Inhibition of Cardiac Potassium Currents by the Vesnarinone Analog OPC-18790: Comparison with Quinidine and Dofetilide

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ABSTRACT
OPC-18790 is a vesnarinone analog currently in clinical trials for treatment of heart failure. In vitro studies have shown that, in addition to its positive inotropic actions, OPC-18790 prolongs cardiac action potentials. Therefore, in this study, the effects of OPC-18790 on cardiac potassium currents were compared with those previously observed for the blockers quinidine and dofetilide in two test systems, i.e., L-cells stably transfected with mammalian cardiac potassium channel clones (Kv1.4, Kv1.5 and Kv2.1) and mouse AT-1 cells, in which the rapidly inactivating component of the cardiac delayed rectifier (I_{Kr}) is the major repolarizing current. In L-cells, 10 to 100 μM OPC-18790 reduced Kv1.4, Kv1.5 and Kv2.1 currents by <30%, whereas quinidine was a more potent blocker (EC_{50} < 10 μM) and the I_{Kr}-specific blocker dofetilide was without effect. In contrast, in AT-1 cells, OPC-18790 blocked I_{Kr} with an EC_{50} (0.96 ± 0.12 μM, n = 10) similar to that of quinidine (0.9 ± 0.2 μM). For both drugs, block was voltage dependent, increasing at positive potentials. OPC-18790 and quinidine showed no frequency dependence, implying block of resting channels and/or very rapid block of open channels; this is in contrast to dofetilide, which displayed slow onset kinetics of block. Thus, we conclude that, 1) unlike quinidine, OPC-18790 does not inhibit currents obtained by expression of the cardiac potassium channel clones Kv1.4, Kv1.5 and Kv2.1; 2) like quinidine and dofetilide, OPC-18790 blocks I_{Kr} in AT-1 cells, but the kinetics of block onset more closely resemble those of quinidine than dofetilide; and 3) block of I_{Kr} appears to be an important mechanism underlying the action potential-prolonging properties of OPC-18790.

Phosphodiesterase inhibition is a therapeutic strategy that may increase cardiac contractility and is therefore under intense investigation for patients with heart failure (Packer, 1993). However, a number of studies have suggested that, although phosphodiesterase inhibitors can transiently improve the symptoms of congestive heart failure, the mortality rate is unaffected or even increased (Nony et al., 1994). Indeed, vesnarinone has been reported to prolong action potential duration in rabbit, guinea pig and human ventricular myocytes (Lathrop et al., 1993), an effect attributed to increased L-type calcium current and/or decreased delayed rectifier current.

OPC-18790 is a vesnarinone analog that is currently in clinical trials for acute i.v. therapy of severe heart failure. Like vesnarinone, OPC-18790 increases action potential duration (Hosokawa et al., 1992) and has been reported to stimulate calcium currents (Wu et al., 1993). In animal models, it increases contractility; at high dosages, it aggravates halothane/adrenaline-induced ventricular tachycardia (Wu et al., 1993b).

A common mechanism for action potential prolongation is block of cardiac potassium currents. The present study was conducted to determine the effect of OPC-18790 on cardiac potassium currents and to compare its effects with those of the antiarrhythmic agents quinidine and dofetilide, which are known to block these currents. Two model systems were used, i.e., mammalian cells stably transfected with cDNAs encoding cardiac potassium channels (Snyders et al., 1993b) and mouse AT-1 cells (atrial tumor myocytes), in which I_{Kr} is the major repolarizing current (Yang et al., 1994b). I_{Kr} is the target of specific methanesulfonanilide inhibitors, such as Packer, 1993b).
of dofetilide (Carmeliet, 1993a; Jurkiewicz and Sanguinetti, 1993). The characteristics of I_{Ks} inhibition by dofetilide in AT-1 cells have been previously reported (Yang et al., 1995), as has block of I_{Ks} in AT-1 cells (Yang and Roden, 1996) and other cardiac myocytes (Carmeliet, 1993b) by relatively low concentrations of quinidine. In addition, the blocking action of quinidine on currents obtained by expression of the human cardiac potassium channel gene Kv1.5 in L-cells has been described (Snyders et al., 1992). Portions of this work have been presented in abstract form (Yang et al., 1994a).

