Detecting Drug-induced QT Interval Prolongation in Healthy Dogs: A Practical Approach

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**Introduction**

Safety assessment of new chemical entities (NCE) is essential to provide Drug Company’s Management with data to inform decision making to discontinue development of unsafe drugs early or to convince the Regulatory Authority that an efficacious NCE is also safe. These considerations imply that a modern company must to create an efficient Safety Pharmacology Department equipped with up to date instruments and validated methodologies. In that process, particular attention must be paid to the risk assessment of drug-induced QT interval prolongation, as biomarker of Torsade de Point (TdP), a potentially lethal arrhythmia. In fact, a specific guideline has been dedicated to this particular aspect (ICH S7B, 2005).

So far, some critical challenges to pre-clinical Cardiovascular Safety Pharmacology (CSF)/Specialists (Guth et al, 2009) exist:
1. The choice of a predictive test system
2. The accuracy in ECG traces recording
3. A reliable waveform measurement and ECG analysis system
4. The correct assessing of QT interval prolongation.

Furthermore, there is little documentation about standardization and validation of currently commercially-available and employed hardware/software (HR/SW) systems to ECG analysis methods in pre-clinical studies (Chui and Vargas, 2009).

Actually, animal models are anymore an issue, due to the wide consensus about dogs and monkeys as suitable animal models (Batey et al., 2002; Holzgrefe et al., 2006). In fact, together with the use of chronically telemetry-instrumentation, their use are recommended by the regulatory guidelines (ICH S7A, 2000; S7B, 2005). In such models, drug-related hemodynamic and ECG changes can be evaluated in the same animal and in real time for long period, without restrain or anaesthesia interference. Because of its advantages, and with the amelioration of implant techniques, telemeter-implanted animals are now the best alternative to “traditional” animal ECGs recording techniques also in term of traces quality. In fact, the first need for a successful electrocardiographic study is availability of high quality ECG traces that allow a precise evaluation of the waveform segments. Of course, the availability of high volume of ECG data due to the digital data recording improvement, pointed out the necessity for investigators of an accurate, efficacious and quick system for ECG analysis. The implementation of potent algorithms for measurement of ECG wave’s segments in commercial SW, releases the company to the necessity of development of “home-made” ECG analysis SW. This availability, together with the impressive increment of personal computers performances, allows investigators to manage huge amount of data in a relatively simple way. As a result, automated ECG analysis is becoming a mainstream method to optimize hemodynamic and ECG data analysis. Commercially available applications offer different approaches to automated ECG analysis for quantifying cardiac intervals, i.e. attribute-based or pattern-recognition-based platforms, or a combination. Recently, a report comparing these approaches concluded that the pattern-recognition-based platform was the most suitable to adapt to ECG morphology changes observed in long term ECG study (Chui and Vargas, 2009).

