The Differential Effect of Nembutal and Ketamine/Xylazine Anesthetic on Dofetilide-Induced QT Interval Prolongation

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Previous work from our group has suggested that anesthetics with additional inherent IKs blockade such as sodium pentobarbital may sensitize the animal to agents that prolong the electrocardiographic QT interval. In the present work, we used dofetilide to assess the vulnerability of both the Nembutal and ketamine/xylazine anesthetized guinea pig. Male guinea pigs (400-550g) were anesthetized with Nembutal (60 mg/kg IP, maintenance dose continuous IV infusion 6 mg/kg/h) or ketamine/xylazine (87/5 mg/kg IP, maintenance dose 44 mg/kg IP) and surgically instrumented with a Millar pressure catheter to measure arterial pressure and heart rate. PR interval, QRS duration, QT/QTc interval and arrhythmogenesis were determined from continuous surface electrocardiograms. Animals anesthetized with Nembutal received vehicle (10% hydroxypropyl beta cyclodextrin in saline) or dofetilide (0, 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg/kg; 0.5 mL/kg), and animals anesthetized with ketamine/xylazine received vehicle (10% hydroxypropyl beta cyclodextrin in saline) or dofetilide (0, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/kg; 2.5 mL/kg) over a period of 5 minutes followed by 10 minute-recovery period. Administration of dofetilide to either Nembutal or ketamine/xylazine anesthetized guinea pigs significantly increased QTcB interval at all doses studied compared to time-matched vehicle control (p< 0.05). QTcB interval maximally increased 22% at a dofetilide dose of 0.08 mg/kg in the Nembutal group. In ketamine/xylazine guinea pigs, a dose of dofetilide five times higher (0.4mg/kg) increased QTcB by only 12% maximally. No changes in mean arterial pressure, HR, QRS interval or PR interval were observed in Nembutal or ketamine/xylazine groups treated with dofetilide compared with each respective vehicle control. Our results demonstrate that sodium pentobarbital anesthetized guinea pigs are more sensitive to QTc interval prolongation; this should be the anesthetic of choice when screening agents for the potential to prolong QTc interval.

Introduction

The anesthetized guinea pig is a widely used model for early screening of drug-candidate effects on cardiovascular function. They are excellent for predicting the potential of a test article to delay ventricular repolarization in humans as they have similar cardiac action potential and ECG characteristics. Many anesthetics have been studied in this model but sodium pentobarbital is the most widely used. Studies have documented that sodium pentobarbital is an antagonist of the inward rectifying cardiac potassium channel IKs. Since the IKs is a component of the guinea pig cardiac repolarization sequence, this species can be exquisitely sensitive to IKs blockade. Because anesthetics can alter the sensitivity of a preparation to test articles it is necessary to assess its vulnerability in the guinea pig QT model. In this study we assessed the hemodynamic effects of increasing doses of dofetilide in the Nembutal and ketamine/xylazine anesthetized guinea pig model. Dofetilide is a Class III antiarrhythmic drug that increases refractoriness by antagonizing IKr. It has been associated with QTc prolongation and TdP arrhythmias in humans.

Objectives

Assess vulnerability of the Nembutal and ketamine/xylazine anesthetized guinea pig for safety pharmacology screening of drugs with potential to prolong the QT interval.

Methods

Surgical Preparation: Male Dunkin Hartley guinea pigs (400-550g) were anesthetized with ketamine/xylazine (87/5 mg/kg IP, maintenance dose 44 mg/kg IP) or Nembutal (60 mg/kg IP, maintenance dose continuous IV infusion 6mg/kg/h). Once consciousness was lost the trachea was isolated and an endotracheal tube was inserted for mechanical ventilation (~60 breaths/min with a tidal volume of ~7-8 mL/kg). A Millar pressure catheter was placed in the carotid artery to measure arterial pressure. A lead II electrocardiogram was monitored throughout the experiment via electrodes placed in the skin of the right arm, left leg and chest of the animal. Body temperature was also monitored throughout the experiment. The jugular vein was cannulated for test compound administration.

Experimental Plan: ECG and blood pressure were continuously monitored throughout the experiment with the NOTOCORD-Hem (Software 4.3 NOTOCORD Inc., Croissy sur Seine, France) data capture system. Individual animals were deemed acceptable for use in the study if they exhibited acceptable hemodynamic parameters during the approximate 15-30 minutes equilibration period. Baseline hemodynamic and electrocardiographic parameters were obtained for 15 minutes followed by escalating doses of vehicle or dofetilide administered into a jugular vein. Guinea pigs anesthetized with Nembutal received vehicle (10% hydroxypropyl beta cyclodextrin in saline) or dofetilide (0, 0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg/kg; 0.5 mL/kg) and animals anesthetized with ketamine/xylazine received vehicle (10% hydroxypropyl beta cyclodextrin in saline) or dofetilide (0, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/kg; 2.5 mL/kg) over a period of 5 minutes followed by 10 minute-recovery period.

Hemodynamic and Electrocardiographic Measurements: Average values taken from 20 second blocks of consecutive cardiac cycles uninterrupted by interference of ectopic beats were used for analysis. Measurements were taken at baseline, and every 1 minute during the monitoring period. The monitoring period began at the initiation of that test period's respective dose. Values from each individual animal were pooled to determine an average for each variable for each group.

