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Differential effects of thioridazine enantiomers on action potential duration in rabbit papillary muscle

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ABSTRACT

The antipsychotic drug thioridazine has potential for treatment of multidrug-resistant microbes including tuberculosis but also causes cardiotoxic QT interval prolongation. Both thioridazine enantiomers have potent antimicrobial effects, but the neuroleptic effect primarily resides with (+)-thioridazine. In this study we for the first time investigate the cardiotoxicity of the isolated thioridazine enantiomers and show their effects on ventricular repolarization. The effects of (+)-thioridazine, (−)-thioridazine, and racemate on the rabbit ventricular action potential duration (APD) were investigated in a randomized controlled blinded experiment. Action potentials were measured in papillary muscles isolated from 21 female rabbits, and the drug effect on 90% APD in comparison with control ($\Delta\Delta$-APD_{90}) was evaluated. Increasing concentrations of (+)-thioridazine and the racemate caused significant dose-dependent $\Delta\Delta$-APD_{90} prolongation, while (−)-thioridazine did not. At 0.5 and 2 Hz pacing, (+)-thioridazine caused 19.5% and 20.1% $\Delta\Delta$-APD_{90} prolongation, the racemate caused 8.0% and 12.9%, and (−)-thioridazine caused 1.5% and 1.1%. The effect of (−)-thioridazine on APD_{90} was significantly less than that of the other drugs at both pacing rates ($P < 0.01$ in all cases), and there was no significant difference between (−)-thioridazine and control. The results of this study indicate that the APD prolonging effect of thioridazine is primarily due to the (+)-thioridazine enantiomer. If these results are valid in humans, (−)-thioridazine may be a safer drug for treatment of multidrug-resistant tuberculosis and other microbes.

1. Introduction

Thioridazine was introduced as an antipsychotic drug in 1959. Subsequently, several side effects were reported including the occurrence of sudden cardiac death, and it became clear that thioridazine causes QT interval prolongation and an increased risk of the potentially fatal ventricular arrhythmia Torsades de Pointes (Harrigan et al., 2004, Salih et al., 2007, Mehtonen et al., 1991, Ray et al., 2001, Reilly et al., 2002, Hennessy et al., 2002). Drug-induced Torsades de Pointes is associated with blockade of the rapid component of the delayed rectifier potassium current (I_{Kr}) (Yap and Camm, 2003), which is indeed blocked by thioridazine (Drolet et al., 1999, Tie et al., 2000, Milnes et al., 2006). Inhibition of I_{Kr} prolongs the ventricular action potential duration (APD), which in the electrocardiogram manifests as QT interval prolongation (Yap and Camm, 2003). Due to these concerns, branded versions of thioridazine were withdrawn in 2005 (Thanacoody, 2011).

Apart from its antipsychotic effects, thioridazine has antimicrobial effects against a range of bacteria including *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus*, *Shigella*, *Klebsiella*, *Pseudomonas*, and *Vibrio parahaemolyticus* (Martins et al., 2008). It also increases the sensitivity of drug resistant microbes, including extensively drug resistant tuberculosis, to previously ineffective drugs (Dastidar et al., 2013, Abbate et al., 2007, Abbate et al., 2012). Introduction of thioridazine as an antimicrobial agent could therefore have great impact in the treatment of drug-resistant bacterial strains.

Thioridazine is a chiral compound administered as a racemic mixture of its (+) and (−) enantiomers. Enantiomers of many chiral compounds have distinct pharmacological characteristics (Baumann et al., 2002), and this is also true for thioridazine. The antipsychotic effect of thioridazine is due to blockade of the D_2

Abbreviations: APD, action potential duration; $\Delta\Delta$-APD, drug effect on APD compared to control; APD_{90}, 90% action potential duration; AAP, amplitude of the action potential; V_{rest}, resting membrane potential; I_{Kr}, rapid component of delayed rectifier potassium current; I_{Ks}, slow component of delayed rectifier potassium current; I_{to}, transient outward current; I_{K1}, inward rectifier current

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dopamine receptor, for which (+)-thioridazine has been shown to have 2.7 times greater affinity compared to (−)-thioridazine (Thanacoody, 2011, Svendsen et al., 1988, Jortani and Poklis, 1993). (−)-thioridazine is also accumulated to a greater extent in human tissue (Jortani et al., 1994). However, the antimicrobial properties of the enantiomers are similar (Kristiansen et al., 2007), and in fact, some experiments find that (−)-thioridazine has stronger antimicrobial effect (Hendricks et al., 2004, Hendricks, 2007). The difference in neuroleptic effect naturally leads to the hypothesis that the enantiomers may also have different cardiotoxicities, as many D_2 receptor blocking agents are known to prolong the QT interval and in some cases cause Torsades de Pointes (Glassman and Bigger, 2001, Ray et al., 2009).

In this study we conducted the first investigation into the effect of the isolated thioridazine enantiomers on cardiac repolarization. The purpose was to test the hypothesis that the thioridazine enantiomers have dissimilar effect on ventricular repolarization in a suitable animal model. Due to the strong link between I_{Kr} current blockade and risk of Torsades de Pointes, APD prolongation due to inhibition of this current is of particular importance. It was reported that a pure I_{Kr} inhibitor causes substantial APD prolongation in the isolated rabbit papillary muscle while pure I_{Ks} blockers do not (Lengyel et al., 2001), and consequently we investigated the effects of the isolated thioridazine enantiomers in this preparation. In this paper we present the results of a randomized controlled blinded experiment producing evidence that the isolated enantiomers have different effects on the APD of the isolated rabbit papillary muscle.

