The Antiestrogen Tamoxifen Blocks the Delayed Rectifier Potassium Current, $I_{Kr}$, in Rabbit Ventricular Myocytes

XIAO-KE LIU, ALEXANDER KATCHMAN, STEVEN N. EBERT and RAYMOND L. WOOSLEY

Department of Pharmacology, Georgetown University Medical Center, Washington, DC

Accepted for publication July 16, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

Tamoxifen is an antiestrogen drug commonly used to treat breast cancer and has been shown to cause prolongation of the electrocardiographic QT interval in humans. Because QT prolongation could influence cardiac arrhythmias, we sought to determine the electrophysiologic mechanism(s) underlying the tamoxifen action. The whole-cell patch-clamp technique was used to study the effect of tamoxifen on the delayed rectifier ($I_{Kr}$), the inward rectifier ($I_{K1}$), the transient outward current ($I_{to}$), and the inward L-type calcium current ($I_{Ca}$) in rabbit ventricular myocytes. By switching to the current-clamp mode, the effect of tamoxifen on action potential duration (APD) was also studied. Tamoxifen blocked $I_{Kr}$ in a time-, concentration- and voltage-dependent fashion. $I_{Kr}$ tail currents were completely blocked by 10 μmol/l tamoxifen with no recovery after 15 min of washout. At +50 mV, tamoxifen 1 and 3.3 μmol/l blocked $I_{Kr}$ by 39.5 ± 1.7% (P < .01) and 84.8 ± 1.3% (P < .01) respectively, while no significant block of $I_{K1}$ or $I_{to}$ was observed. Significant block of $I_{Ca}$ by tamoxifen was also observed at concentrations greater than 1 μmol/l, with almost complete inhibition at 10 μmol/l. Tamoxifen showed no significant effect on APD at concentrations up to 3.3 μmol/l. We conclude that tamoxifen potency blocks both $I_{Kr}$ and $I_{Ca}$ at clinically relevant concentrations. The observed QT prolongation by tamoxifen in humans may be a result of its predominant effect on $I_{Kr}$. Inhibition of $I_{Kr}$, in conjunction with other QT-prolonging factors in patients could increase their risk of developing torsades de pointes-type cardiac arrhythmias.

Tamoxifen is one of the most effective and frequently prescribed drugs to treat breast cancer (Jordan, 1992). A recent clinical observation indicated that tamoxifen prolongs the QT interval in human subjects (Trump et al., 1992). Prolongation of cardiac repolarization and the QT interval has clinical importance because under certain situations it may confer a class III antiarrhythmic effect; however, it has also been shown to increase the risk of developing a complex form of potentially lethal ventricular arrhythmia known as TdP (Ben-David and Zipes, 1993; Carlsson et al., 1990; Roden et al., 1986).

Crucial to generation of TdP is prolongation of the QT interval and APD that permits EADs to occur (Zeng and Rudy, 1995). Because potassium currents are major determinants of cardiac repolarization and because shortening of the action potential suppresses EADs in isolated myocytes (Bouchard et al., 1995), modulation of cardiac repolarizing potassium channels may be critical in modulating EADs. Clinically, drugs that prolong the QT interval by blocking cardiac potassium channels, especially the rapid component of the delayed rectifier current, $I_{Kr}$, are often associated with acquired TdP arrhythmias (Carlsson et al., 1990; Roden et al., 1986; Follmer et al., 1990; Woosley, 1996). In the present study, we evaluated the potential cardiac electrophysiologic effects of tamoxifen at clinically relevant concentrations (Murphy et al., 1987) using the whole-cell patch-clamp technique in rabbit ventricular myocytes.

