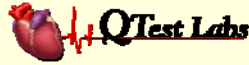


ANALYSIS OF DYNAMIC RESTITUTION OF ECG PARAMETERS IN THE RABBIT LANGENDORFF HEART EXPOSED TO DRUGS THAT LENGTHEN OR ABBREVIATE QT

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ABSTRACT

Dynamic restitution has been used to characterize ECG parameters but not for cardiac electrograms recorded from Langendorff preparations. This study was designed to determine if expressions of dynamic restitution detect effects of drugs that either prolong or abbreviate QT in the rabbit Langendorff preparation. Dynamic restitution was characterized as hysteresis of beat-to-beat QT-TQ relationships, an expression known to predict arrhythmogenic liability *in vivo*. Rabbit hearts perfused according to the methods of Langendorff were exposed to vehicle or to escalating concentrations of dofetilide (that prolongs QT) and pinacidil (that shortens QT). RR, QT, QTcF, B, TQ and QT/TQ were measured from bipolar transventricular electrograms during baseline and exposure to drugs. Plots of each parameter versus concentration, or versus time for vehicle, were made. Opposite changes were produced by dofetilide and pinacidil; QT, QTcF and QTcB lengthened significantly for dofetilide and shortened significantly for pinacidil. The QT/TQ ratio lengthens for dofetilide and shortens for pinacidil. Thus effects on expressions of dynamic restitution in the *in vitro* Langendorff preparation mimic effects observed *in vivo*.

INTRODUCTION

Cardiac electrical restitution refers to the relationship between an action potential duration (APD) and its preceding diastolic interval (DI) which could be analogous to the relationship between QT and TQ measured from electrocardiogram (Fossa et al., 2005). The relationship of QT-TQ is influenced by species, disease state, autonomic tone, and sudden changes in heart rate (Fossa, 2008). The study of restitution has been shown to predict vulnerability to ventricular tachycardia and ventricular fibrillation (Fossa et al., 2008). Recent studies showed that changes of preceding TQ intervals, the lower limit of TQ interval for 95% of the cardiac cycles, the percentage of cardiac cycles with QT/TQ ratio greater than 1, and the 98th percentile of the QT/TQ ratio are correlated with vulnerability to reentrant arrhythmia in both animals and humans receiving torsadogens (Fossa et al., 2007; Fossa, 2008). While there is much information on restitution parameters studied in *in vivo* animal models and humans, no study has been done on the isolated, perfused rabbit heart. If the same *in vivo* examination of beat-to-beat QT-TQ relationship is applied to the isolated heart, the arrhythmic vulnerability could possibly be defined in the *in vitro* setting. This study was designed to determine if expressions of dynamic restitution detect effects of drugs that either prolong or abbreviate QT in the rabbit Langendorff preparation. To examine this concept, isolated, perfused rabbit hearts were used. Repolarization was changed with either dofetilide (I_{Kr} blocker) to lengthen QT interval, or pinacidil ($I_{K(ATP)}$ opener) to shorten QT interval.

MATERIALS AND METHODS

Ten male rabbits, weighing between 3-4 kg, were anesthetized with ketamine/xylazine, and after heparinization, hearts were removed quickly and were suspended on a Langendorff perfusion apparatus.

Modified Krebs-Henseleit solution, gassed with 95% oxygen and 5% carbon dioxide, with pH between 7.34 and 7.69, temperature between 35°C and 37°C, was used.

Bipolar transventricular electrograms, with clear onset of QRS and end of T wave were recorded from electrodes held gently on the epicardium of the right atrium and left ventricle. A metal cannula with a rubber balloon on the tip filled with saline was inserted through the mitral orifice so that the balloon lay within the left ventricle. Monophasic action potentials (MAP) were recorded by pressing a Ag-AgCl electrode against the left ventricular epicardium.

Recordings were made on an EMKA IOX Data Acquisition Unit. After control (vehicle) measurements were obtained, three hearts were perfused with escalating concentrations of dofetilide (10^{-9} – 10^{-5} M), and five hearts were perfused with escalating concentrations of pinacidil (10^{-8} – 10^{-5} M). Two additional hearts were used for vehicle control (0.1% DMSO). Each concentration of dofetilide and pinacidil was perfused for 15 min with data collected over the final 3-5 minutes (500 cardiac cycles) of each 15 min epoch. The vehicle hearts were perfused for 75 minutes to match the perfusion time of each test article.