2. Materials and methods

2.1. Animals and experimental preparation

Transmembrane action potential recordings were carried out in right ventricular papillary muscles isolated from female New Zealand white rabbits weighing between 1.5 and 2.5 kg ($N=21$). Animals were kept for acclimation in species specific housing for 2 weeks at the Biomedical Research Laboratory at Aalborg University Hospital, Denmark. Environmental controls maintained a 12–12 h light–dark cycle, 20 ± 2 °C temperature, and approximately 50% relative humidity. Water and pellets were supplied ad libitum. Carrots, apples, and hay were supplied as well. The experimental procedures were carried out under the approval of the Danish Animal Welfare Council, and animal care, transport, and handling was carried out in accordance with Danish guidelines by qualified personnel. A superfusate was used in the experiments containing (in mM): NaCl 117.6, KCl 5.0, $MgCl_2$ 1.0, $CaCl_2$ 2.0, NaH_2PO_4 0.4, $NaHCO_3$ 24.0, and glucose 11.0. The solution was equilibrated to a pH value of 7.4 with carbogen gas (95% O_2 and 5% CO_2) at the temperature of use (5° or 37 °C). Anesthesia was performed using a combination of Hypnorm (0.5 ml kg^{-1}) and Midazolam (0.5 ml kg^{-1}), which was followed by a blow to the neck. When the rabbit displayed no response to stimuli, the chest was opened by a midsternal incision. The heart was rapidly excised and placed in superfusate cooled to 5 °C. The apex of the heart was cut to open the right ventricle, and the lateral ventricular wall was cut from apex to base. The right ventricular papillary muscles were excised from the ventricle and mounted in a bath with fine pins through the base of the muscle and the chordae tendineae. The bath was placed in an experimental setup with superfusate recirculated by a peristaltic pump operating at a flow rate of 3.0–3.5 ml min^{-1}. The solution was gradually heated, continuously gassed with carbogen, and pumped to the bath, where the temperature was maintained between 36 and 37 °C and the pH between 7.35 and 7.45. A single papillary muscle was selected for measurement with preference given to muscles of the elongated type with a diameter of 1 mm or less. The muscle was stimulated at the base using a square pulse of 4 ms duration and amplitude of twice the stimulation threshold of visible contraction.

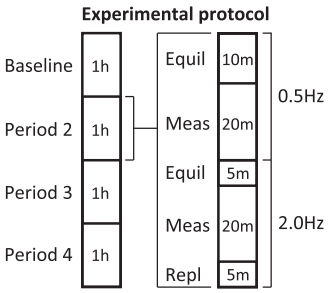

Fig. 1. Experimental time protocol. Left: 1 h time blocks. Right: subdivision of each time block. For non-control experiments, the drug concentration during period 2, 3, and 4 was 0.1, 1, and 10 mg L^{-1} respectively. Equil: equilibration. Meas: measurement. Repl: replacement of solution.

2.2. Experimental design

The researchers were blinded to the identity of the drugs during data acquisition and analysis. Action potentials were recorded at stimulation frequencies of 0.5–2 Hz at baseline (no drug applied) and drug concentrations of 0.1, 1.0, and 10.0 mg L^{-1}. This dose range is in good agreement with the range over which thioridazine was shown to prolong APD and inhibit I_{Kr} and I_{Ks} in isolated guinea pig heart and ventricular myocyte preparations (Drolet et al., 1999). The muscle was allowed 90 min of equilibration at 1 Hz stimulation prior to baseline measurements. Drug concentrations were always increased and wash out was not attempted. To ensure comparability between groups and to enable correction for time-dependent action potential changes, every experiment followed a strict time schedule (Fig. 1). For each level of drug concentration, 1 h of measurement time was allocated. To avoid change in superfusate composition due to evaporation, the superfusate was replaced before measurement at baseline and with each change in concentration. The new superfusate was pre-equilibrated with carbogen and contained the appropriate drug concentration. Stimulation at 0.5 Hz was initiated and 10 min of equilibration followed. Measurements were carried out for 20 min, and 2 Hz stimulation commenced. A 5 min equilibration period was allowed and measurements were carried out for 20 min. Preliminary experiments indicated that APD adaptation occurred over approx. 5 min for concentration change and 3 min for frequency change. For each combination of frequency and concentration it was sought to obtain stable measurements of at least 30 s duration from eight different cells located between the apex and middle of one side of the muscle. However, if the contraction of the muscle was unusually strong, and stable impalement was difficult to maintain, measurements from fewer cells were sometimes made in order to maintain the time schedule. The rabbits were randomly assigned to 4 groups: control ($N=5$), (+)-thioridazine ($N=4$), (−)-thioridazine ($N=6$), and racemate ($N=6$). One out of 5 rabbits receiving (+)-thioridazine was excluded as the muscles obtained from this rabbit displayed substantially diminishing contraction strength, diminishing plateau amplitude, and APD shortening before drug application.

2.3. Data analysis

Following each stimulus pulse, 400 ms of data were recorded at a sample rate of 2 kHz. Signals were analyzed and features were