimic increased alfuzosin plasma levels in the presence of a potent inhibitor of liver metabolism of alfuzosin, like ketoconazole) (<http://products.sanofi-aventis.us/uroxatral/uroxatral.html>), alfuzosin prolonged QTc, although the extent of the prolongation depended on the method of correction. For TCQS, the threshold of positivity for mean QTc was 5 ms, and the upper bound for the 95% confidence interval was less than 10 ms. For alfuzosin, the mean corrected QT interval by Fridericia's formula was 7.7 ms, and the upper bound of the 95% confidence interval was 13.5 ms (<http://products.sanofi-aventis.us/uroxatral/uroxatral.html>). The positive TCQS (<http://www.ich.org/LOB/media/MEDIA1476.pdf>) was contrasted with the negative nonclinical data included in the NDA submission. These contained an IC<sub>50</sub> value of 83  $\mu$ M for K<sub>v</sub>11.1 block, a piglet cardiac Purkinje fiber (PF) action potential (AP) assay demonstrating no effect of 1  $\mu$ M alfuzosin and a large safety margin (Table 1). In this study, we show that alfuzosin does, in fact, produce positive nonclinical signals for repolarization risk and provide a mechanism for the positive TCQS. We claim that there is no absolute primacy for TCQS and that both nonclinical and clinical assays are necessary for QT risk assessment.

## Materials and Methods

### Animals

Two adult Hartley guinea pigs and 11 New Zealand white rabbits of either gender were maintained in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited facilities at Case Western Reserve University (Cleveland, OH) and Northeastern Ohio Universities College of Medicine (Rootstown, OH), respectively. All procedures for harvesting tissues were approved by the Institutional Animal Care and Use Committee of each facility.

### Patch-Clamp Assays

Methods for voltage clamp of cells, solution preparation, drug application, data acquisition, and analysis hardware and software were essentially performed as described previously (Kirsch et al., 2004). HEK293 cells were stably transfected with hK<sub>v</sub>11.1 (hERG,

KCNH2), hK<sub>v</sub>4.3, hK<sub>v</sub>7.1/hKCNE1 (hK<sub>v</sub>LQT1/hminK), or hNa<sub>v</sub>1.5 cDNA (Kuryshv et al., 2001; Lacerda et al., 2001). Guinea pig cardiac myocyte L-type calcium channel currents (I<sub>Ca,L</sub>) were recorded from acutely isolated, enzymatically dispersed guinea pig ventricular myocytes (Kuryshv et al., 2005), which were prepared from adult animals of either gender. Unless otherwise indicated, chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Alfuzosin was obtained from SynFine Research (Richmond Hill, ON, Canada). Single-channel data were analyzed with Clampfit 9.2 (Molecular Devices, Sunnyvale, CA). Experiments were performed at room temperature.

**Patch-Clamp Solutions.** With the exception of hNa<sub>v</sub>1.5 and I<sub>Ca,L</sub> recordings, alfuzosin concentrations were prepared by diluting dimethyl sulfoxide (DMSO) stock solutions into a HEPES-buffered physiological solution (HB-PS) (Kirsch et al., 2004). The pipette solution for whole-cell recordings of hK<sub>v</sub>11.1 and hK<sub>v</sub>4.3 potassium channels was prepared as described previously (Kirsch et al., 2004). For hNa<sub>v</sub>1.5-channel recordings, alfuzosin concentrations were prepared by diluting DMSO stock solutions into the following low-sodium HB-PS solution (sodium channel-isolating physiological saline): 40 mM NaCl, 97 mM L-aspartic acid, 4.0 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 5 mM HEPES, and 10 mM glucose, and pH was adjusted to 7.4 with *N*-methyl-D-glucamine. The intracellular pipette solution for hNa<sub>v</sub>1.5 measurements was as follows: 130 mM cesium aspartate, 5 mM MgCl<sub>2</sub>, 5 mM EGTA, 2 mM Na<sub>2</sub>ATP, 0.1 mM GTP, and 10 mM HEPES, and pH was adjusted to 7.2 with CsOH. Guinea pig myocytes were superfused with a calcium channel-isolating external solution of the following composition (calcium channel-isolating physiological saline): 137 mM NaCl, 5.4 mM CsCl, 1.8 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM HEPES, and 10 mM glucose, and pH was adjusted to 7.4 with NaOH. The internal solution for I<sub>Ca,L</sub> measurements was designed to prevent outward currents through sodium and potassium channels, and it had the following composition: 130 mM cesium methanesulfonate, 20 mM tetraethylammonium chloride, 1 mM MgCl<sub>2</sub>, 10 mM EGTA, and 10 mM HEPES, and pH was adjusted to 7.2 with methanesulfonic acid. Internal solutions for I<sub>Ca,L</sub> and hK<sub>v</sub>7.1/hKCNE1 measurements were supplemented with ATP, GTP, phosphocreatine, and creatine phosphokinase to prevent rundown of calcium channels (Bean, 1992). The pipette solutions were prepared in batches, aliquoted, and stored frozen at  $-20^{\circ}\text{C}$  ( $-80^{\circ}\text{C}$  for solutions containing enzyme), and they were freshly thawed each day of use. Measurements were made at room temperature.