Materials and Methods

AT-1 cell culture methods. The detailed methods have been reported previously (Yang et al., 1994b). In brief, AT-1 cells were isolated from s.c. tumors we propagated in C57BL/6Jfemale mice (The Jackson Laboratory, Bar Harbor, ME). To isolate cells, live, whole mice were placed in 70% ethanol for sterilization. The tumor mass was excised, rinsed with PBS, minced finely and placed for 1 hr at 37°C, with gentle rocking, in PBS containing penicillin/streptomycin (100 U/ml penicillin and 100 μg/ml streptomycin; Gibco) and 0.1% collagenase. The cell suspension was centrifuged, washed with PBS, resuspended and then plated at a density of 250 to 325 × 10^5 cells/ml in 10-mm Primaria dishes (Falcon). The medium [PC1 (Ventrex Laboratories), which included penicillin/streptomycin, 10% fetal bovine serum and 10 mM dexamethasone] was changed every other day until cells were used. For electrophysiological studies, cells were removed from the culture dish by 2-min exposure to a trypsin-containing solution (0.125% in calcium/magnesium-free Hank’s solution), decanted into sterile culture tubes (without trypsin) and maintained at room temperature for 2 to 4 hr before study.

L-cell culture. The methods used to establish expression of cardiac potassium channel genes in cloned mouse fibroblasts (Ltk- cells, or L-cells) have been described previously (Snyders et al., 1992, 1993a). Transfected cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% horse serum and 0.25 mg/ml G418 (GIBCO, Grand Island, NY), in a 5% CO2 atmosphere. The cultures were passaged every 3 to 5 days, by brief trypsinization. The transfection vector included a dexamethasone-inducible promoter. Therefore, before electrophysiological experiments, subconfluent cultures were incubated with 2 μM dexamethasone for 24 hr. The cells were then removed from the dish with a cell scraper, and the cell suspension was stored at room temperature and used within 12 hr for the experiments described here.

Electrophysiological methods. Electrophysiological recordings were performed at room temperature (22–23°C) using an Axopatch-1A patch-clamp amplifier (Axon Instruments, Inc., Foster City, CA), in the whole-cell configuration of the patch-clamp technique. After the whole-cell configuration was established, the capacitive transients elicited by symmetrical 10-mV voltage-clamp steps from –80 mV were recorded at 50 kHz (filtered at a bandwidth of 10 kHz, –3 dB) for calculation of capacitive surface area; capacitance and series resistance compensation were then optimized. To record potassium currents, the extracellular solution was normal Tyrode’s solution, containing 130 mM NaCl, 4 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 10 mM glucose, with the pH adjusted to 7.35 with NaOH. The intracellular pipette filling solution contained 110 mM KCl, 5 mM tetrabutylammonium, 1.2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid, 5 mM KATP, 1 mM MgCl2 and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and the solution was adjusted to pH 7.2 with KOH, yielding a final intracellular K+ concentration of ~145 mM. L-type calcium currents were blocked with 0.5 μM nisoldipine. A holding potential of –40 mV was used in AT-1 cells to inactivate inward currents through sodium or T-type calcium channels, as well as rarely observed transient outward components (Yang et al., 1995). For L-cell experiments, negative holding potentials (~80 to ~100 mV) could be used, because sham-transfected cells display no endogenous currents. OPC-18790 was provided by Otsuka America Pharmaceutical Co. (Rockville, MD). Drug (0.1–100 μM) from a 10 mM stock solution (3.8 mg/ml) in lactic acid was added to Tyrode’s solution to yield the final concentration in each experiment. The pH of the drug-containing solution was adjusted to 7.35. There was no effect of low concentrations (0.1–1.0%) of lactic acid alone in these studies.

Data analysis. To compare current densities among cells, currents are reported as current per unit capacitance (picampere per picofarad) after linear leak subtraction and normalization to cell surface area determined by measurement of capacitance, as described above. The drug concentration blocking 50% of the current, IC_{50}, was determined using a Hill function, y = 1/(1 + ([D]/IC_{50})), where [D] is the drug concentration. Mono- or biexponential functions were fit to data as previously described (Yang et al., 1994b). Comparisons were performed by Student’s t test. Results are reported as mean ± 1 S.E.