Despite dramatic improvement of data analysis power, the issue of a method to assess QT interval prolongation, however, is not yet resolved and there is no general consensus about the best method to use. Numerous factors are implicated in duration and variability of ventricular repolarization. This has obvious consequences on the understanding of pharmacologically induced modifications. The QT interval duration in fact, is strongly dependent on heart rate (HR) and thus, directly varies with changes in the RR interval. Noteworthy is the fact that QT interval rate-adaptation is not precisely regulated on a beat-to-beat basis. In fact, it is strongly influenced by the preceding HR history, a well-described phenomenon referred as “QT hysteresis” (Pueyo et al, 2003). Moreover, QT interval duration is also partly conditioned by autonomic activity oscillations (Ahnve & Vallin, 1982), electrolyte disorders (Jackman et al. 1988), exercise, changes in cardiac after load, stressful situations, etc.
For these reasons, linear regression based-formulae had been proposed to correct the QT interval (QTc) for HR changes since 1920 (Bazett, 1920; Fridericia, 1920). However, despite the general consensus in clinical practice to use these, and others, formulae generally they provide satisfactory QTc only within a narrow range of heart rates (60/80 bpm). In the animal models like dog, however, usually a wider and less stable RR intervals range than in humans is present (<60 up to >150 bpm). As consequence, QTc formulae like Bazett or Fridericia, or other single-coefficient models, fail to properly describe the QT/RR relationship in the dog. Consequently, this unresolved imprecision in QT rate-correction methodology has fostered newer mathematical approaches. A number of different ECG analysis approaches based on individual correction, proposed by many authors (Batey et al., 2002; Fossa et al., 2002; Matsunaga et al., 1998; Holzgreve et al., 2006), are currently available. Compared to standard formula-based approaches, it is now clear that individually tailored QTc offers improved, but still imperfect solutions (Batchvarov et al., 2002; Malik, et al., 2002) and often, they are too complex to apply in routine testing, specifically for the heavy data processing and analysis, and/or sometime, the need of development of specific computer applications. One of the alternative methods recently under consideration is dynamic beat-to-beat QT/RR relationship (QTbtb) analysis (Fossa et al., 2002; Batey and Doe, 2002). Using all sequentially collected beats from ECG, this method of analysis allows to compare QT intervals to individual cardiac cycles, from all normal autonomic states, at similar RR intervals, thereby eliminating potential sources of error from the use of correction functions. Recently, the efficacy of this method to differentiate changes of QT interval duration due to heart rate or autonomic state, from impaired repolarization, it has been reported (Fossa et al, 2005). But in the lack of a “golden standard”, and in the absence of consensus about the best QT correction method(s), QT interval measurement and analysis method choice play the role of key issue in daily CSF Specialist work. At the actual State-of-Art, in vitro data plus the combination of different in vivo QT analysis methodologies could give un-ambiguous answer to the Company Management if a NCE can be developable and/or fulfil the safety standard required by Regulatory Authority.

Aims and Objectives

Aim of this report is to describe our experience about the implementation of a modern and efficient CSF laboratory taking into account all the above reported considerations, with particular attention to develop, standardize and validate a relatively simple, powerful and user friendly practical data analysis procedure. Such a procedure utilizes commercially available SW with built in a combination of QT-RR relationship, on a Beat-to-Beat basis, and individual- and classical QT rate-correction based analysis. The hardware/software set up and analysis method (HW/SW System) were validated trough the comparison of the effects on QT interval induced by 2 well-known QT interval prolonging drugs of two different therapeutical classes: the antiarrhythmic sotalol (Sot) and the H1 antagonist astemizole (Ast) (Hashimoto et al, 1998; Snook et al, 1998). Finally, the system was tested “on the road” performing a GLP study on a NCE, ST 1326, a Sigma-Tau, S.p.A. drug candidate.

Materials and Methods

All aspects of the study concerning animal care were performed in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication 85-23, revised 1985) and the Italian regulatory system (D.L. 116, Art. 6 of 27 Jan 1992) under the control of Sigma-Tau veterinarians. All experiments were conducted in compliance with GLP rules and internal SOPs using GLP validated computer system and procedures.

Animals and housing conditions: Eleven male Beagle dogs bred for scientific research (11 to 13 kg body weight upon arrival, Harlan, Natisone, UD, Italy) were used. The animal room had the following environmental conditions: temperature: 18 ± 2 °C, relative humidity: 55 ± 10%, about 15-20 filtered air changes/hour and a 12-hour circadian cycle of artificial light (7:00 am – 7:00 pm). Animal room cleaning from 8:00 to 10:00 am. The animals were fed ( Tekland 2021 Dog Maintenance; Harlan, Natisone, UD Italy) once a day at 09:00 am. Drinking water was given ad libitum through an automatic system.