**K<sub>v</sub>11.1 Assay.** HEK293 cells stably expressing hK<sub>v</sub>11.1 were held at  $-80$  mV. Onset and steady-state block of hK<sub>v</sub>11.1 current due to alfuzosin was measured using a pulse pattern with fixed amplitudes (depolarization,  $+20$  mV for 2 s; repolarization,  $-50$  mV for 2 s) repeated at 10-s intervals. Peak tail current was measured during the 2-s step to  $-50$  mV.

**Na<sub>v</sub>1.5 Assay.** For concentration-response measurements, cells stably expressing hNa<sub>v</sub>1.5 were held at  $-80$  mV. Onset and steady-state activation of hNa<sub>v</sub>1.5 current due to alfuzosin was measured using a pulse pattern with fixed amplitudes (hyperpolarization,  $-120$  mV for 200 ms; depolarization,  $-15$  mV for 10 ms) repeated at 10-s intervals. Peak inward current was measured during the step to  $-15$  mV. The protocol for measurements of recovery from inactivation is described in the legend to Fig. 6.

TABLE 1  
Alfuzosin concentrations measured in human plasma and hK<sub>v</sub>11.1 safety ratio

Dose	C <sub>max</sub>	C <sub>max</sub> Free <sup>a</sup>	C <sub>max</sub>	C <sub>max</sub> Free <sup>a</sup>	30 × C <sub>max</sub> Free	Safety Ratio (K <sub>v</sub> 11.1 IC <sub>50</sub> )/(C <sub>max</sub> Free)
	ng/ml		$\mu$ M			
10 mg	21.8 <sup>b</sup>	2.18	0.056	0.0056	0.1676	2488
40 mg	38.9 <sup>c</sup>	3.89	0.100	0.0100	0.2997	1392

<sup>a</sup> Alfuzosin is 82 to 90% protein bound in plasma.

<sup>b</sup> A single 10-mg dose of C<sub>max</sub> is 13.6 ng/ml, and the repeat oral dosing is 1.2 to 1.6 higher. Taking the upper limit (1.6) makes the C<sub>max</sub> for repeat oral dosing 21.8 ng/ml.

<sup>c</sup> Single dose.

**K<sub>v</sub>4.3 Assay.** HEK293 cells stably expressing hK<sub>v</sub>4.3 were held at  $-80$  mV. Onset and steady-state block of hK<sub>v</sub>4.3 current due to alfuzosin was measured using a pulse pattern with fixed amplitudes (depolarization,  $+20$  mV for 300 ms) repeated at 15-s intervals. Peak current and current after 70 ms were measured during the step to  $+20$  mV.

**K<sub>v</sub>7.1/KCNE1 Assay.** Cells stably expressing hK<sub>v</sub>7.1/hKCNE1 were held at  $-80$  mV. Onset and steady-state block of hK<sub>v</sub>7.1/hKCNE1 current was measured using a pulse pattern with fixed amplitudes (depolarization,  $+20$  mV for 2 s; repolarization,  $-40$  mV for 0.5 s) repeated at 15-s intervals. Current was measured at the peak of current at  $-40$  mV.

**Calcium Current Assay.** Cells were held at  $-40$  mV to inactivate sodium channels. Onset and steady-state block of I<sub>Ca,L</sub> current due to alfuzosin was measured using a pulse pattern with fixed amplitudes (depolarization, 0 mV for 300 ms) repeated at 20-s intervals. Peak current was measured during the step to 0 mV.

## Purkinje Fiber AP Assay

**Purkinje Fiber Preparation.** Purkinje fibers were excised from adult rabbit ventricles by a procedure adapted from the standard methods for canine Purkinje fiber dissection (Gintant et al., 2001). On the day of testing, a rabbit was heparinized (sodium heparin, 1000 U/kg i.v.) and anesthetized (i.v.) with ketamine (37 mg/kg), xylazine (2.5 mg/kg), and acepromazine (1 mg/kg). Purkinje fibers were mounted in a Plexiglas chamber (approximate volume, 2 ml), affixed to a heated platform, and superfused at approximately 4 ml/min with standard Purkinje Fiber Tyrode (PFT) solution warmed to  $37 \pm 1^\circ\text{C}$ . Alfuzosin concentrations were prepared by diluting DMSO stock solutions into the following standard PFT solution: 131 mM NaCl, 4.0 mM KCl, 2.0 mM CaCl<sub>2</sub>, 0.5 mM MgCl<sub>2</sub>, 18.0 mM NaHCO<sub>3</sub>, 1.8 mM NaH<sub>2</sub>PO<sub>4</sub>, and 5.5 mM glucose. Before use, the PFT solution was aerated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> (pH 7.2 at room temperature) and warmed to  $37^\circ\text{C}$ .

**Purkinje Fiber Electrophysiology Protocols.** Membrane potentials were recorded using conventional intracellular microelectrodes filled with 3 M KCl solution connected to an electrometer amplifier (IE 210; Warner Instruments, Hamden, CT). Action potentials were evoked by repetitive electrical stimuli (0.1–3-ms duration, approximately  $1.5\times$  threshold amplitude) generated by a photo-isolated stimulator (S-900; Dagan Corporation, Minneapolis, MN). Analog signals were low-pass filtered at 20 kHz before digitization at 50 kHz (DT3010; Data Translation, Inc., Marlboro, MA) and stored on a hard disk using a PC-compatible computer controlled by Notocord-Hem 3.5 software (Notocord Systems SA, Croissy-sur-Seine, France).