Data analysis

ECG parameters:

The RR, PR, QRS, and QT intervals were measured, at the targeted time points, from beats during sinus rhythm. QTc was calculated by the cube root correction method of Fridericia (1920) and by square root correction method of Bazett (1920).

MATERIALS AND METHODS

Restitution parameters:

TQ 5th percentile (TQ_{min}): The TQ interval of the ECG was calculated as the RR interval minus the QT interval of the previous beat. TQ 5th percentile is the measure of the lower limit for 95% of the beats.

Percentage of beats with QT/TQ ratio greater than 1 (%QT/TQ ratio>1): The percentage of beats with a QT/TQ ratio greater than 1 reflects the relative time spent on the restitution curve where stability is not as certain.

Upper 98th percentile of the QT/TQ ratio (QT/TQ_{max}): The upper 98th percentile of the QT/TQ ratio reflects the magnitude of the steepness of the restitution relationship.

RESULTS



There were no drug effects on PR interval or QRS duration. Arrhythmia (ventricular fibrillation) developed in only 1 heart, that perfused with 10^{-5} M pinacidil (Figure 1). There were changes manifested by trends (e.g., RR interval, TQ interval) of statistical significance (e.g., QT, QTcB, QTcF, APD_{30} , dP/dt_{min} , dP/dt_{max}) in all other parameters.

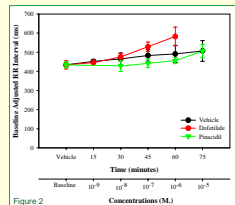


Figure 2

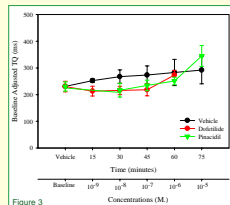


Figure 3

Plots of baseline adjusted mean values and standard error of the mean for RR (Figure 2) and TQ (Figure 3) intervals from an isolated heart perfused with vehicle, dofetilide, and pinacidil vs concentration of test articles or time for vehicle are shown. Each value represents the mean \pm SEM of an average of 500 cardiac cycles from a different rabbit heart recorded between the 10^{th} - 15^{th} minute of each escalating dose of test articles or equivalent volumes of vehicles. RR and TQ intervals did not differ significantly among group or with time. Although there were no differences of statistical significance, it appears that both dofetilide (at 10^{-6} M) and pinacidil (at 10^{-6} M) prolonged both RR and TQ. This cardiodeceleration is consistent with the known effect of dofetilide and the relationship between RR and TQ was expected since reduction in heart rate is normally much more an effect on TQ than on QT.

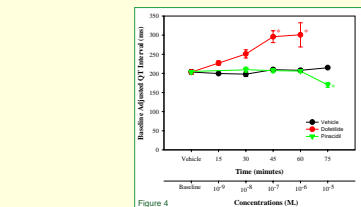
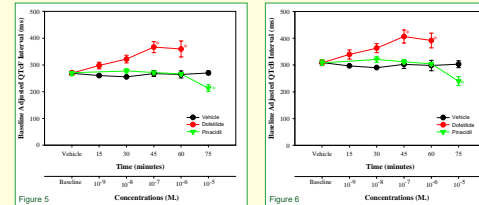


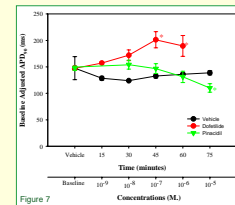
Figure 4

Plots of baseline adjusted mean values and standard error of the mean for QT interval for isolated heart perfused with vehicle, dofetilide, and pinacidil vs concentration of test articles or time for vehicle are shown (Figure 4). Asterisk ($p < 0.05$) shows where significant differences occurred from baseline in the same group and where difference occurred between test article heart and vehicle heart at the same concentration. Each value represents the mean \pm SEM of an average of 500 cardiac cycles from a different rabbit heart recorded between the 10^{th} - 15^{th} minute of each escalating dose of test articles or equivalent volumes of vehicles. QT lengthened significantly for dofetilide and shortened significantly for pinacidil.

