Evaluation of terfenadine and ketoconazole-induced QT prolongation in conscious telemetered guinea pigs[#]

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Abstract:

Terfenadine and ketoconazole are the most widely used positive reference agents in non-clinical cardiac repolarization safety studies. The aim of the present study was to evaluate the effects of terfenadine, ketoconazole and their combination on QT prolongation using conscious guinea pigs. Conscious telemetered guinea pigs were orally administered terfenadine (50 mg/kg), ketoconazole (200 mg/kg) or a combination of the two, and effects on QT were recorded using a telemetry system. The QT correction was carried out with Bazett's formula to eliminate confounding effect of HR. Neither terfenadine nor ketoconazole produced any effect on the RR and QT intervals, QRS complex or heart rate (HR). However, a combination of terfenadine and ketoconazole significantly prolonged the RR and QT intervals and decreased HR in a time-dependent manner. This study demonstrated that the combination of terfenadine and ketoconazole produces QT prolongation in conscious telemetered guinea pigs.

Key words:

ECG, QT, telemetry method, terfenadine and ketoconazole

Introduction

Non-clinical assays are not sensitive enough to accurately predict repolarization liabilities in man. For this reason, a thorough QT/QTc study is now required for virtually all newly developed pharmaceutical agents [4, 5, 23]. S7A guidelines of the International Conference of Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use states a preference for *in vivo* experimental models using unstressed animals for safety pharmacology studies [2, 25]. Guinea pigs have been found to be one

of the preferred and attractive *in vivo* models to evaluate the effect of drugs on QT interval. Guinea pigs are small in size and have an ionic current profile similar to that of humans (except for the I_{to} channel) during the ventricular action potential [14]. Although QT prolongation and/or torsades de pointes caused by co-administration of terfenadine and ketoconazole has been studied in animals and humans [16, 17], most of the studies were done in anesthetized animals [1, 4, 5]. With the use of a telemetry system, experimentation can be performed on conscious unstressed animals [7, 13, 19–21]. Few studies have reported the ef-

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fects of drug-induced QT interval prolongation in conscious guinea pigs using a telemetry system [7, 21]. Among them, a high dose of ketoconazole (400 mg/kg, *po*) administered to guinea pigs induced a significant prolongation of the QTc interval [7]. The subsequent administration of terfenadine (120 mg/kg, *po*) 120 min after ketoconazole administration did not show any further increase in QTc interval. In the present study, conscious telemetered guinea pigs were orally administered a lower dose of ketoconazole (200 mg/kg), terfenadine (50 mg/kg) or a combination of the two, and their effects on QT interval, RR interval and heart rate were evaluated.

Materials and Methods

Animals

The guinea pigs (Dunkin Hartley; 850–1000 g) were procured from the Central Animal Facility, National Institute of Pharmaceutical Education and Research (NIPER). The animal experimental protocol was approved by Institutional Animal Ethics Committee, NIPER. Animals were housed in individual cages and maintained on a 12 h dark-light cycle. The temperature of the animal room was regulated at $23 \pm 2^\circ\text{C}$, and the relative humidity was maintained at $55 \pm 15\%$. Vitamin-C supplemented water and a diet consisting of standard guinea pig pellets, gram and cabbages were supplied *ad libitum*. The animals were given free access to food and water prior to and during electrocardiograms (ECG) recording periods.

Drugs and solutions

Terfenadine and sodium chloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Carboxymethylcellulose (CMC) was used as a vehicle and was obtained from Merck, India. Ketoconazole was obtained from Micro Labs, India. Xylazine hydrochloride was obtained from Indian Immunologicals Ltd., India. Ketamine hydrochloride was obtained from Themis Medicare Ltd., India, and ampicillin was obtained from Cadila Pharmaceuticals Ltd., India. For oral administration, terfenadine (50 mg/kg dosed in a volume of 1 ml/kg) and ketoconazole (200 mg/kg dosed in a volume of 1 ml/kg) were dissolved in CMC.

Transmitter implantation

An ECG transmitter (TA11M2-C50 PXT, Data Sciences International Inc., St. Paul, MN) was implanted into a subcutaneous pocket made in the right or left flank of guinea pigs under anesthesia (combination of 5 mg/kg of xylazine hydrochloride and 40 mg/kg of ketamine hydrochloride; *ip*). A telemetry system (Data Sciences International Inc., St. Paul, MN) was used to record ECG in guinea pigs. A dorsal-precordial configuration with bipolar leads for ECG was implanted with the negative pole between the scapulas and the positive pole close to the sternum.