Purkinje fibers were continuously paced at a basic cycle length (BCL) of 1 s during a stabilization period of at least 25 min before obtaining control AP responses. Acceptable fibers were continuously stimulated at a BCL of 1 s for 20 min. At the end of this period, baseline action potential duration (APD) rate dependence under control conditions was measured using stimulus pulse trains consisting of approximately 50 pulses at a BCL of 1 and 0.5 s. After returning to a BCL of 1 s, a test article at the lowest concentration was applied for 20 min to allow equilibration, and the stimulus trains were repeated. The entire sequence (20 min of equilibration followed by two cycles of stimulus trains at decreasing BCL) was repeated at increased alfuzosin concentrations. The average responses from the last five recorded action potentials from each stimulus train were analyzed for each test condition.

## QT in Isolated, Perfused Rabbit Heart

Alfuzosin concentrations were prepared daily by diluting stock solutions in Krebs-Henseleit (KH) solution: 129 mM NaCl, 3.7 mM KCl, 1.3 mM CaCl<sub>2</sub>, 0.64 mM MgSO<sub>4</sub>, 2.0 mM sodium pyruvate, 17.8 mM NaHCO<sub>3</sub>, and 5 mM glucose. The solution was aerated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>, pH 7.3 to 7.45. On the day of testing, a rabbit was heparinized (sodium heparin, 500 U/kg i.v.) and anes-

thetized with sodium pentothal (50 mg/kg i.v.). The heart was rapidly removed via a midsternal thoracotomy and placed in chilled oxygenated (95% O<sub>2</sub> + 5% CO<sub>2</sub>) KH solution. The heart was mounted in a Langendorff heart perfusion apparatus and perfused with KH solution ( $37^\circ\text{C}$ ) in a retrograde fashion through the aorta at a constant pressure. A polyethylene drainage tube was inserted into the left ventricle via the left atrial appendage to diminish the metabolic effects of myocardial work on the coronary vasomotor tone and to continuously assess the competency of the aortic valves. The A-V node was ablated to slow the intrinsic heart rate to a ventricular escape less than 60 beats/min. The heart was immersed in a thermostatically controlled bath into which the heart's effluent drained. The heart was rejected if visual inspection revealed signs of clotting or if the coronary flow was less than 20 ml/min.

**Stimulus Generation and Data Acquisition.** The heart was paced by repetitive electrical stimuli (0.1–5-ms duration, approximately  $1.5\times$  threshold amplitude). A bipolar, insulated (except at the tip) electrode was used to deliver pulses generated by a stimulator (Master 8; A.M.P.I., Jerusalem, Israel). ECG signals were conditioned by an AC-coupled preamplifier (Grass Model P511; Astro-Med, Inc., West Warwick, RI) with low-pass filtering to achieve a bandwidth of 10 to 300 Hz. Analog signals were digitized at 1 kHz and stored on a hard disk using a PC-compatible computer controlled by LabView software (version 7.1; National Instruments, Austin, TX).

**Test Procedures.** The concentration-response relationship was determined by continuous pacing at a basic cycle length of either 0.5 or 1 s throughout the experimental period. A stabilization period of at least 30-min duration was obtained before the start of measurements. Baseline control data were acquired at the end of the vehicle control period at BCLs of 1 and 0.5 s. Alfuzosin concentrations were sequentially applied in ascending order for exposure periods of at least 15 min/concentration to allow equilibration with the tissue. The average responses (at BCLs of 1 and 0.5 s) from four to five hearts were analyzed for each alfuzosin concentration.

**Data Analysis.** The QT interval was determined using the tangent method (Surawicz and Knochel, 1984; Sides, 2002; Al-Khatib et al., 2003). QRS duration and P-P intervals were measured from the ECG tracings. The mean  $\pm$  S.E.M. values were calculated from the last four beats in each equilibration period.