Surgical Preparation: Overnight-fasted dogs were pre-anesthetized with intramuscular injection of Zoletil 100®(tiletamine hydrochloride and zolazepam hydrochloride; Virbac – Carros, France). After tracheal intubation, the surgical anesthesia level was obtained and maintained by ventilation with isoflurane (1.5-2.5%) in an oxygen-nitrous oxide mixture. Body temperature was maintained at 37.5 ± 1 °C. A left thoracotomy was performed in the 4th/5th intercostal...
space. Each dog was instrumented with radiotelemetric devices (Mod. TL11M2-D70-CTP, DSI, St Paul, MN). Briefly: dogs underwent left thoracotomy to expose aortic arch and heart. Sensor 1 was inserted into aorta to monitor systemic blood pressure, sensor 3 was inserted into left ventricle through (radiotelemetry pressure data are not a part of current study and are not reported herein). ECG lead placement was epicardial, thus the two cables of sensor 2 (ECG sensor) were secured, the positive ECG electrode was sutured directly to the left ventricular epicardium near the ventricular apex, while the negative lead was secured to the mediastinal reflection near the base of the right atrial appendage (LEAD II like configuration).

Finally, the telemetric transducer body was fastened into subcutaneous task and secured.

In four out of eleven dogs, a titanium vascular access port (Harvard Apparatus, Inc., MA, USA) was also implanted for continuous iv infusion. After all surgical procedures, the antibiotic Spectrum® (ceftazidima 1 g per day, Sigma Tau, Pomezia, Italy) and Contramal® (tramadol HCl 50 mg bis die, Formenti, Mi, Italy) were administered for four days. The animals were allowed to recover for at least two weeks before experimental procedures.

Data Acquisition Laboratory: Hardware and Software: The Telemetric Acquisition Laboratory consists of two areas, the first for animal housing and signals detection (Animal Room) and the second for data monitoring and recording computer system (Data Room). Dogs, throughout the study period, were housed individually in stainless-steel cages (l 110 x w 225 x h 300 cm) each equipped with two receivers (RMC-1, DSI, St Paul, MN, USA) to cover the entire cage area. Each receiver, as well as the Ambient Pressure Reference module (APR-1, DSI, St Paul, MN, USA), positioned in the Animal Room is wired with the Data Exchange Matrix (DEM, DSI, St Paul, MN, USA) connected to the HW/SW data acquisition system: HP Workstation xw4100 (Hewlett-Packard) plus Dataquest A.R.T. V 4.1 Gold, accessorized with Diagnostics, Security Manager and GLP Auditor modules (DSI, St Paul, MN, USA), positioned in the adjacent Data Room. All digital signals were stored on hard disks configured as RAID 1 (Mirroring) to improve data security. The system was GLP validated.

Telemetric ECG and hemodynamic monitoring: During the study ECG sampling rate was 1 KHz. The high sampling rate for the ECG was specifically important as it implies a < 2 ms interval resolution, minimizing the recording error. All data are filtered through a 1 – 100 Hz band pass filter which is coded into transmitters, and is not modifiable by the end user.

All experimental sessions started at 11:00 am of first day and ended after 24-26 hours of continued data acquisition. To obtain discrete sampling periods, signals were sampled and recorded continuously for 5 min every 1 min, thus 10 steps per hour were collected, each of them consisted of approximately 300-500 consecutive cardiac cycles.

System and method validation

Experimental protocol: The day before starting the first experimental session, dogs underwent a 24 h parameters recording to obtain individual reference values (pre-study Session). The data from each dog were averaged to obtain the mean values over 24 h in free-moving, untreated conditions. Further, the presence of circadian rhythmicity was simply investigated by dividing the acquired data in 2 time period defined as: Day (11:00 am to 7:00 pm), and Night (7:00 pm to 7:00 am) and the values of each period averaged to obtain the diurnal and nocturnal mean values.

Drugs dose levels and treatment schedule: Placebo (Gelatin capsules), sotalol (3, 10, and 30 mg/kg) and astemizole (1, 3 and 10 mg/kg) were orally administered. Sotalol and astemizole were chosen on the basis of their documented capability of inducing QT interval prolongation and/or TdP in animals and humans (Hashimoto et al, 1998; Snook et al, 1998). Then each dog was randomly assigned to placebo or test items treatment following a randomized 7x7 Latin Square experimental design. A washout period of at least 1 week between the seven experimental sessions was applied. On the day of dosing, telemetric signals from each animal were monitored continuously for at least 2 h pre-dose (11:00 am – 1:00 pm; BASAL), and up to 20 h after drugs administration (DRUG ADMINISTRATION).

