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SPECIAL REPORT

# The role of ketoconazole in the QTc interval prolonging effects of $H_1$-antihistamines in a guinea-pig model of arrhythmogenicity

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We have carried out experiments to re-evaluate the influence of ketoconazole (400 mg $kg^{-1}$,p.o.) on the effects of ebastine, terfenadine and loratadine on the QTc interval in conscious guinea-pigs. Following a previously described protocol of oral drug administration, but using telemetric recording of the ECG, we have found that the prolongation of the QTc interval attributed to ebastine and terfenadine is in fact entirely due to ketoconazole, and that neither terfenadine, ebastine nor loratadine produce any additional effects on subsequent administration.

**Keywords:** QTc interval; ketoconazole; ebastine; loratadine; terfenadine

**Introduction** Since the relationship between the use of some non-sedative $H_1$-antihistamines (especially in combination with cytochrome P450 inhibitors such as ketoconazole) and prolongation of the QTc interval, occasionally leading to life-threatening arrhythmias (e.g. 'torsades de pointes'), appeared in the literature (Monahan *et al.*, 1990; Nightingale, 1992), much effort has been expended in the search for experimental models capable of predicting the proclivity for compounds to produce such effects under clinical conditions. The mechanism of action seems not to be related to antihistamine activity but to the blockade of the delayed rectifier $K^+$ currents (Rampe *et al.*, 1993; Crumb *et al.*, 1994). Recently, Hey *et al.* (1996) have described experiments in which they administered antihistamines orally to guinea-pigs pretreated with ketoconazole (400 mg $kg^{-1}$, p.o.) and concluded that, at doses that were without effect in the absence of ketoconazole, both terfenadine and ebastine, but not loratadine, caused significant increases in the QTc interval. Since the dose of ketoconazole used in this study was more than double its $LD_{50}$ in the guinea-pig (Heel, 1982), the objective of the present study was to see whether ketoconazole, at such a high dose, itself increases the QTc interval significantly which would thereby allow a different interpretation of the results presented in the aforementioned study.

**Methods** Male Dunkin-Hartley guinea-pigs (450-550 g) were anaesthetized with thiopentone sodium (40 mg $kg^{-1}$, i.p.) and telemetric ECG transducers (type TA10CA-F40 from DataSciences) were implanted subcutaneously in the interscapular region with the two tips sutured in the right shoulder (-ve electrode) and left flank (+ve electrode), respectively. Animals were housed in an air-conditioned room at $20 \pm 3°C$ with $55 \pm 5\%$ humidity and were used following a post-surgery recovery time of at least 1 week. A DataSciences Telemetry Recording System was used for capturing the ECG waveforms of the guinea-pigs via the LabPRO, version 3.01, programme. ECG data were recorded for 1 h before and 4 h after ketoconazole dosing. Waveforms (duration 10 s; sampling frequency 1000 Hz; filter cut-off 50 Hz) were captured from each animal at 10 min intervals. QT and R-R measurements were made manually on at least three cycle waveforms taken every 10 min and the QTc was calculated by use of Bazett's formula: $QTc = QT/\sqrt{RR}$. Animals were fasted overnight and dosed with vehicle (methylcellulose 0.5% + Tween 80 0.1% in water; 5 ml $kg^{-1}$), terfenadine (120 mg $kg^{-1}$, p.o.), ebastine (20 mg $kg^{-1}$, p.o.) or loratadine (20 mg $kg^{-1}$, p.o.), 2 h after ketoconazole (400 mg $kg^{-1}$, p.o.) pretreatment. Animals pretreated with vehicle were used as controls. Drug effects on the QTc interval were calculated as the change in ms from baseline values. Upon conclusion of the recording period, the animals were killed by an overdose of barbiturate. The results shown in the text and figure are expressed as mean values $\pm$ s.e.mean. Main evaluation criteria were based on two global measures (before and after treatment with vehicle, loratadine, terfenadine or ebastine). Hypothesis tests and confidence intervals were made following LSD-ANOVA comparisons with SAS-PROC GLM (Mathews *et al.*, 1989). Drugs used were ebastine (Almirall); ketoconazole, terfenadine and loratadine (Impex Química, Llissá del Vallés, Spain).

**Results** Whereas vehicle treatment had no significant effect on the QTc interval, ketoconazole produced a significant prolongation of rapid onset (30 min) and long duration (>4 h). A maximum increase of $58 \pm 11$ ms was observed about 4 h after treatment (Figure 1). All pairwise comparisons between the controls and the four ketaconazole treated groups were statistically significant at the $\alpha < 0.001$ level. Ebastine, loratadine and terfenadine failed to induce further prolongations of the QTc interval different from vehicle when administered 2 h after ketoconazole ($P > 0.05$). The baseline values before ketoconazole treatment were $279 \pm 7$ ms, $264 \pm 6$ ms, $270 \pm 6$ ms, $279 \pm 7$ ms and $272 \pm 6$ ms for control, vehicle, terfenadine ebastine and loratadine, respectively.