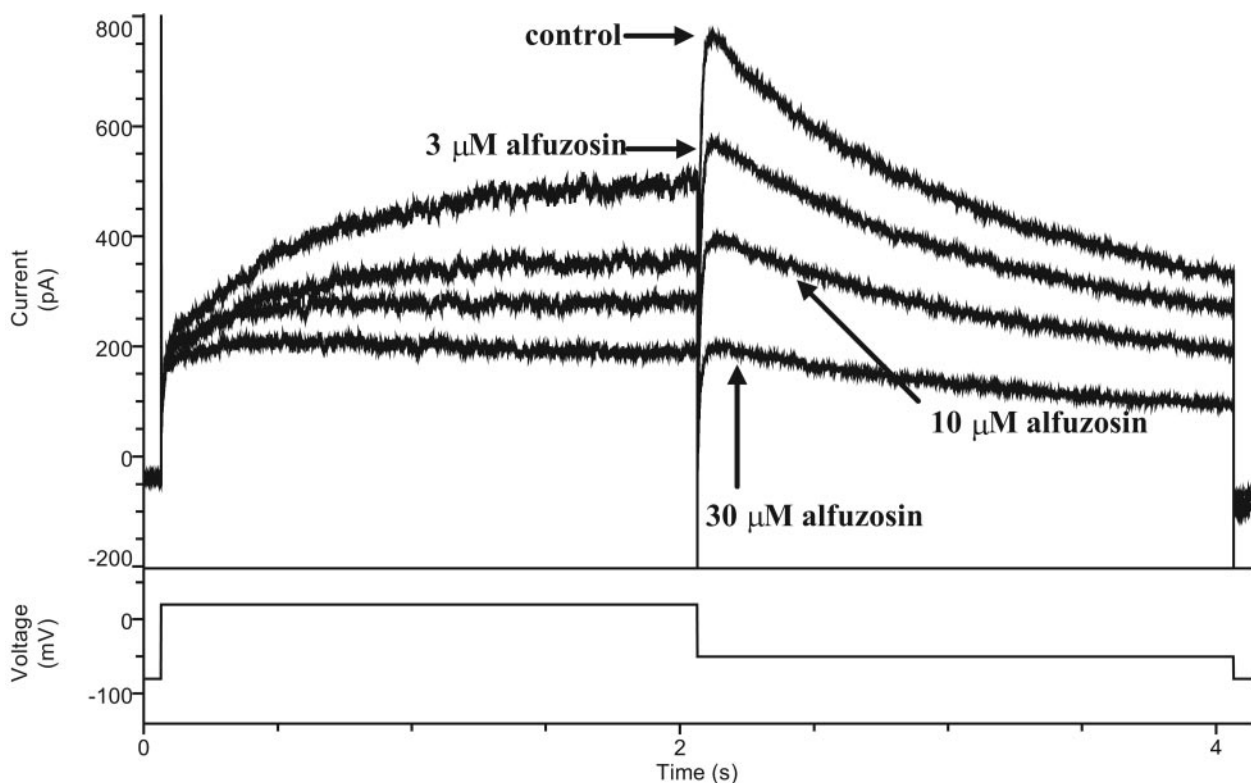
## Statistical Analysis

Data were reported as the mean  $\pm$  S.E.M. and were calculated with Microsoft Excel 2003 (Microsoft, Redmond, WA). Statistical analyses were performed with SAS JMP 5.0.1 software (SAS Institute, Cary, NC). Changes in paired data were evaluated for statistical significance using a two-tailed Student's *t* test for paired samples with significance at  $p < 0.05$ .

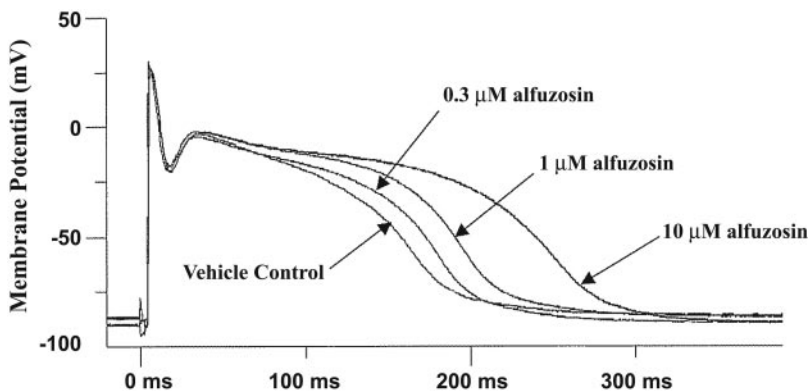
## Results

**K<sub>v</sub>11.1 Concentration Response to Alfuzosin.** hK<sub>v</sub>11.1 current (Fig. 1) was used as a surrogate for I<sub>Kr</sub>, the rapidly repolarizing cardiac potassium current. The concentration-response relationship for block of hK<sub>v</sub>11.1 peak tail currents was fit with an IC<sub>50</sub> value of 13.9  $\mu\text{M}$ . The safety margin for hK<sub>v</sub>11.1 repolarization risk was greater than 1000 (Table 1).

**Prolongation of Rabbit Purkinje Fiber Action Potential.** Alfuzosin produced a concentration-dependent increase in rabbit Purkinje fiber APD parameters (Fig. 2) measured at a basic cycle length of 2 s. The effect was greater on APD<sub>60</sub> than APD<sub>90</sub>, and late AP plateau potential was more positive (Table 2; Fig. 2). The increase of APD was larger at slower rates and showed reverse-use dependence. Prolongation of APD was statistically significant ( $p < 0.05$  using the Dunnett's test). Mean time differences from control at a BCL of



**Fig. 1.** Block of  $hK_{v11.1}$  current by alfuzosin. Sample of  $hK_{v11.1}$  current records [current (pA); time (s)] during application of control and alfuzosin solutions at the indicated concentrations. A steady state was maintained for at least 30 s before and after application of each alfuzosin concentration. Peak tail current was measured until a new steady state was achieved. Voltage wave form [voltage (mV); time (s)] stimulus used to activate  $hK_{v11.1}$  currents is shown in the bottom panel. Cells stably expressing  $hK_{v11.1}$  were held at  $-80$  mV. Onset and steady-state block of  $hK_{v11.1}$  current due to alfuzosin was measured using a pulse pattern with fixed amplitudes (depolarization,  $+20$  mV for 2 s; repolarization,  $-50$  mV for 2 s) repeated at 10-s intervals. Peak tail current was measured during the 2-s step to  $-50$  mV. Records were not corrected for capacity transient or leak current.



**Fig. 2.** Alfuzosin delays cardiac repolarization. Alfuzosin was sequentially applied at increasing concentrations to a rabbit Purkinje fiber. Superimposed records before (control) and after equilibration with 0.3, 1, and 10  $\mu$ M alfuzosin. Temperature =  $37 \pm 1^\circ\text{C}$ , BCL = 2 s. Alfuzosin prolonged APD and increased plateau potential at all of the tested concentrations at this cycle length.