Methods

Isolation of ventricular cells. Rabbit (3–4 months old, weight 3–3.5 kg; HRP Inc., Denver, PA) ventricular myocytes were isolated using a modification of the method previously described (Giles and Imaizumi, 1988). Briefly, rabbit hearts were removed and mounted on the Langendorff perfusion system. The following procedure was used: (1) perfusion with normal Tyrode’s solution for 10 min; (2) perfusion for ~20 min with a Ca$^{2+}$-free Tyrode’s solution; (3) perfusion for 30 to 35 min with Tyrode’s solution containing 40 U/ml of collagenase II ( Worthington Biochemical, Freehold, New Jersey) and 50 μmol/l CaCl$_2$. The ventricles were then minced and gently stirred in Tyrode’s solution containing 100 μmol/l CaCl$_2$. After stirring for 5 to 10 min, a large number of single ventricular cells was obtained. The resulting cell suspension was then filtered through a nylon mesh. Cells were collected by centrifugation at 50×g and then resuspended in Tyrode’s solution containing 250 μmol/l Ca$^{2+}$. The cells were again collected by centrifugation, and then resuspended in Tyrode’s solution containing 1 mmol/l Ca$^{2+}$. After a third centrifugation, the cells were resuspended in DMEM culture medium sup-

Received for publication March 12, 1998.

ABBREVIATIONS: $I_{Kr}$, delayed rectifier; $I_{K1}$, inward rectifier; $I_{to}$, transient outward current; $I_{Ca}$, inward L-type calcium current; TdP, torsades de pointes; APD, action potential duration; EAD, early afterdepolarization.
plemented with 10% bovine serum (Hyclone Labs, Logon, UT). The myocytes were immediately seeded onto laminin-coated glass microcoverslips at a density of 10^4 rod-shaped cells/cm^2 and allowed to attach. The cells were stored in a humidified incubator in 5% CO_2, 95% air at 37°C. Only cells within the first 36 hr after isolation were used.

**Whole-cell patch-clamp.** The patch-clamp technique was used to record the membrane currents and action potentials in single ventricular myocytes. Command voltage pulses were generated using PCLAMP 6.0.2 Software connected to an interface (Axon Instruments, Foster City, CA), an IBM-compatible Pentium computer, and an Axopatch 200A amplifier. Membrane potentials and current signals were monitored on an oscilloscope (Model 5103, Tektronix, Beaverton, Oregon) and stored in the lab computer. Pipettes with tip resistance of 1 to 4 MΩ were pulled from borosilicate glass (World Precision Instruments, Sarasota, Florida) and filled with an intracellular solution containing (mmol/l) KCl 125, NaCl 10, CaCl_2 1, Mg-ATP 5, EGTA 10, HEPES 10, cAMP 0.1, adjusted with KOH to

---

**Fig. 1.** Recordings of I_{Kr}, I_{to} (A and B) and I_{K1} (C) in the same cell before and after 5-min superfusion with 5 μmol/l E-4031. A, I_{Kr} and I_{to} currents before and after superfusion with E-4031. E-4031 abolished the I_{Kr} tail current and also reduced the time-dependent I_{Kr} current without affecting the transient outward current (I_{to}) or the holding current. B, E-4031-sensitive currents obtained by digital subtraction of currents after E-4031 from currents before E-4031. The inward rectification of the time-dependent I_{Kr} currents at very positive potentials compared with the tail currents, C, I_{K1} current before and after superfusion with E-4031. E-4031 showed little effect on the inward I_{K1} current recorded at −120 mV. The outward holding currents that represent the amplitude of I_{K1} at −40 mV are superimposed.

---

**Fig. 2.** Recordings of I_{Kr}, I_{to} (A and B) and I_{K1} (C) in the same cell before and after 5-min superfusion with 10 μmol/l tamoxifen. A, I_{Kr} and I_{to} currents recorded before and after superfusion with tamoxifen. Tamoxifen abolished the I_{Kr} tail current and also reduced the time-dependent I_{Kr} current, without affecting the transient outward current (I_{to}). B, Tamoxifen-sensitive currents obtained by digital subtraction of currents before and after tamoxifen superfusion. Note the inward rectification of the time-dependent I_{Kr} currents at very positive potentials compared with the tail currents and their similarity to the E-4031 sensitive currents. C, I_{K1} current recorded before and after superfusion with tamoxifen. Tamoxifen showed no block of I_{K1} inward current. The outward holding currents that represent the amplitude of I_{K1} at −40 mV are superimposed.
pH 7.2. A holding potential of –40 mV was used to inactivate fast sodium and T-type calcium currents. The external solution was Tyrode’s solution containing (mmol/l) NaCl 137, KCl 5.4, HEPES 10.0, MgCl$_2$ 1.0, CaCl$_2$ 2.0, glucose 10.0, and was adjusted with NaOH to pH 7.4 (NaOH). Cd$^{2+}$ (0.2 mmol/l) was used to block the L-type calcium channel (ICa) and to shift the I-V relationship of Ito and IKr to more positive potentials (Daleau et al., 1997; Agus et al., 1991). This allows 1) separation of IKr and the outward portion of IK1, especially at membrane potentials positive to –30 mV and 2) marked increase of Ito availability at a holding potential of –40 mV. For action potential recording, Cd$^{2+}$ was omitted from the extracellular solution and the voltage-clamp mode was switched to current clamp mode.

