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Original article

Interferences of the autonomic nervous system with drug induced QT prolongation: A point to consider in non-clinical safety studies

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ABSTRACT

Introduction: QT interval assessment by telemetry has become one of the most useful models in testing strategies adopted for detection of drug induced QT prolongation in non-clinical safety pharmacology studies. This study reports experimental data showing that the autonomic nervous system might influence drug induced QT prolongation. **Methods:** Animals were instrumented with telemetric transmitters and epicardial ECG leads. Effects on QT interval of reference drugs such as thioridazine and terfenadine were analysed with different approaches, the Holzgrefe's probabilistic method, the QT shift method and an individual analysis of beat-to-beat QT/RR pair distribution visualised as points-cloud. **Results:** Two cases of unexpected absence of QT interval prolongation are reported with thioridazine and terfenadine in conscious beagle dogs under conditions of concomitant tachycardia. The pro-arrhythmic properties of these two molecules were unmasked by co-treatment with sympatholytic agents, atenolol and clonidine respectively suggesting that sympathetic activation and/or parasympathetic withdrawal might impair a drug induced QT prolongation. **Discussion:** The apparent absence of changes in the QT interval due to novel drug candidates should be interpreted cautiously under conditions of concomitant tachycardia or elevated heart rate levels in non-clinical safety studies.

1. Introduction

QT interval prolongation is currently considered to be an important surrogate for the evaluation of risk of life threatening conditions, such as polymorphic ventricular tachycardia, Torsades de Pointes, and other forms of malignant ventricular arrhythmias (Hanson et al., 2006; Sides, 2002). Numerous improvements have been introduced in non-clinical safety studies for evaluation of drug induced QT prolongation. Since the QT interval duration is dependent upon heart rate, QT rate correction formulas are used to assess possible drug effect on the duration of ventricular repolarisation. Major improvements have been achieved in this field. Indeed, the QT correction formulas initially proposed for man, such as Bazett's or Fridericia's formulas have been demonstrated as more or less inappropriate depending on animal species (Batey & Doe, 2002; Matsunaga et al., 1998). More reliable methods have been published in the past decade, most of which are based on an individual QT rate correction derived from an individual analysis of the QT/RR relationship (Miyazaki & Tagawa, 2002; Ollerstam et al., 2007; Spence, Soper, Hoe, & Coleman, 1998). Recently, an important methodological refinement has been identified, the probabilistic method (Holzgrefe et al., 2007; Holzgrefe, Cavero, Buchanan, Gill, & Durham, 2007; Holzgrefe, Cavero, & Gleason, 2007). This is the method that we now use routinely in our laboratory in association with the QT shift method (Champeroux et al., 2009), since both methods enable not only reliable calculations of drug effect on QT interval but also improved sensitivity of detection at levels close to those required in clinical QT thorough studies whilst requiring only a small number of animals. Conditions of measurements have also been considerably improved, in particular due to the use of telemetry. Continuous recording of ECGs for long periods (e.g. 24 h) is now possible in conscious telemetered animals (Gauvin, Tilley, Smith, & Baird, 2006a,b). The epicardial placement of electrodes in large species can also be considered as a source of improvement in ECG analysis in safety pharmacology studies (Holzgrefe et al., 2007; Holzgrefe, Cavero, Buchanan et al., 2007; Holzgrefe, Cavero, & Gleason, 2007). Indeed, the quality of ECG signal is so much improved that beat-to-beat analysis over a 24-h period is now possible even in difficult species such as cynomolgus monkeys, enabling thorough analysis of a larger amount of data and application of the probabilistic method (Holzgrefe et al., 2007; Holzgrefe, Cavero, Buchanan et al., 2007; Holzgrefe, Cavero, & Gleason, 2007).

All these technological refinements should facilitate improved predictivity for non-clinical safety studies to accurately detect drug induced QT prolongation. However, the predictivity of these studies cannot be improved simply by attention to methodological aspects

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since other factors linked to the pharmacological properties of drugs must be also taken into consideration. For example, recently, Van der Linde, Van Deuren, Teisman, Towart, and Gallagher (2008) pointed out the importance of taking into account possible drug effect on body temperature after demonstrating that slight hyperthermia or hypothermia might have a noticeable impact on the QT interval duration. Thus both false positive and negative results could arise, depending on any effects on body temperature. Likewise, the general electrophysiological profile of the molecule evaluated from in vitro or ex vivo studies (patch-clamp and action potential studies) should also be taken in careful consideration in the interpretation of in vivo studies. Indeed, QT prolongation is in most cases attributed to I_{Kr} blocking properties. However, some compounds may have complex electrophysiological patterns that limit capability to prolong the QT interval when compared to pure I_{Kr} blockers. In a previous report (Champeroux et al., 2005), we demonstrated that this is the case for approximately 80% of torsadogenic agents and suggested that the electrophysiological pattern of these torsadogenic agents was related to both I_{Kr} and I_{Na} blocking properties at least. Furthermore, while the I_{Kr} blocking component generally prolongs the cardiac action potential duration and consequently the QT interval duration, the I_{Na} blocking component tends to shorten the cardiac action potential and the QT interval. Consequently, we observe that some torsadogenic molecules such as terfenadine might cause only a weak or moderate QT prolongation, also explaining the lack of correlation between the QT interval prolongation capability and the risk of Torsades de Pointes. In non-clinical safety studies, the risk of not detecting a torsadogenic pattern is relatively high if the methodology used is not sufficiently sensitive and reliable. This point is particularly important if QT interval assessment studies are primarily directed at detection of drugs which could cause Torsades de Pointes and only secondarily at drugs which could cause QT prolongation.