1 s corresponding to statistically significant increases of  $APD_{60}$  in Table 2 for alfuzosin at 0.3, 1, and 10  $\mu$ M were  $10.9 \pm 3.3$ ,  $17.0 \pm 4.8$ , and  $41.7 \pm 10.2$  ms, respectively. For  $APD_{90}$ , corresponding time differences for alfuzosin at 1 and 10  $\mu$ M were  $15.1 \pm 4.4$  and  $47.2 \pm 10.2$  ms, respectively. At a BCL of 0.5 s, corresponding  $APD_{60}$  and  $APD_{90}$  mean time differences for alfuzosin at 10  $\mu$ M were  $22.7 \pm 7.1$  and  $26.4 \pm 5.5$  ms, respectively.

**Prolongation of QT in Isolated Rabbit Heart.** The effects of alfuzosin on the QT interval measured in a paced Langendorff-perfused rabbit heart model (CT-QT) showed a concentration-dependent increase of QT, similar to the effect of alfuzosin on rabbit Purkinje fiber APD (Fig. 3). The effect on QT was detected at 300 nM concentration and was statistically significant ( $p < 0.05$ ).

**Effects on  $hK_{v4.3}$ ,  $hK_{v7.1}/hKCNE1$ , and  $I_{Ca,L}$  Currents.**  $hK_{v4.3}$  current was used as a surrogate for  $I_{to}$ , the transient outward potassium current. Alfuzosin had no significant effect on  $hK_{v4.3}$  currents at 10  $\mu$ M concentration (increased current by  $6.1 \pm 2.5\%$ ,  $n = 2$ ; data not shown).  $hK_{v7.1}/hKCNE1$  current was used as a surrogate for  $I_{Ks}$ , the slowly activating component of the cardiac-delayed rectifier potassium current, and alfuzosin had no significant effect on this current (data not shown). Alfuzosin had no significant effect on  $I_{Ca,L}$  currents at 10  $\mu$ M concentration (data not shown).

**Effects on Cardiac Sodium Currents.** Because alfuzosin had no effect on  $hK_{v11.1}$ ,  $hK_{v4.3}$ , or  $hK_{v7.1}/hKCNE1$  potassium currents and no effect on L-type calcium currents, we hypothesized that it might increase sodium current. In

TABLE 2

Alfuzosin effects on rabbit cardiac AP parameters at 0.3, 1, and 10  $\mu\text{M}$ 

Alfuzosin	BCL	APD <sub>60</sub>		APD <sub>90</sub>		RMP <sup>a</sup>		APA		V <sub>max</sub>	
		s	$\Delta$ %	$\Delta$ %	$\Delta$ mV	$\Delta$ mV	$\Delta$ %	$\Delta$ %	$\Delta$ %		
0.3 $\mu\text{M}$	0.5		6.1 $\pm$ 2.8		3.2 $\pm$ 1.9		-1.4 $\pm$ 0.8		1.4 $\pm$ 1.6		5.2 $\pm$ 7.7
	1		7.8 $\pm$ 2.2*		3.9 $\pm$ 1.4		-1.2 $\pm$ 0.7		2.4 $\pm$ 0.8		5.5 $\pm$ 2.3
1.0 $\mu\text{M}$	0.5		6.0 $\pm$ 4.2		4.3 $\pm$ 2.8		-0.2 $\pm$ 0.8		-2.4 $\pm$ 3.5		-0.4 $\pm$ 10.7
	1		11.5 $\pm$ 3.2*		7.8 $\pm$ 2.3*		-0.1 $\pm$ 0.7		-1.6 $\pm$ 3.3		4.2 $\pm$ 12.0
10.0 $\mu\text{M}$	0.5		15.6 $\pm$ 4.7*		14.3 $\pm$ 2.8*		1.1 $\pm$ 2.3		-3.9 $\pm$ 3.0		-11.4 $\pm$ 10.9
	1		28.3 $\pm$ 6.2*		24.7 $\pm$ 5.1*		0.9 $\pm$ 1.9		-2.3 $\pm$ 2.7		-1.9 $\pm$ 9.5

\* Statistically significant ( $p < 0.05$ ) Student's  $t$  test for paired data ( $n = 3-5$ ). Alfuzosin-dependent changes in AP parameters were calculated as the difference from control in each fiber and expressed in the measurement units or as a percentage of change.