This configuration has demonstrated a well-defined end of T wave in conscious male guinea pigs [19], and the ends of T waves were observed clearly in drug-treated animals. After implantation, all animals were administered a single dose of the antibiotic ampicillin (30 mg/kg, *sc*). Animals were allowed to recover for a week before experimentation.

Treatment schedules

Animals were orally administered vehicle (0.5% CMC), terfenadine (50 mg/kg), ketoconazole (200 mg/kg) or a combination of terfenadine and ketoconazole.

Recording and analysis of waveforms

The ECG waveforms were recorded from the telemetry system following treatment. ECG recording were carried out for 360 min after drug administration, including the time during the dosing procedure (approximately 25–60 s). The recording was started 20 min post-treatment to give the animal time to recover from the stress caused by treatment. Baseline ECG data were recorded for a few minutes prior to the administration of the compounds and continued 360 min post-dose. The order of drug administration was randomized.

ECG signals were collected using the Dataquest ART data acquisition system and PONEMAH software (Data Sciences International Inc., St. Paul, MN). Raw data were converted into a compatible format and data in terms of RR, QRS interval, HR and QT interval with QT correction by Bazett's formula [3] were derived with PONEMAH software. We calculated the slopes for each regression line of QTc *vs.* RR interval in different groups using different correction

formulas. Using Bazett's correction formula, the slope of QTc vs. RR interval was closest to 0 with $RR^{1/2}$ (slope: 0.004). Thus, Bazett's correction was determined to be the best formula to correct QT for the RR interval in all groups.

Statistical analysis

All values are expressed as the mean $\pm$ SEM. Differences between various groups were compared by one-way analysis of variance (ANOVA), followed by Dunnett's *post-hoc* test. Values of $p < 0.05$ were considered to be significant.

Results

ECG morphology in guinea pig

A typical ECG and drug-induced QT prolongation recorded by the telemetry system are shown in Figure 1.

The ECGs recorded with lead apex base system showed the positive T waves. All animals showed clear morphology of the T wave, even if the QT interval was prolonged. In this study, we only included the results when the end point of the T waves did not fuse with the next P wave.

Drug-induced alterations in heart rate

Although terfenadine and ketoconazole administration did not produce any effects by themselves, heart rate was significantly decreased ($p < 0.05$) by the combination of these two drugs (Tab. 1).

Drug-induced alterations in QT and RR intervals

Terfenadine and ketoconazole alone had no apparent effect on the QT and RR intervals. The combination of both drugs, however, altered the QT and RR intervals (Tab. 1). The QT interval was 119 ± 3.4 ms immediately following terfenadine administration and 124 ± 5.3 ms after six hours. The QT interval was 126 ± 8.9 ms immediately following ketoconazole administra-

Fig. 1. The left panel shows representative recordings immediately following treatment with vehicle (A), terfenadine alone (B), ketoconazole alone (C), or ketoconazole + terfenadine (D). The right panel shows the representative recordings of the same groups after 180 min of treatment. Vertical calibration = 1 mV and horizontal calibration = 1 s for all recordings

Tab. 1. These data show the drug-induced alterations of RR (ms), HR (bpm), QRS (ms), QT (ms) and QTc-Bazett's at different time intervals in the presence of terfenadine alone (TER, n = 6), ketoconazole alone (KETO, n = 4), ketoconazole + terfenadine (n = 6) and vehicle (control; n = 9). Values are expressed as the mean $\pm$ SEM. * p < 0.05 vs. the 0-min time interval. Statistical analysis was performed with one-way ANOVA, followed by Dunnett's test. RR – R-R segment (ms), HR – heart rate (bpm), QRS – Q-R-S segment (ms), QT – Q-T segment (ms), QTc – QT correction using Bazett's formula