Statistical Methods: Hemodynamic parameters were analyzed using an analysis of variance (ANOVA) model at each timepoint. Baseline data were analyzed with an ANOVA model with an effect for treatment. The model for each subsequent timepoint included baseline as a covariate and an effect for treatment. Baseline was defined as the value at time 0. Comparisons were considered significant at the 0.05 level. All statistical analyses were conducted with SAS® version 9.2.

Experimental Design

Anesthesia - Nembutal *(60 mg/kg IP, maintenance dose continuous IV infusion 6mg/kg/h)*

Group 1 Dose 2-Dose 7 - **Vehicle 0.5 mL/kg**

Group 2 Dose 2-Dose 7 - **Dofetilide (0.0025, 0.005, 0.01, 0.02, 0.04, and 0.08 mg/kg)**

Anesthesia - ketamine/xylazine *(87/5 mg/kg IP, maintenance dose ketamine 44 mg/kg IP)*

Group 3 Dose 2-Dose 7 - **Vehicle 2.5 mL/kg**

Group 4 Dose 2-Dose 7 - **Dofetilide (0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/kg)**

Effects of Anesthetic on QT Interval in the Guinea Pig

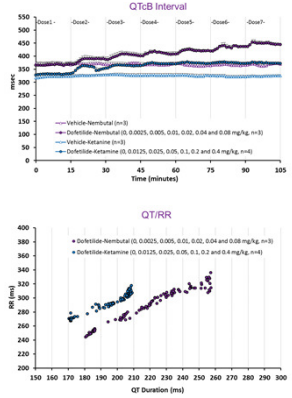

Figure 1. A- *Effects of dofetilide on QTcB duration in ketamine and Nembutal anesthetized guinea pigs. Values are expressed as mean±SEM. Data were analyzed by using an analysis of variance (ANOVA) model at each timepoint. Comparisons were considered significant at the 0.05 level. All statistical analyses were conducted with SAS® version 9.2.* ***B-*** *QT versus RR interval plot of dofetilide treated ketamine and Nembutal anesthetized guinea pigs. Each data point is an average values taken from 20 second blocks of consecutive cardiac cycles uninterrupted by interference of ectopic beats, measurements were taken at baseline, and every 1 minute during the monitoring period.*

Effects of Anesthetic on PR Interval and QRS Duration in the Guinea Pig

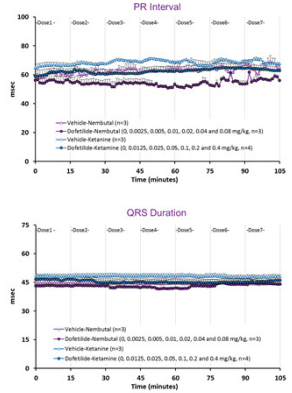

Figure 2. *Effects of dofetilide on PR interval and QRS duration in ketamine and Nembutal anesthetized guinea pigs. Values are expressed as mean±SEM. Data were analyzed by using an analysis of variance (ANOVA) model at each timepoint. Comparisons were considered significant at the 0.05 level. All statistical analyses were conducted with SAS® version 9.2.*

Effects of Anesthetic on Mean Arterial Pressure And Heart Rate in the Guinea Pig

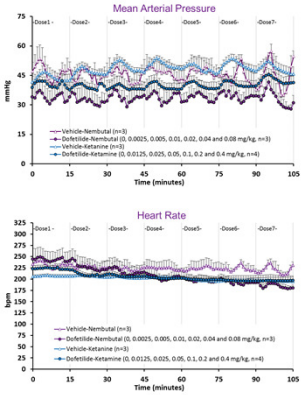

Figure 3. *Effects of dofetilide on mean arterial pressure and heart rate in ketamine and Nembutal anesthetized guinea pigs. Values are expressed as mean±SEM. Data were analyzed by using an analysis of variance (ANOVA) model at each timepoint. Comparisons were considered significant at the 0.05 level. All statistical analyses were conducted with SAS® version 9.2.*

Summary

- **Administration of dofetilide to either Nembutal or ketamine/xylazine anesthetized guinea pigs significantly increased QTcB interval at all doses tested compared to time-matched vehicle control (p< 0.05).**
- **In the Nembutal group, QTcB interval (Δ-Δ% vs Vehicle) maximally increased by 22% at a dofetilide dose of 0.08 mg/kg.**
- **In ketamine/xylazine group, QTcB interval (Δ-Δ% vs Vehicle) maximally increased by 12% at a dofetilide dose of 0.1 mg/kg. No further increase in QTcB was observed at 0.2 and 0.4 mg/kg of Dofetilide.**
- **No changes in mean arterial pressure, HR, QRS interval or PR interval were observed in Nembutal or ketamine/xylazine groups treated with dofetilide compared with each respective vehicle control.**
- **No changes in any of the parameters evaluated were observed in Nembutal vehicle group compared to ketamine/xylazine vehicle group.**

Conclusion

In summary, the choice of anesthetic appears to influence the maximum QTc increase in anesthetized guinea. Sodium pentobarbital anesthetized guinea pigs are more sensitive to QTc interval prolongation; and this should be the anesthetic of choice when screening agents for the potential to prolong QTc interval.