<sup>a</sup> RMP, resting membrane potential; APA, action potential amplitude; V<sub>max</sub>, action potential upstroke velocity.

whole-cell currents, alfuzosin at 300 nM and higher produced a statistically significant ( $p < 0.05$ ) increase ( $330 \pm 13$  pA/9  $\pm$  5% at 0.3  $\mu\text{M}$ ,  $n = 2$  and  $269 \pm 68$  pA/11  $\pm$  2% at 10  $\mu\text{M}$ ,  $n = 3$ ) of peak current amplitude relative to control. Alfuzosin decreased the time to peak. We hypothesized that alfuzosin could modulate sodium channel gating in a manner similar to the hereditary long QT syndrome 3 (LQT3). The LQT3 arises from a low frequency of atypical sodium channel openings characterized by the failure of inactivation to close the channel after it is opened, thereby increasing inward current during the AP plateau and delaying repolarization (Bennett et al., 1995; Dumaine et al., 1996). The increase of late openings seen in LQT3 gating was distinct in unitary sodium current recordings (Dumaine et al., 1996), and we performed similar studies. Figure 4 shows superimposed records with pronounced bursting activity after application of alfuzosin. Figure 5 shows averaged currents with an increase relative to control of early peak current and late current (Table 3). Alfuzosin at 10  $\mu\text{M}$  increased the number of openings in a burst from  $3 \pm 1$  to  $7 \pm 6$  ms ( $n = 2$ ) and burst duration from  $6.3 \pm 4.2$  to  $18.3 \pm 16.8$  ms ( $n = 2$ ). Alfuzosin at 300 nM increased late-opening NP<sub>o</sub> by  $52 \pm 10\%$  ( $n = 3$ ), and the increase was statistically significant ( $p < 0.05$ ) (Table 3). Macropatch average peak currents in alfuzosin decayed faster, crossed over control current, and had a shorter time to

peak (Fig. 5). The average increase of macropatch peak sodium current after exposure to 300 nM alfuzosin was  $55 \pm 5\%$  ( $n = 6$ ) and was statistically significant ( $p < 0.05$ ).

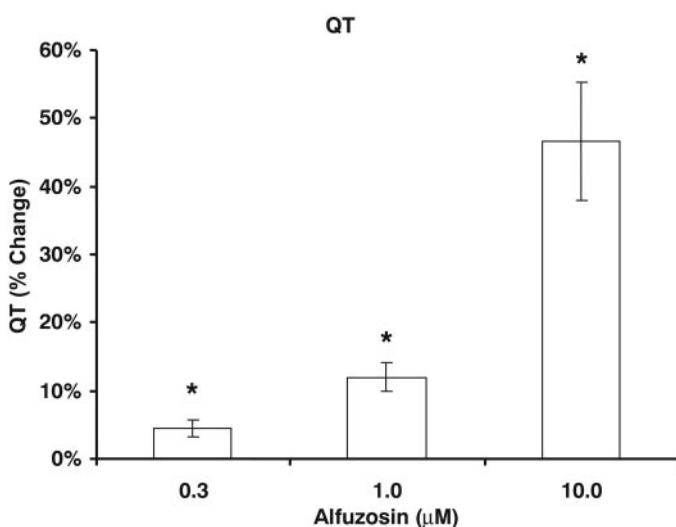
Because alfuzosin altered inactivation gating of single-sodium channels, we looked at recovery from inactivation in whole-cell currents. Consistent with an increase in late channel openings, alfuzosin increased the rate of recovery from inactivation measured with a double-pulse procedure (Wan et al., 2001). Alfuzosin at 300 nM concentration significantly shortened the slow time constant for recovery from inactivation, increasing the number of channels available for activation at late times during the voltage step (Fig. 6). This result was consistent with the increase of channel openings seen in the overlaid single-channel records and averaged late currents and with the increased late AP-plateau potential and APD prolongation (Fig. 2). Alfuzosin only had small effects on activation and inactivation gating of sodium channels (data not shown).

## Discussion

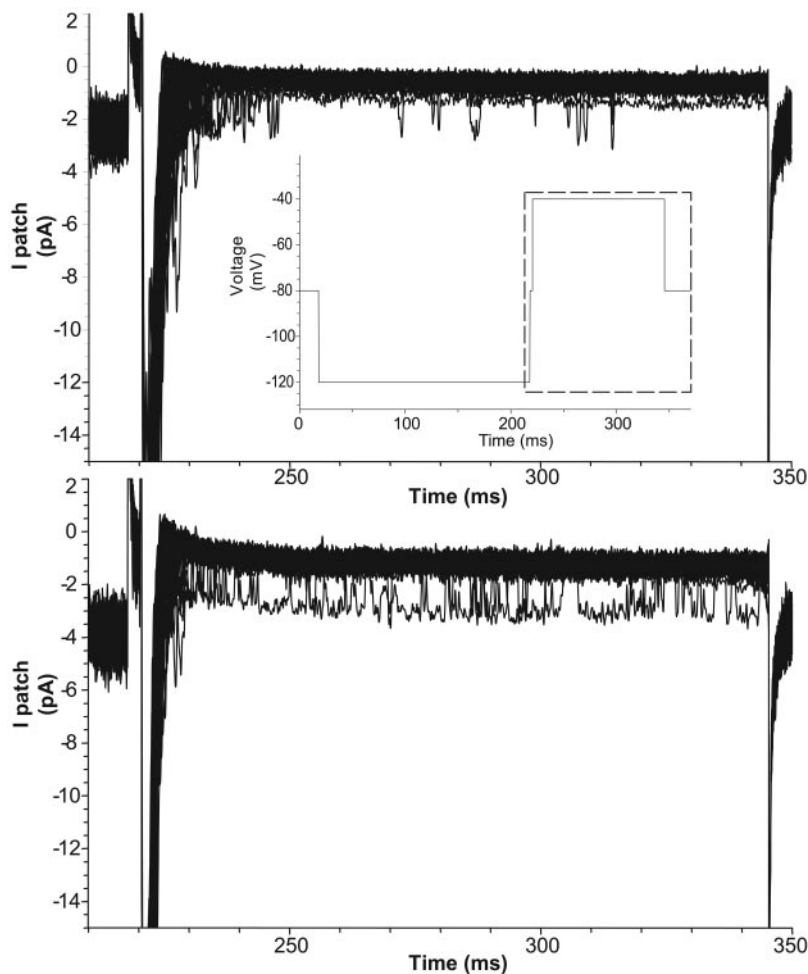
Unlike the conclusion of the FDA and the drug's sponsor, our results showed that alfuzosin tests were positive for delayed repolarization in nonclinical studies. We found significant prolongation of APD in rabbit Purkinje fibers and significant QT prolongation in Langendorff-perfused rabbit hearts at basic cycle lengths of 1 and 0.5 s. The piglet Purkinje fiber measurements included in the NDA submission did show prolongation of APD at a basic cycle length of 4 s, which was reversed to APD shortening at a basic cycle length of 1 s. This result was interpreted by Sanofi-Synthelabo as a reverse use-dependent effect of alfuzosin. Our data from rabbit Purkinje fibers showed reverse-use dependence with basic cycle lengths of 1 and 0.5 s. The rabbit PF preparation more accurately predicts the QT risk of non-antiarrhythmic drugs than the canine PF preparation (Lu et al., 2001), and Purkinje fibers from these two species have been tested much more extensively than piglet PFs. The QT prolongation seen in the paced, isolated Langendorff-perfused rabbit heart was consistent with the Purkinje fiber results and showed a detectable effect at 300 nM.