Results

Effects in L-cells. Previous studies showed that quinidine blocked Kv1.5 expressed in L-cells with an EC_{50} of ~6 μM (Snyders et al., 1992). In the same system, a high concentration of OPC-18790 (100 μM) reduced steady-state Kv1.5 current by 12 ± 1% (n = 4), without markedly altering its kinetics (fig. 1). Expression of Kv2.1 cDNA also resulted in a slowly inactivating, delayed rectifier phenotype; as with Kv1.5, 100 μM OPC-18790 had little effect on steady-state Kv2.1 current, reducing it by 23 ± 3% (n = 3). Whereas expression of Kv1.5 or Kv2.1 resulted in a noninactivating or slowly inactivating current, expression of Kv1.4 resulted in a rapidly inactivating current (fig. 1), whose amplitude was also only slightly decreased (by 28 ± 3%, n = 3) by 100 μM OPC-18790. This high concentration did appear to slow Kv1.4 inactivation. Under control conditions, inactivation during a pulse to +50 mV was biexponential, with time constants of 15.7 ± 0.2 and 51.3 ± 0.9 msec; with 100 μM OPC-18790, the time constants were significantly (P < .001) longer (18.4 ± 0.5 and 80.1 ± 3.9 msec).

The effects of 20 μM quinidine on currents in cells expressing Kv1.5, Kv1.4 and Kv2.1 are shown in figure 2. As previously reported (Snyders et al., 1992), quinidine reduced Kv1.5 by >50%. Peak Kv1.4 currents were reduced to a similar extent (34 ± 1%, n = 3) as with 100 μM OPC-18790. Unlike with OPC-18970, inactivation was slightly accelerated. Time constants for inactivation after a pulse to +50 mV were 19.3 ± 0.4 and 84.6 ± 2.8 msec in the absence of quinidine and 8.5 ± 0.6 and 72.2 ± 3.1 msec (both P < .05) in the presence of drug. Quinidine was a potent inhibitor of Kv2.1 currents, reducing them by 84 ± 2% (n = 3) during pulses to +50 mV. In AT-1 cells and in other species, the EC_{50} for dofetilide block of IKs is in the nanomolar range. However, even at a concentration of 10 μM, dofetilide did not produce any effects on any of the three cloned potassium channels studied.

Effects on IKs. Figure 3A shows IKs traces obtained from a holding potential of –40 mV, followed by a 1-sec depolarizing step to +20 mV and a step back to –40 mV. In the absence of drug, a prominent, time-dependent, activating current was seen during the depolarizing pulse, with a slowly deactivating tail current after the pulse. The magnitude of the tail current is determined by the number of channels

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open at the end of the depolarizing pulse. The “hook” at the onset of the tail current is thought to represent recovery from fast inactivation, as described for $I_{K_r}$-like currents in other systems (Shibasaki, 1987; Sanguinetti et al., 1995; Snyders and Chaudary, 1996; Liu et al., 1996). Figure 3B shows that OPC-18790 is a relatively potent $I_{K_r}$ blocker, with an $EC_{50}$ of $0.96 \pm 0.12 \mu M$ ($n = 10$), which is virtually identical to the value we previously derived for quinidine ($1.0 \pm 0.4 \mu M$) under the same conditions (Yang and Roden, 1996).

Block of activating current by OPC-18790 increased during the activating pulse, i.e. block was time dependent at plateau potentials (fig. 3, C and D). In addition, as with quinidine and dofetilide, $I_{K_r}$ inhibition by OPC-18790 was voltage dependent and more prominent at positive potentials. As discussed below, both of these findings suggest block of open or inactivated channels as the mechanism underlying OPC-18790 block of $I_{K_r}$.