Myocytes adhering to glass coverslips were placed in a small chamber mounted on the stage of an inverted microscope and superfused at 1.5 ml/min at room temperature (22–24°C). Only rod-shaped single cells that were quiescent and exhibiting well defined, cross-striations were studied. After establishment of whole-cell configuration and measurement of cell capacitance, series resistance ($\leq$10 M$\Omega$) was compensated by 50% to 70%. Junction potentials under these conditions were –3 mV and not corrected. IKr currents were elicited from a holding potential of –40 mV by a series of 1.5-sec test pulses from –10 to +50 mV in 10 mV increments. Membrane potential was then held at –30 mV for 2 sec before returning to the holding potential in order to observe IKr tail currents. The current-voltage (I-V) relationship for IKr was constructed by measuring the tail currents. In the presence of 0.2 mmol/l Cd$^{2+}$, the same protocol for IKr also activated Ito due to the marked shift of Ito activation to more positive potentials (Agus et al., 1991).

The inward-rectifier K$^{+}$ currents (IK1) were elicited from a holding potential of –40 mV by a series of 250-msec test pulses ranging from –120 to –10 mV in 10-mV increments. The amplitude of IK1 at each voltage was determined by measuring the peak current relative to zero current. IK1 was recorded as previously described (Osaka and Joyner, 1991). A Na$^{+}$ and K$^{+}$-free extracellular solution was used, containing (mM): N-methyl-D-glucamine (NMG) chloride 130, CaCl$_2$ 1.8, CsCl 20, MgCl$_2$ 0.53, HEPES 5, glucose 5, pH 7.4 with NMG. Pipette solution contains (mmol/l): CsOH 110, aspartic acid 90, CsCl 20, HEPES 10, EGTA 10, MgATP 5, and GTP(Tris) 0.4, pH 7.2 with CsOH. ICa was elicited by series of depolarization steps of 200 ms duration applied in 10-mV increments from a holding potential of –40 mV.

**Drugs and chemicals.** Tamoxifen was purchased from Sigma Chemical (St. Louis, MO), dissolved in ethanol and stored in aliquots at –20°C until used. E-4031 and dofetilide were kindly provided by Eisai Ltd. (Ibaraki, Japan) and Pfizer Central Research (Groten, CN) respectively. All other chemicals were obtained from Sigma Chemical.

**Data analysis and statistics.** Patch-clamp data were normalized for total cell capacitance to allow comparison between cells of various sizes. The paired Student’s $t$ test was used to compare the potassium current amplitude before and after tamoxifen treatment. Data are reported as mean ± S.D., and differences between values were considered statistically significant when $P < .05$.

### Results

**Effect of E-4031 on IKr, Ito, and IK1.** IKr is one of the major repolarizing currents, and its block has been implicated in TdP (Carlsson et al., 1990; Roden et al., 1986; Follmer and Colatsky, 1990; Woosley, 1996). To evaluate whether tamoxifen affects IKr, we first sought to establish the presence of IKr in our rabbit ventricular myocytes. Figures 1, A and B, show the membrane currents elicited by a 1.5-sec voltage-clamp step from –40 mV to different test potentials ranging from –10 to +50 mV in the same cell before (A) and after (B) 5-min exposure to 10 μmol/l E-4031, a highly selective IKr blocker (Clay et al., 1995; Sanguinetti et al., 1990). Under control conditions, a slowly activating outward current flowed during depolarization, followed by an outward tail current that has been shown to represent the gradual