The prolongation of APD and QT was not due to block of hK<sub>v</sub>11.1 current, which usually accounts for 95% plus of drug-induced delayed repolarization. Likewise, hK<sub>v</sub>4.3, hK<sub>v</sub>7.1/hKCNE1, and L-type calcium currents were not affected. Previous experiments showed that alfuzosin does not interrupt trafficking of hK<sub>v</sub>11.1 protein to the cell surface (Wible et al., 2005).

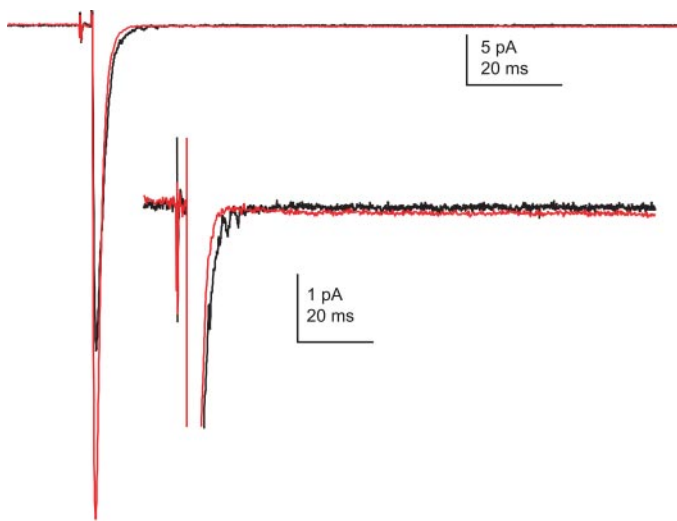
Our results showed that alfuzosin delays cardiac repolar-



**Fig. 3.** Alfuzosin prolongs QT. QT prolongation by alfuzosin was concentration-dependent in Langendorff-perfused rabbit hearts paced at a basic cycle length of 1 s ( $n = 3-5$ ). The AV node was crushed to suppress sinus rate. The percentage change of QT was measured relative to control in each heart. QT increased significantly (\*,  $p < 0.05$ ) at all concentrations (Student's paired  $t$  test).



**Fig. 4.** Alfuzosin increases opening probability of late cardiac sodium channels. Unitary currents are recorded from an HEK293 cell stably expressing hNa<sub>v</sub>1.5. Top, 47 superimposed control records; bottom, 50 superimposed records after equilibration with 10 μM alfuzosin. Stimulation frequency is 0.4 Hz (2.5-s interval). Solutions used for recording were high potassium-depolarizing solution in bath and HB-PS in pipette (room temperature). Voltage protocol shown in the inset is as follows: holding potential, -80 mV; conditioning voltage steps, -120 mV for 200 ms; second voltage step, -80 mV for 2.5 ms; test voltage step to -40 mV for 125 ms; and return to -80 mV (holding potential). Single-channel currents are displayed during the portion of the voltage protocol within the dashed-line bounding rectangle.



**Fig. 5.** Alfuzosin increases early and late sodium current. The main figure shows the average of control (47 current records; black lines) and 10 μM alfuzosin (106 current records; red lines) responses of hNa<sub>v</sub>1.5 channels from the cell-attached patch shown in Fig. 4 and obtained with the voltage protocol shown and described in Fig. 4. Alfuzosin increased the early mean peak sodium current (main figure) and the late mean sodium current (inset) relative to control. The inset shows currents from the main figure at a higher vertical resolution. Mean current records were corrected for leak and capacity current transients. Currents shown were evoked by the stimulus wave-form components within the dashed-line bounding rectangle of the inset shown in Fig. 4.