Another important reason that may affect the predictivity of non-clinical studies is whether or not a drug has the capability of interacting with the autonomic nervous system, in particular with the cardiac rhythm whether directly or indirectly. This aspect if present, should lead to more cautious interpretation of the risk for QT prolongation in both safety pharmacology and toxicity studies. Indeed, most drugs causing QT prolongation are, at a minimum, I_{Kr} inhibitors (Redfern et al., 2003). An important property of these potassium channel inhibitors is the reverse use dependence of their I_{Kr} blocking effect (Hondeghem & Snyders, 1990). This means that their inhibitory effect is dependent upon the cardiac rhythm. It increases when heart rate is lower, also explaining why bradycardia enhances drug induced QT prolongation and consequently, it is a risk factor for Torsades de Pointes. On the other hand, the I_{Kr} inhibition decreases when heart rate is accelerated. Transposed to the QT interval, this means that drug induced QT prolongation is similarly rate dependent such that heart rate elevation could theoretically reduce drug induced QT prolongation. This is exactly what we have seen over a long period in the studies that we have conducted in our laboratory. Does this reduce the predictivity of non-clinical studies? The answer is yes. The present study is intended to share some experimental data on this topic and to report some examples of reference data obtained with thioridazine and terfenadine.

2. Methods

All experiments were subjected to rigorous ethical review and utilised GLP-validated computer procedures. The standard study protocol was that routinely used for non-clinical safety pharmacology studies at CERB.

2.1. Surgical procedures

Beagle dogs aged 9–22 months (10–15 kg) were fitted with radio telemetry transmitters (model TL11M3D70PCTP or TL11M2D70PCT, Data Sciences International). Dogs were premedicated with acetylpromazine (0.05 mg/kg, s.c.) and buprenorphine (0.01 mg/kg, s.c.). Anaesthesia was induced by thiopental (15–20 mg/kg, i.v.) and maintained with isoflurane 0.5–1.5% in oxygen. A left thoracotomy was performed between the fourth and the fifth intercostal space. One transmitter electrode was sutured directly to the left ventricular epicardium near the apex while the second electrode was sutured to the pericardium above the right atrium to approximate a limb Lead II electrocardiogram. The body of the transmitter was placed into the peritoneal cavity and secured to the abdominal wall. Analgesic treatment with buprenorphine was continued for a minimum of two days after the surgery to alleviate any post operative pain.

2.2. Housing and dosing

A minimum period of 3 weeks was allowed for recovery from the surgery. During the telemetry recording periods, animals were housed in individual stainless steel cages of appropriate size. Environmental parameters were recorded continuously and maintained within a fixed range, room temperature at 15–21 °C, at 45–65% relative humidity. Drinking water was provided *ad libitum*. Solid diet (300 g) was given daily in the morning. All treatments with drugs were performed between 3.00 p.m. and 3.30 p.m. A minimum washout period of one week or more depending upon the half-life of elimination was allowed between each dosing. Experiments were performed on groups of six animals (3 males and 3 females).

2.3. Telemetry ECG data acquisition

ECG waveforms were continuously recorded at a sampling rate of 500 Hz using the ART™ acquisition software release 4.1 (Data Sciences International). Cardiac conduction times including QT interval were calculated from a beat-to-beat analysis using internal software developed in RPL (RS/1 programming language, RS/1 release 6.3, Applied Materials), GLP-validated. Epicardial placement of ECG electrodes was shown to provide high quality ECG signals (Holzgrefe et al., 2007; Holzgrefe, Cavero, Buchanan et al., 2007; Holzgrefe, Cavero, & Gleason, 2007) even during activity period of animals enabling a fully automated beat-to-beat analysis of cardiac conduction times over the whole period of recording, i.e. 24 h (Champeroux et al., 2009). Validation of the correct location of cardiac wave markers was performed according to a standardised procedure which covers the whole 24-h period. In most cases, the percentage of errors in location of the end of the T wave was less than 3%.

2.4. Probabilistic method

All calculations were processed from the beat-to-beat signal. No binary method based on logging rates was employed meaning that no averaging of the source signal was applied (Holzgrefe et al., 2007; Holzgrefe, Cavero, Buchanan et al., 2007; Holzgrefe, Cavero, & Gleason, 2007). The QT/RR relationship was built individually from a 24-h treatment-free period following the principles of the probabilistic method described by Holzgrefe et al. (2007), Holzgrefe, Cavero, Buchanan et al. (2007), Holzgrefe, Cavero, and Gleason (2007) using 10 ms RR increments. For calculations of QT and RR values recorded at time points post-dosing, the probabilistic method was applied by processing all QT/RR pairs present at time point +/−10 consecutive minutes. Using this time range and taking into account the heart rate levels of Beagle dogs, the minimum number of QT/RR pairs required, i.e. 250 for dogs was reached even when a compound causes bradycardia, conforming to the principles of Holzgrefe's probabilistic method.

Baseline values were computed from all QT/RR pairs recorded during a 1-h period before dosing.