**Fig. 3.** Time-dependent block of IKr by tamoxifen. IKr currents were recorded in the same cell before drug administration, after 3-, 5- and 9-min superfusion with 1 μmol/l tamoxifen.
decay of $I_{Kr}$ (Follmer et al., 1990; Clay et al., 1995; Sanguinetti et al., 1990). The initial peak in the time-dependent outward current was due to the rapid activation and inactivation of $I_{Kr}$, which is sensitive to 4-aminopyridine (data not shown). E-4031 abolished the tail current on repolarization and also reduced the time-dependent outward current, without affecting the initial peak ($I_{to}$) or the holding current ($I_{K1}$). Shown in B are the E-4031-sensitive currents obtained by digital subtraction of currents in the bottom tracings from currents in the top tracings in A. Compared with the tail current, the time-dependent current demonstrated marked inward rectification at very positive potentials. $I_{to}$ was not present in the E-4031-sensitive currents, indicating that E-4031 had no effect on the $I_{K1}$ outward current.

**Time-dependent block of $I_{Kr}$ by tamoxifen.** To further characterize the observed inhibition of $I_{Kr}$ by tamoxifen, we examined whether this inhibition is time dependent. Figure 3 depicts a typical experiment performed in the same cell before drug administration (control), or after 3-, 5- and 9-min superfusion with 1 μmol/l tamoxifen. As shown in figure 3, block of $I_{Kr}$ by tamoxifen is time dependent and has a slow onset. Further block can still be observed after superfusion for 5 min. In contrast, $I_{Kr}$ was readily recorded from control myocytes (no exposure to tamoxifen) for at least 10 min without any sign of run-down. In the absence of drug, the amplitude of $I_{Kr}$ measured 10 min after membrane rupture was 103.4 ± 6.15% of that measured immediately after membrane rupture ($n = 3, P > .05$).

**Effect of tamoxifen on the I-V relationship of $I_{Kr}$.** We next evaluated the effect of tamoxifen on the I-V relationship of $I_{Kr}$. Figure 4, A and B, demonstrates the effects of 1 and 3.3 μmol/l on the I-V relationship of $I_{Kr}$, measured after 5-to-7-min infusion of tamoxifen. Tamoxifen markedly reduced $I_{Kr}$ current amplitude in a concentration-dependent fashion. A typical voltage-dependent block of $I_{Kr}$ is shown in figure 4. Note the greater block at more positive potentials. At the test potential of +50 mV, tamoxifen (1 and 3.3 μmol/l) blocked $I_{Kr}$ by 39.5 ± 1.3% (P < .01) and 84.8 ± 1.3%, respectively (P <
.01), whereas no significant block of \( I_{K1} \) was observed at 3.3 \( \mu \)mol/l (5.5 ± 0.9% test potential \(-120 \text{ mV}, n = 4, P > .05\)).

**Comparison of \( I_{Kr} \) block by tamoxifen and quinidine.**

Quinidine is a drug that has been frequently associated with TdP (Roden et al., 1986). To compare the block of \( I_{Kr} \) by tamoxifen to that produced by quinidine, we performed the protocol shown in figure 5, using either 10 \( \mu \)mol/l tamoxifen or 10 \( \mu \)mol/l quinidine. Tamoxifen completely blocked the \( I_{Kr} \) tail current with no recovery observed after 5-min washout, whereas the same concentration of quinidine (10 \( \mu \)mol/l) only partially blocked the \( I_{Kr} \) tail currents, with recovery within 3-min washout. In other experiments, complete recovery of \( I_{Kr} \) from quinidine block was usually observed after 3-min washout, whereas no recovery from tamoxifen could be detected even after 15-min washout (data not shown). Figure 5C compares the percentage inhibition of \( I_{Kr} \) by 3.3 \( \mu \)mol/l tamoxifen and 3.3 \( \mu \)mol/l quinidine. Data are expressed as mean ± S.D., \( n = 4, **P < .01 \).