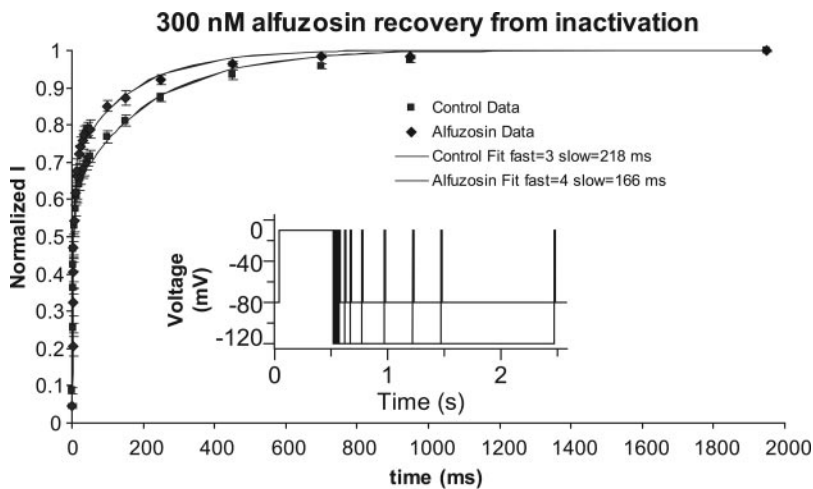
TABLE 3

Alfuzosin stimulation of late sodium current

Unitary currents were recorded from HEK293 cells stably expressing hNa<sub>v</sub>1.5 with the cell-attached configuration of the patch-clamp method. Patches contained multiple sodium channels, and openings were evoked with the voltage protocol described in the legend to Fig. 4. NP<sub>o</sub> for late openings was measured over the last 65 ms of a 125-ms step to -40 mV. Alfuzosin was applied to the bath at 300 nM and 10 μM concentrations and increased late openings. The mean increase at 300 nM was 52 ± 10% (n = 3) and was statistically significant (p < 0.05).

Cell	Alfuzosin μM	Control NP <sub>o</sub>	Alfuzosin NP <sub>o</sub>	Change %
a	10	0.0024	0.0117	396
b	10	0.0376	0.0613	63
c	0.3	0.0093	0.0160	72
d	0.3	0.0085	0.0122	43
e	0.3	0.0335	0.0477	42

ization due to an agonist effect on sodium current. Alfuzosin is a rare example of a drug that increases QT interval in this manner. Alfuzosin significantly increased peak sodium current. Alfuzosin also reduced the time to peak current (p = 0.05). We interpreted this to indicate that alfuzosin reduced the latency to first opening of sodium channels. A reduced latency to first opening could result in more overlapping channel openings earlier, increasing peak current but also increasing the peak current decay that we observed due to fewer first openings after the peak. These effects explain the rabbit Purkinje fiber data that showed an increase in APD<sub>60</sub> at 300 nM alfuzosin. Our single-channel data and recovery from inactivation data showed that alfuzosin increases the



**Fig. 6.** Alfuzosin shortens recovery from inactivation. The main figure shows normalized recovery from inactivation data ( $n = 6$ ) in control (filled square symbols) and after exposure to 300 nM alfuzosin (filled diamond symbols). The control and 300 nM alfuzosin data were fit with a sum of two exponential functions (solid curved lines through the data). Fit parameter values in control were as follows: fast amplitude, 0.62; fast tau, 3.3 ms; slow amplitude, 0.38; and slow tau, 218 ms. Fit parameter values in 300 nM alfuzosin were as follows: fast amplitude, 0.70; fast tau, 4.4 ms; slow amplitude, 0.30; slow tau, 166 ms. The alfuzosin-mediated faster recovery from inactivation reflected in the smaller slow-time constant value was statistically significant at  $p < 0.05$ . The inset shows the voltage protocol used to obtain recovery data. The holding potential was  $-80$  mV, the conditioning pulse potential was  $-15$  mV for 500 ms, and a variable duration step to  $-120$  mV was followed by a 10-ms step to  $-15$  mV to measure recovery of initial current during the 500-ms-conditioning step. Pulse sequences were repeated at 8-s intervals.

availability of sodium channels at long times similar to the duration of the cardiac action potential and the QT interval. The resulting increase of late inward current was consistent with the increase of late AP plateau potential, APD, and QT.

Our experiments with alfuzosin showed that thorough non-clinical testing of cardiac depolarization and repolarization abrogated the false-negative result of concern to the FDA. By extension, we argue that nonclinical QT risk will be observed for other unnamed drugs that the FDA refers to in support of mandatory TCQS if appropriate, accurate, nonclinical tests were done. In conclusion, what is required is standardization of the nonclinical assays recommended in the International Conference on Harmonization of Technical Requirements for Registration at Pharmaceuticals for Human Use (ICH) S7B (www.ICH.org) guidance similar to the standardization for clinical QT measurements in the ICH E14 guidance.

Clinical QT is a weak surrogate for torsades de pointes (Sides, 2002). It is likely that the FDA threshold values for positive thorough clinical QT studies will result in far too many false-positives and seriously impair drug development and approval. Because nonclinical tests can include all known mechanisms for QT prolongation risk, the FDA is premature in assigning primacy to the TCQS. A more balanced approach that takes into account both nonclinical and clinical studies (namely, the European and Japanese regulatory bodies) seems to be more appropriate at this time.

#### Acknowledgments

We thank Sharon Beringuel for preparation of cell cultures, Bryce Waldal for sodium channel measurements, and Lisa Dewey for performing Purkinje fiber measurements.

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