Parameters/treatment	Time interval							
	0	30	60	120	180	240	300	360
RR (ms)								
Control	252 $\pm$ 11	254 $\pm$ 13	248 $\pm$ 9	248 $\pm$ 9	250 $\pm$ 9	246 $\pm$ 8	259 $\pm$ 6	246 $\pm$ 6
TER	228 $\pm$ 12	221 $\pm$ 11	229 $\pm$ 12	234 $\pm$ 12	242 $\pm$ 12	246 $\pm$ 14	245 $\pm$ 13	236 $\pm$ 11
KETO	231 $\pm$ 6	255 $\pm$ 16	269 $\pm$ 18	269 $\pm$ 16	268 $\pm$ 19	287 $\pm$ 24	249 $\pm$ 13	246 $\pm$ 4
TER + KETO	232 $\pm$ 13	267 $\pm$ 23	306 $\pm$ 25	329 $\pm$ 25	401 $\pm$ 48*	511 $\pm$ 60*	576 $\pm$ 68*	504 $\pm$ 64*
HR (bpm)								
Control	243 $\pm$ 9	241 $\pm$ 10	245 $\pm$ 8	244 $\pm$ 8	243 $\pm$ 8	246 $\pm$ 8	232 $\pm$ 6	245 $\pm$ 6
TER	271 $\pm$ 15	278 $\pm$ 13	269 $\pm$ 14	263 $\pm$ 14	254 $\pm$ 13	252 $\pm$ 13	252 $\pm$ 13	256 $\pm$ 11
KETO	261 $\pm$ 7	238 $\pm$ 14	225 $\pm$ 14	225 $\pm$ 12	227 $\pm$ 15	214 $\pm$ 17	243 $\pm$ 14	244 $\pm$ 4
TER + KETO	241 $\pm$ 25	234 $\pm$ 20	221 $\pm$ 24	187 $\pm$ 15	162 $\pm$ 22*	132 $\pm$ 25*	119 $\pm$ 25*	134 $\pm$ 24*
QRS (ms)								
Control	23.4 $\pm$ 0.5	23.4 $\pm$ 0.4	23.5 $\pm$ 0.4	23.0 $\pm$ 0.4	23.6 $\pm$ 0.4	23.0 $\pm$ 0.4	23.0 $\pm$ 0.3	23.8 $\pm$ 0.3
TER	21.7 $\pm$ 0.5	20.8 $\pm$ 0.8	22.0 $\pm$ 0.8	22.0 $\pm$ 0.8	22.0 $\pm$ 0.8	22.0 $\pm$ 0.8	23.0 $\pm$ 1.0	22.0 $\pm$ 0.8
KETO	23.7 $\pm$ 0.5	25.0 $\pm$ 0.0	25.0 $\pm$ 0.5	25.0 $\pm$ 0.3	24.6 $\pm$ 0.8	25.0 $\pm$ 0.5	24.3 $\pm$ 0.6	26.6 $\pm$ 0.6
TER + KETO	21.8 $\pm$ 0.3	22.50 $\pm$ 0.7	23.2 $\pm$ 0.4	24.2 $\pm$ 1.1	23.6 $\pm$ 1.1	23.3 $\pm$ 0.6*	24.2 $\pm$ 0.6*	25.2 $\pm$ 0.7
QT (ms)								
Control	132 $\pm$ 5	135 $\pm$ 9	131 $\pm$ 6	127 $\pm$ 4	127 $\pm$ 5	132 $\pm$ 6	136 $\pm$ 5	133 $\pm$ 5
TER	121 $\pm$ 5	119 $\pm$ 3	117 $\pm$ 2	118 $\pm$ 2	126 $\pm$ 6	127 $\pm$ 7	124 $\pm$ 5	124 $\pm$ 5
KETO	128 $\pm$ 1	126 $\pm$ 9	131 $\pm$ 6	133 $\pm$ 6	136 $\pm$ 5	130 $\pm$ 5	127 $\pm$ 6	127 $\pm$ 6
TER + KETO	127 $\pm$ 14	138 $\pm$ 14	149 $\pm$ 15	163 $\pm$ 11*	199 $\pm$ 15*	216 $\pm$ 20*	238 $\pm$ 25*	251 $\pm$ 18*
QTc-Bazett's (ms)								
Control	264 $\pm$ 7	260 $\pm$ 7	263 $\pm$ 8	255 $\pm$ 7	254 $\pm$ 8	263 $\pm$ 9	269 $\pm$ 9	267 $\pm$ 9
TER	253 $\pm$ 8	254 $\pm$ 9	246 $\pm$ 8	246 $\pm$ 8	258 $\pm$ 9	256 $\pm$ 9	252 $\pm$ 6	254 $\pm$ 8
KETO	263 $\pm$ 4	260 $\pm$ 6	252 $\pm$ 4	257 $\pm$ 8	264 $\pm$ 7	245 $\pm$ 11	256 $\pm$ 11	257 $\pm$ 11
TER + KETO	233 $\pm$ 14	242 $\pm$ 7	254 $\pm$ 14	277 $\pm$ 17*	303 $\pm$ 18*	315 $\pm$ 8*	326 $\pm$ 12*	340 $\pm$ 12*

tion and 127 $\pm$ 6.2 ms after six hours. The QT interval with the combination of both drugs was 138 $\pm$ 14 ms immediately following administration and increased to 251 $\pm$ 18 ms after six hours. We observed significant increases in QTc only with administration of the combination of the two drugs (p < 0.05). Bazett's formula was used to correct the QT value (QTc) of the control group which was initially 264 $\pm$ 7 ms and remained unaltered after six hours. The QTc values for

terfenadine immediately after administration and six hours later were 254 $\pm$ 9 ms and 254 $\pm$ 8 ms, respectively. The QTc values for ketoconazole immediately after administration and six hours later were 260 $\pm$ 6 ms and 257 $\pm$ 11 ms, respectively. The QTc value for the combination of both drugs was 242 $\pm$ 7 ms immediately following administration, and it increased to 340 $\pm$ 12 ms after six hours. Terfenadine or ketoconazole alone did not result in any significant change in HR,