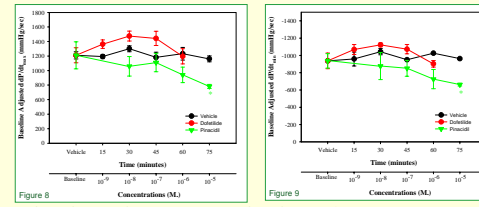
RESULTS



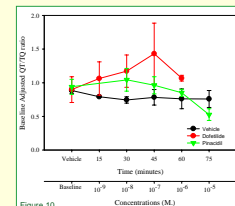
QTcF and QTcB (Figures 5 and 6) prolonged for dofetilide and shortened for pinacidil. These are known effects of these drugs.



APD_{30} (Figure 7) prolonged significantly for dofetilide and shortened for pinacidil. These changes in APD_{30} are consistent with changes in QT.



dP/dt_{max} (Figure 8) decreased and dP/dt_{min} (Figure 9) increased significantly for pinacidil at the 10^{-5} M concentration. This indicates slight negative inotrope and lusitrope. Negative inotrope is a known effect of pinacidil.



Plots of baseline adjusted mean values and standard error of the mean are shown for QT/TQ ratio (Figure 10) for isolated heart perfused with vehicle, dofetilide, and pinacidil vs concentration of test articles or time for vehicle. Each value represents the mean \pm SEM of an average of 500 cardiac cycles from a different rabbit heart recorded between the 10^{th} - 15^{th} minute of each escalating dose of test articles or equivalent volumes of vehicles. There was no significant change for QT/TQ ratio but the ratio tended to increase for dofetilide whereas it appears to decrease for pinacidil when compared to baseline or vehicle at the same time.



The authors gratefully acknowledge the monumental contributions of Dr. A.A. Fossa for development of this technology.

RESULTS

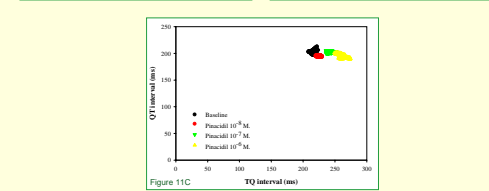
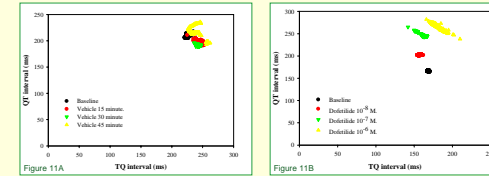


Figure 11 shows comparison of the QT-TQ interval relationships in isolated rabbit hearts given either vehicle(A), dofetilide(B), or pinacidil(C). Each concentration for each test article or control hearts contains at least 500 cardiac cycles. Notice that dofetilide causes upward shift and increased heterogeneity of the restitution cloud when compared to vehicle or pinacidil.

DISCUSSION & CONCLUSIONS

- All hearts perfused with vehicle or dofetilide survived exposures through 75 minutes of perfusion, and no heart developed ventricular arrhythmia including torsades de pointes or ventricular fibrillation. However, one of 5 hearts perfused with 10^{-5} M pinacidil developed ventricular fibrillation.
- ECG parameters remained unchanged when hearts were perfused with vehicle, whereas QT, QTcB, QTcF, APD_{30} were statistically significant prolonged for hearts perfused with dofetilide, a known torsadogen. On the other hand, QT, QTcB, QTcF, and APD_{30} were significantly abbreviated at 10^{-5} M of pinacidil.
- No statistically significant changes on restitution parameters (QT/TQ ratio, TQ_{min} , %QT/TQ ratio>1, QT/TQ ratio_{max}) occurred with hearts perfused with vehicle, dofetilide, or pinacidil, however there was a trend for QT/TQ to become less than 1.0 for pinacidil (10^{-9}) and to become >1.0 for dofetilide (10^{-7}). It is proposed that had a larger n been used, the trends would no doubt have become significant; therefore dofetilide would be considered a torsadogen (as it is) and pinacidil likely to produce reentrant arrhythmia (as it did in 1 rabbit). The power for this analysis was 0.66
- Pinacidil caused a significant decrease in both dP/dt_{max} (inotrope) and an increase in dP/dt_{min} (negative lusitrope). This may have been a consequence of either calcium kinetics or of calcium binding to troponin-C.
- This study demonstrated that basic ECG and MAP parameters (QT, QTcB, QTcF, and APD_{30}) can be used as indicators for predicting QT shortening and lengthening due to test articles.

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