Importance of Species Selection in Arrythmogenic Models of Q-T Interval Prolongation

In a recent article by Ohtani and colleagues (4), prolongation of the Q-T interval in an anesthetized-rat electrocardiogram (ECG) model was used to determine the arrhythmogenic potential of macrolide antibiotics. Prolongation of the Q-T interval on an ECG has been linked to the rare (but potentially fatal) ventricular arrhythmia known as Torsade de Pointes. In vitro studies suggest that most Q-T-prolonging drugs block the delayed rectifier potassium current (iKr) carried by the pore-forming subunit encoded by hERG (human ether-a-go-go-related gene) in humans (6). While hERG-like mRNA and functional iKs current have been found in many species including dogs, guinea pigs, and rabbits (3), little (if any) functional iKs or hERG-like current is found in the rat ventricle (8). Therefore, we were surprised at the sensitivity of this rat ECG model to the selected macrolide antibiotics, given that these drugs do not elicit iKs blocking until supratherapeutic concentrations are achieved (7). To clarify these issues, we chose to compare the effects of erythromycin and two established iKr-blocking drugs on repolarization of rat ventricular muscle, because Q-T prolongation in vivo is reflective of action potential prolongation.

We used standard microelectrode techniques (2) to evaluate the effects of erythromycin (at a concentration achieved by Ohtani et al. in plasma [3.6 μM]) as well as two standard iKs blockers, dofetilide (10 nM) and E-4031 (1.0 μM), at concentrations 2.5-fold greater than those shown to block 50% of native iKs current (1, 5). These concentrations have also been shown to significantly prolong the action potential duration (APD) in other species prominently expressing iKs (5). In brief, male CD rats (300 to 350 g; Charles River Labs) were anesthetized, hearts were removed, and ventricular papillary muscles were excised and placed in a warmed (37°C) superfusion chamber known as Torsade de Pointes. In vitro studies suggest that most Q-T-prolonging drugs block the delayed rectifier potassium current (iKr) carried by the pore-forming subunit encoded by hERG (human ether-a-go-go-related gene) in humans (6). While hERG-like mRNA and functional iKs current have been found in many species including dogs, guinea pigs, and rabbits (3), little (if any) functional iKs or hERG-like current is found in the rat ventricle (8). Therefore, we were surprised at the sensitivity of this rat ECG model to the selected macrolide antibiotics, given that these drugs do not elicit iKs blocking until supratherapeutic concentrations are achieved (7). To clarify these issues, we chose to compare the effects of erythromycin and two established iKr-blocking drugs on repolarization of rat ventricular muscle, because Q-T prolongation in vivo is reflective of action potential prolongation.

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The typical effects of erythromycin, E-4031, and dofetilide on rat ventricular muscle action potentials are illustrated in Fig. 1. Rat papillary muscle repolarization is very sensitive to erythromycin (Fig. 1B) but not the iKr-blocking drug E-4031 (Fig. 1A). Figure 1C summarizes results obtained from 15 preparations (10 hearts); drugs are arranged on the abscissa in the order of increasing potency for native-iKr blockade. Whereas erythromycin elicited 76.6% ± 6.4% APD prolongation at concentrations substantially lower than those required for iKr blockade, the iKr blockers dofetilide and E-4031 elicited much smaller prolongation (2.4% ± 2.9% and 1.8% ±

FIG. 1. Effects of select drugs on rat ventricular repolarization. (A and B) Action potential traces from rat papillary muscle preparations. In each panel, pairs of superimposed traces show effects obtained during continuous recordings before and after drug exposure. Panel A highlights the lack of effect of E-4031 (1.0 μM) on APD, while panel B shows prominent prolongation of APD with erythromycin (3.6 μM) exposure. (C) Effects of erythromycin (Ery.), E-4031, and dofetilide on APD. Drugs are arranged on the abscissa in the order of increasing potency for blocking native iKr. While erythromycin significantly prolongs the APD at a concentration far below that which blocks iKr (IC50 = 100 μM [7]), high concentrations of iKr-blocking agents E-4031 and dofetilide (2.5-fold above the 50% inhibitory concentration for blocking native iKr [1, 5]) minimally prolong repolarization. n = 5 per group; the data are means ± standard errors of the means. Statistical significance was defined as P < 0.05 (+) (paired t-tests).
2.1%, respectively) at concentrations 2.5-fold the 50% inhibitory concentration for iKr blockade. The greater APD prolongation of rat papillary muscle with erythromycin compared to potent iKr-blocking drugs suggests that iKr is not the current predominantly affected by erythromycin in the rat papillary muscle.

In conclusion, this in vitro study corroborates the findings of Ohtani et al. regarding the sensitivity of the rat in vivo ECG model for erythromycin. However, the lack of effects of high concentrations of known iKr-blocking drugs on the APD corroborates the reported paucity of iKr in the rat ventricular muscle and suggests that currents other than iKr are responsible for delaying repolarization with erythromycin. Thus, the present studies suggest that the rat ECG model is inappropriate for evaluating the proarrhythmic potential of noncardiovascular drugs that prolong the Q-T interval by blocking iKr. Further studies are necessary to determine the ionic current(s) responsible for delayed repolarization in the rat myocardium seen with macrolide antibiotics.

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Editor’s Note: The authors of the published article declined to respond.