QRS, QT and RR intervals, whereas the combination of both drugs resulted in increased QT and RR intervals compared with control (Tab. 1).

Discussion

The purpose of this study was to evaluate the terfenadine- and ketoconazole-induced QT prolongation in conscious telemetered guinea pigs. Guinea pigs have received attention with regard to *in vivo* QT assessment of newly discovered drugs. Studying characteristics of QT interval in conscious guinea pigs can be a good choice because it minimizes anesthesia and stress-induced alterations [8]. This study demonstrated that the administration of terfenadine (50 mg/kg) or ketoconazole (200 mg/kg) alone did not have any effects on the QT and RR intervals, QRS, HR and drug-induced QTc prolongation in telemetered conscious guinea pigs. However, a combination of terfenadine and ketoconazole significantly prolonged the RR and QT intervals and decreased the HR in a time-dependent manner. The QT intervals vary with heart rate; they decrease with tachycardia and increase in bradycardia. Different correction formulas can be used to compensate for variations in heart rate. In this study, Bazett's formula was found to be the most appropriate for detecting QTc prolongation [3].

Terfenadine is a second generation histamine receptor antagonist that is widely used as a non-sedating antihistamine. These second generation drugs are pharmacologically different from first-generation antihistamines because they have preferential affinity for peripheral H_1 receptors vs. brain H_1 and cholinergic receptors [22]. Ketoconazole is a synthetic antifungal drug that blocks potassium rectifier currents. Ketoconazole prolongs the QT interval by directly blocking I_{Kr} and delaying cytochrome P450-dependent metabolism of other drugs that also prolong the QT interval [12, 15, 17]. Inhibition of cytochrome P450-dependent metabolism of terfenadine had a significant effect on QT prolongation because terfenadine alone did not increase the QT. Indeed, this effect may be due to the presence of ketoconazole because the serum level of terfenadine increased and the concentrations of metabolite decreased, which suggested that ketoconazole inhibited terfenadine metabolism [16]. Terfenadine at 0.3 mg/kg (*iv*) produced no effect on

the QT interval, but at 1 and 3 mg/kg, it significantly prolonged the QT interval in dogs [24]. In guinea pigs, a 10 mg/kg intravenous infusion of terfenadine also resulted in QT prolongation, and an oral dose of terfenadine (50–60 mg) in combination with ketoconazole (200 mg/kg) caused immediate prolongation [11]. Our data support the findings that the combination of terfenadine (50 mg/kg) and ketoconazole (200 mg/kg) resulted in immediate QT prolongation. In addition, we found that neither drug increased QT prolongation by itself in telemetered conscious guinea pigs. Mammalian cytochrome P450 enzymes involved in the metabolism of different drugs, including terfenadine, are sensitive to ketoconazole. Inhibition of these enzymes slows down the metabolism of terfenadine and increases the toxic effects of the drug. The combination of terfenadine and ketoconazole showed the maximum effect on QT prolongation.

This combination could be used as a positive reference for the validation of animal models to screen new chemical entities (NCEs) for their effects on electrical activity of the heart. Although the guinea pig ventricle lacks I_{to} , the guinea pig has been suggested as a useful animal model for the detection of prolonged QT intervals and drug-induced proarrhythmia liability [6, 9, 18]. One of the advantages of using a telemetry system for QT evaluation is the possibility of recording the ECG continuously over a long period of time. The generation of continuous ECG data allows for the detection of the effects of test drugs on the QT interval (and to evoke arrhythmias) as well as an examination of other drug-induced effects. For example, we could examine the effects of drugs on the function of the autonomic nervous system. It has been reported that sympathetic/parasympathetic activity modulates the QT interval and ventricular arrhythmia susceptibility [10, 18].

In conclusion, our study revealed that a combined oral dose of terfenadine and ketoconazole produced QT prolongation in conscious telemetered guinea pigs. Furthermore, telemetered, conscious, unrestrained guinea pigs seem to be a sensitive model that demonstrates high predictive power for the evaluation of QT risks of NCEs.

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