**Effect of tamoxifen on APD and \( I_{Ca} \).** We next examined whether tamoxifen causes prolongation of APD. Figure 6 shows action potentials recorded before and after 4-min exposure to 3.3 \( \mu \)mol/l tamoxifen. Surprisingly, although tamoxifen inhibited \( I_{Kr} \) by ~84.8% at this concentration (fig. 4), no significant prolongation of APD was observed. APD measured at 90% repolarization (APD\(_{90}\)) before and after 4- to 5 min superfusion of tamoxifen (3.3 \( \mu \)mol/l) was 341 ± 49 ms and 332 ± 19 ms respectively (\( n = 16, P > .05 \)). Because under control conditions, no significant shortening of APD was observed in the initial 10 min after cell membrane rupture, the absence of APD prolongation by tamoxifen was not secondary to a “rundown” phenomenon.

This unexpected effect on APD and previous reports of \( I_{Ca} \)...
blockade by tamoxifen (Song et al., 1996) prompted us to study the possible effect of tamoxifen on the cardiac inward $I_{Ca}$. Consistent with earlier studies by Song et al. (1996) in vascular smooth muscle cells, we also observed a potent effect of tamoxifen in cardiac rabbit ventricular myocytes. Significant inhibition of $I_{Ca}$ was observed at tamoxifen concentrations greater than 1 µmol/l, with almost complete inhibition observed at 10 µmol/l (fig. 7).

Discussion

The major finding of the present study is that the commonly prescribed antiestrogen drug tamoxifen potently blocks the rapid component of the delayed rectifier current, $I_{Kr}$, in a voltage-, concentration- and time-dependent fashion. The potassium channel-blocking effect of tamoxifen seems to be specific for $I_{Kr}$ because no significant effect was observed on $I_{K1}$ or $I_{Ko}$ at concentrations up to 10 µmol/l (fig. 2). Tamoxifen blocks $I_{K1}$ with a potency even greater than quinidine, a drug that has been shown to block $I_{Kr}$ and is associated with a high incidence of drug-induced TdP (Roden et al., 1986). This is, to our knowledge, the first study showing that tamoxifen is a potent and selective $I_{Kr}$ blocker.

Outward potassium channels are the major determinants of the repolarization phase of the cardiac action potential. In the cardiac ventricular myocytes, the transient outward current $I_{To}$, the delayed rectifier $I_{Kr}$ and the inward rectifier $I_{K1}$ mediate different phases of the repolarization process (Giles and Imaizumi, 1988; Sanguinetti et al., 1990; Surawicz, 1992). The contribution of $I_{K1}$ and $I_{Kr}$ to the APD is well established and reduction of $I_{K1}$ or $I_{Kr}$ will cause APD and QT prolongation (Giles and Imaizumi, 1988; Sanguinetti and Jurkiewicz, 1990; Surawicz, 1992). On the other hand, although $I_{To}$ has been shown to play an important role in determining phase 1 repolarization, there is still some uncertainty regarding its contribution to normal APD (Giles and Imaizumi, 1988; Litovsky and Antzelevitch, 1988; Kaab et al., 1996). Interestingly, to our knowledge, all the drugs that are clinically associated with QT prolongation and acquired TdP invariably block $I_{Kr}$. The reason why $I_{Kr}$ is sensitive to block by so many drugs is still not well understood.

$I_{Kr}$ has been reported to consist of two components, $I_{Kr}$ and $I_{Kr1}$ in guinea pig, dog and human ventricles (Sanguinetti and Jurkiewicz, 1990; Gintant, 1996; Li et al., 1996). In the rabbit ventricular myocytes, $I_{Kr}$ has been reported to be absent (Giles and Imaizumi, 1988), consist of only one component (Clay et al., 1995) or consist of two components (Salata et al., 1996). In our experiments, $I_{Kr}$ can be consistently recorded with a clear tail in all of the untreated cells we studied. Our previous studies (Liu et al., 1998) have indicated that the major delayed rectifier current that contributes to APD in normal rabbit ventricular myocytes is $I_{Kr}$.