The voltage dependence of $I_{K_r}$ block by quinidine and by OPC-18790 was qualitatively similar to that we previously reported for dofetilide (Yang et al., 1995). In previous studies of dofetilide block of $I_{K_r}$, we and others (Carmeliet, 1993a) demonstrated use-dependent block by washing drug in during a period of prolonged quiescence and then resuming stimulation. In the absence of drug, tail currents were similar whether measured during stimulation or after quiescence. With dofetilide, tail currents declined slowly after resumption of stimulation, with a time constant of $4.2 \pm 0.5$ sec ($n = 3$), in agreement with data reported by others (Carmeliet, 1993b). When the same experiment was repeated with quinidine or with OPC-18790 (fig. 4), a different result was obtained; tail currents were inhibited with even the first pulse after the period of quiescence, and no incremental block was observed with additional pulses. This implies drug block of resting states and/or rapid onset of drug block during the first pulse after quiescence; the observed voltage dependence of block argues for the latter mechanism.
Discussion

Effects of OPC-18790 on cardiac potassium currents. In this study, we have demonstrated that OPC-18790 is only a very weak blocker of the potassium currents obtained by expression of the cardiac potassium channel genes Kv1.4, Kv1.5 and Kv2.1. This is in contrast to quinidine, which blocks these currents at concentrations similar to those observed in clinical therapy. Moreover, the concentrations of quinidine required to block IKr are also in, or indeed below, the range that is usually associated with clinical effects in humans (Yang and Roden, 1996). It is, however, well recognized that some patients develop marked QT prolongation and the polymorphic ventricular tachycardia torsades de pointes even at “subtherapeutic” plasma quinidine concentrations (Koster and Wellens, 1976; Roden et al., 1986). As discussed below, the low EC50 for quinidine block of IKr may be especially relevant to this form of quinidine toxicity. OPC-18790 also blocked IKr, at concentrations similar to those required for quinidine block. However, it differed from the IKr-specific blocker dofetilide, in that its use-dependent IKr-blocking properties were more similar to those observed with quinidine than with dofetilide. Thus, in these studies, OPC-18790 exhibited a profile of block of cardiac potassium currents different from those of other potassium channel blockers, i.e., quinidine and dofetilide, whose effects we have assessed in these test systems.

Drug block of IKr. We have found that IKr block by quinidine and OPC-18790 is time and voltage dependent; these features are very similar to those we and others have previously reported for dofetilide and other methanesulfonanilides. Block of activating currents increased with pulse duration, and block was enhanced at very positive potentials. These characteristics indicate that the drugs do not block channels in the closed state. A contemporary model for IKr gating includes at least one open and one inactivated state (Shibasaki, 1987; Sanguinetti et al., 1995; Trudeau et al., 1995; Snyders and Chaudary, 1996), as follows:

Closed ⇔ open ⇔ inactivated
It is thought that the tail current hook observed immediately after a repolarizing voltage-clamp step represents channels recovering very rapidly from inactivation into the open state and then undergoing much slower deactivation to the closed state. Within this context, quinidine, OPC-18790 and dofetilide block either the open or the inactivated state. The observed voltage dependence suggests preferential binding to the inactivated state or voltage-dependent open channel block.

Implications for OPC-18790. Meta-analysis of the effects of phosphodiesterase inhibitors in patients with congestive heart failure suggests that these drugs, as a class, increase the mortality rate (Nony et al., 1994). However, clinical trials with vesnarinone have hinted that, at lower dosages, it might decrease the mortality rate, possibly as a result of its action potential-prolonging actions, because the latter can produce arrhythmia suppression. Importantly, action potential prolongation may also exert modest positive inotropic actions, thought to be attributable to increases in intracellular calcium levels resulting from delayed repolarization. The identification of mutations in \textit{HERG}, the gene that appears to encode \textit{I_{Kr}}, in the congenital long-QT syndrome naturally raises the question of whether block of \textit{I_{Kr}} is likely to be a safe antiarrhythmic strategy. In clinical trials, high dosages of OPC-18790 have been reported to cause torsades de pointes. Clinical trials that are currently in progress with both vesnarinone and OPC-18790 should help further test the concept that the combination of phosphodiesterase inhibition and action potential prolongation results in improved outcomes for patients with heart failure, as long as dosages that result in marked QT prolongation and torsades de pointes can be avoided.

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References


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