**Discussion** The present study has shown that ketoconazole, administered orally at a dose of 400 mg $kg^{-1}$ in conscious guinea-pigs, induces a significant prolongation of the QTc interval of rapid onset and long duration. Furthermore, the subsequent administration of ebastine, loratadine or terfenadine (20, 20 and 120 mg $kg^{-1}$, p.o., respectively) 2 h after the ketoconazole dose failed to cause any further increase in the QTc interval different from that seen with vehicle. These findings are clearly at variance with those recently described by Hey *et al.* (1996) in whose hands ketoconazole administered orally at a dose of 200 mg per guinea-pig ($\approx 500$ g) is described as not affecting the QTc interval, whereas ebastine and terfenadine, but not loratadine (given orally at the dose of 10, 60 and 10 mg/guinea-pig, respectively, 2 h after ketoconazole) produced an almost immediate and long lasting increase. A possible explanation for the discrepancy during the ketoconazole pretreatment period is that in the Hey *et al.* (1996) study the animals were not telemetrically equipped and the period of

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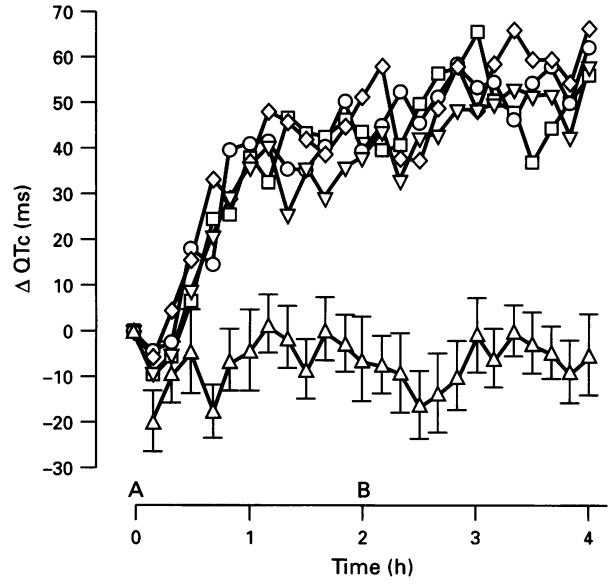


**Figure 1** Effects of vehicle (▽; 5 ml kg$^{-1}$; $n=10$), ebastine (□; 20 mg kg$^{-1}$, p.o.; $n=10$), terfenadine (◇; 120 mg kg$^{-1}$, p.o.; $n=10$) and loratadine (○; 20 mg kg$^{-1}$, p.o.; $n=10$) in guinea-pigs pretreated with ketoconazole (400 mg kg$^{-1}$, p.o.) on the QTc intervals of the ECGs. Animals pretreated with vehicle (△; $n=10$) were used as controls. Symbols represent mean of QTc differences from their respective baseline values. For reasons of clarity, error bars are only shown for vehicle-treated animals not receiving ketoconazole. No statistical differences were found between the three ketaconazole treated groups ($P>0.05$). Ketoconazole (or vehicle) were administered at (A). Vehicle, ebastine, terfenadine and loratadine were administered 2 h afterwards (B).

time needed from ketoconazole dosage to the first ECG measurement was sufficiently long for the drug to have already exerted most of its effect on the QTc interval. Furthermore, the animals were restrained, and hence stressed, and ECG measurements might have been influenced by catecholamine release. It also seems possible, taking into account the similarity between the baseline QTc interval values in all of their groups of animals, that having concluded that ketoconazole was without effect, these baseline values were obtained before ketoconazole administration. Nevertheless, none of these hypotheses explain why these authors found loratadine to have no effect on the QTc interval in animals pretreated with ketoconazole.

Ours is the first study showing that ketoconazole increases the QTc interval in a pharmacological model, albeit at toxicological doses. Thus, the $LD_{50}$ of ketoconazole in male guinea-pigs is 178 mg kg$^{-1}$ (Heel, 1982), a dose less than half of that used by both Hey *et al.* (1996) and ourselves, and in a preliminary study we did in fact observe that animals died 24–48 h following ketoconazole treatment at 400 mg kg$^{-1}$. It is also clear that a much lower dose would be sufficient to inhibit the CYP 3A4 enzyme (Honig *et al.*, 1993). It seems obvious, therefore, that for the guinea-pig model to be at all meaningful in terms of measuring the proclivity for QTc-related cardiotoxicity of antihistamine in the presence of ketoconazole and other cytochrome P450 inhibitors, the doses used should be sufficient to inhibit adequately the enzyme without demonstrating any inherent cardiotoxicity *per se*, and that under these conditions terfenadine should produce dose-dependent prolongations of the QTc as a positive control.

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