Tamoxifen has been shown to prolong the QT interval in human subjects (Trump et al., 1992). The present study suggests that the tamoxifen-induced QT prolongation may be related to a direct block of cardiac $I_{Kr}$ current. The tamoxifen plasma concentration was greater than 5 µmol/l when QT prolongation was observed clinically by Trump et al. (Trump et al., 1992). As shown in figure 4, tamoxifen was even more potent than quinidine in blocking $I_{Kr}$, causing more than 80% block of $I_{Kr}$ at 3.3 µmol/l. Although no $I_{Kr}$ recovery from tamoxifen block could be observed even after an extended washout period, the possibility that the block we observed was due to “rundown” of $I_{Kr}$ can be excluded because block of $I_{Kr}$ by quinidine can completely recover after ≥5-min washout.

Interestingly, a recent clinical study using F-18 fluorotamoxifen showed significant cardiac uptake of tamoxifen, presumably due to intracellular accumulation of tamoxifen in cardiac myocytes (Inoue et al., 1997). In the present study, $I_{Kr}$ block by tamoxifen had a slow onset and no recovery was observed after an extended washout period. These results may indicate that tamoxifen blocks $I_{Kr}$ through an intracellular site. However, further studies are needed to elucidate the exact mechanism of the potent $I_{Kr}$ block by tamoxifen.

One unexpected finding of this study is that, although we demonstrated a potent inhibition of $I_{Kr}$ by tamoxifen, it had no significant effect on APD in rabbit ventricular myocytes. This may be due to the fact that tamoxifen is also a potent calcium channel blocker, as documented by Song et al. (1996) and confirmed by our current study (fig. 7). Because blocking of $I_{Ca}$ will lead to a shortening of the APD, this effect may largely counteract the $I_{Kr}$ blocking effect of tamoxifen which would otherwise lead to a prolongation of APD in single rabbit cardiomyocyte and prolongation of QT interval in whole heart. The obvious discrepancy between the current study in rabbit (no effect on APD) and the observations in humans (QT prolongation) may result from different relative contributions of $I_{Kr}$ or $I_{Ca}$ to the APD and/or different relative potencies of tamoxifen in blocking $I_{Kr}$ vs. $I_{Ca}$ in difference species. The net effect of tamoxifen on the APD in a certain

Fig. 7. Effect of tamoxifen on the L-type $I_{Ca}$. $I_{Ca}$ was recorded in the same cell before tamoxifen administration; after 1-, 2-, 3- and 4-min superfusion of 10 µmol/l tamoxifen, and after 2-, 4-, 8- and 16-min washout. Note the marked inhibition of $I_{Ca}$ and partial recovery after washout.
species would therefore depend on both the relative contribution of the $I_{Kr}$ vs. $I_{Ca}$ to the APD and the relative potency of tamoxifen in blocking $I_{Kr}$ vs. $I_{Ca}$. It is also possible that other ionic currents not examined in this study such as chloride current (Vanderberg et al., 1994), may mediate the differential effects of tamoxifen in different species. Further studies using human ventricular myocytes are needed to resolve these issues.

At the present time, there are no clinical data available regarding the risk of developing TdP in patients after tamoxifen administration. Nevertheless, the current finding has important clinical implications. Therapeutic plasma concentrations of tamoxifen (Murphy et al., 1987) in patients are similar to concentrations that produce potent block of $I_{Kr}$ in isolated rabbit ventricular myocytes (i.e., low micromolar range, see fig. 4). In addition, tamoxifen is frequently used to treat breast cancer in female patients, whereas recent clinical observations and experimental data have indicated that female gender is associated with a higher risk of developing drug-induced TdP (Makkar et al., 1993; Lehmann et al., 1996; Liu et al., 1997). Although the effect of tamoxifen on $I_{Ca}$ may potentially ameliorate its $I_{Kr}$ blocking effect, caution should still be taken when administering tamoxifen to patients in situations where other risk factors for TdP also exist, such as hypokalemia, bradycardia, congenital long QT syndrome or coadministration of other drugs that may also delay cardiac repolarization.

References


