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

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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 potently 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.

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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.
implemented with 10% bovine serum (Hyclone Labs, Logan, UT). The myocytes were immediately seeded onto laminin-coated glass microcoverslips at a density of 10⁴ rod-shaped cells/cm² and allowed to attach. The cells were stored in a humidified incubator in 5% CO₂, 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, CaCl₂ 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. Note 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.
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, MgCl₂ 1.0, CaCl₂ 2.0, glucose 10.0, and was adjusted with NaOH to pH 7.4 (NaOH). Cd²⁺ (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, Cd²⁺ 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 (>10 MΩ) 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 Cd²⁺, the same protocol for IKr also activated IK1 due to the marked shift of IK1 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.

IKr 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, CaCl₂ 1.8, CsCl 20, MgCl₂ 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. IKr 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.

**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 5 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

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**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 $I_{K_r}$ (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 $I_{K_r}$, 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 E-4031 has no effect on the tail current (Liu et al., 1998). These features of the delayed rectifier current (inward rectification of the time-dependent current, complete block of the tail current by E-4031, dofetilide or removal of extracellular $K^+$) also abolished the tail current (Liu et al., 1998). These features of the delayed rectifier current (inward rectification of the time-dependent current, complete block of the tail current by E-4031, dofetilide or removal of extracellular $K^+$) are consistent with the previous description of $I_{K_r}$ in rabbit and other species (Clay et al., 1995; Sanguinetti et al., 1990).

In the same cell shown in figure 1, A and B, we also measured $I_{K1}$ current before and after E-4031 exposure. Figure 1C demonstrates the $I_{K1}$ current elicited by a 250-msec hyperpolarization to $-120$ mV from a holding potential of $-40$ mV before and after E-4031 superfusion. Little effect was observed on the $I_{K1}$ inward current, although $I_{Kr}$ was completely blocked in the same cell. The outward holding currents that represent the amplitude of $I_{K1}$ at $-40$ mV before and after E-4031 superfusion were superimposable, indicating that E-4031 had no effect on the $I_{K1}$ outward current.

**Effect of tamoxifen on $I_{K1}$**. We next tested the effect of tamoxifen on the three major potassium currents using the same protocol as shown in figure 1. Shown in figure 2 are the $I_{Kr}$, $I_{to}$ and $I_{K1}$ currents elicited in the same cell before and after 5-min exposure to 10 μmol/l tamoxifen. Similar to E-4031, tamoxifen abolished the $I_{Kr}$ tail current and also reduced the time-dependent current without affecting $I_{to}$ or the holding current (fig. 2A). The solvent for tamoxifen, ethanol, had no effect on $I_{K1}$ at the concentration (≤1%, v/v) used to dissolve tamoxifen (data not shown). Figure 2B depicts the tamoxifen sensitive currents obtained by digital subtraction of currents in the bottom tracings from currents in the top tracings in A. There is a striking similarity between the tamoxifen sensitive current and the E-4031-sensitive current shown in figure 1B. In both figures 1B and 2B, the time-dependent current demonstrated strong inward rectification at very positive potentials compared with the tail current, whereas $I_{to}$ was not present in either the tamoxifen or the E-4031-sensitive current. Figure 2C shows the $I_{K1}$ current measured before and after 5-min exposure to 10 μmol/l tamoxifen in the same cell shown in figure 2, A and B. Like E-4031, tamoxifen produced no inhibition of the $I_{K1}$ inward current at $-120$ mV. In fact, $I_{K1}$ inward current was even slightly larger after tamoxifen treatment in this cell (fig. 2C). The outward holding currents representing the amplitude of $I_{K1}$ at $-40$ mV were superimposable, indicating that tamoxifen 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 $39.5 ± 1.7$% ($P < .01$) and $84.8 ± 1.3$%, respectively ($P < .01$).
whereas no significant block of $I_{K1}$ was observed at 3.3 mmol/l (5.5 ± 0.9% test potential -120 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 μmol/l tamoxifen or 10 μmol/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 μmol/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 5-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 μmol/l tamoxifen and 3.3 μmol/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 μmol/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 μmol/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_{Kr} 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 (Rodent 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_{K} 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_{K} and I_{K1} to the APD is well established and reduction of I_{K} or I_{K1} 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_{Kr}, 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
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