Data analyses: Raw data were analysed on a separate data analysis HW/SW system: HP Workstation xw4600 (Hewlett-Packard) with hard disks configured as RAID 1 (Mirroring), and Dataquest A.R.T. Analysis Only V 4.1 with File Format Utility module (DSI, St Paul, MN, USA) plus ECG AUTO2 V2.4.0.29 with Files Converter Utility v2.3.0.3 (EMKA Tech., Paris, Fr).

Study’s parameters: Digital ECG raw data were
converted in a ECG AUTO2 readable file format and HR, cardiac intervals (PR, RR, QRS and QT), QT-RR relationship and rate-corrected QT intervals, by the means of 3 different formulae: Van de Water, Matsunaga and individually rate-corrected by regression (QTcReg) formula (2), were calculated.

**QT/RR relationship and QTcReg formula**: Individual QTbtt was studied for each dog, in both pre-study and in pre- and post-treatment condition, applying the “2-parameters non-linear fitting” formula of Carmeliet [1]

\[ a^*(1-esp(f(RR))) \]  

where a = measured QT, and f = coef. f of regression (Fig. 2). A fitting r2 value >0.600 was considered satisfactory. The QT interval values, obtained for each dog, at three RR reference (RRref) intervals: 600 (HR=100 bpm), 800 (HR=75 bpm) and 1000 ms (HR=60 bpm), were reported in a table (i.e. Table 2). The QT value at RRref of 1000 ms, obtained for each dog from 24 h pre-study Session data analysis, was also assumed as QTref value to build the individual QTcReg formula [2].

\[ QTcReg = QT + QTref - (QT * 1-f * RR) \]  

where QTref is QT value at reference RR interval, and f = coef. f of regression.

**ECG analysis procedures**: Briefly: ECG traces quality was inspected on monitor screen by a skilled operator. ECG AUTO2 processes data using shape-recognition techniques and a library of model waveforms as reference. The reference library is built, enriched and edited by the operator. Library of representative ECG complex waveforms, derived by all recordings, is iteratively built for each dog. When the recognition performances appears satisfactory (Fig. 1), the analytical process progresses in four consecutive steps:

1. **Step 1**: Definition of the list of parameters to measure (Tuning analysis) and identification of the ECG segments (i.e. daytime, pre-dosing, dosing, etc.) to be processed (Protocol writing).
2. **Step 2**: The pre-study Session QTbtt ECG analysis and build of the individual QTcReg formula
3. **Step 3**: Treatment Sessions ECG analysis
4. **Step 4**: Review and save of the results: the accepted analysis results were saved on files, printed and archived.

**Statistical analysis**: The individual data are averaged and mean ± standard deviation reported. Statistical analysis of QTbtt was performed by paired student t test or one way ANOVA followed by Dunnett’s multiple comparison test. Statistical analysis of QTc time course was performed by two way repeated measure ANOVA followed by Dunnett’s multiple comparison test. A value of p < 0.05 was considered as statistically significant.

**“On the road” validation**

Finally, to test the system and methods under operative conditions, a GLP CSF study was performed on a Sigma Tau NCE under evaluation, ST1326.

**Animals preparation, dose levels and treatment schedule**: A group of 4 dogs was prepared as above described. Route, duration and doses of test item were chosen on the basis of putative route, doses and duration of administration in humans. ST1326 (1, 3, and 6 mg/kg/24 h) or vehicle (physiologic saline) was administered to each dog on the basis of individual body weight as continuous 24 h i.v. infusion by the means of a portable mini-pump (Dakmed II ) accommodated in a lateral pocket of a dog jacket. Each dog was randomly assigned to vehicle or ST1326 in a crossover sequence, according to 4 x 4 Latin square design, a washout period of at least two weeks between each of the four experimental sessions were respected.

In a second group of 4 animals, randomly chosen in the group for the validation study, Placebo (gelatin capsules) or Sotalol (3, 10 and 30 mg/kg) as positive control, was administered orally following a similar treatment schedules as aforementioned.

**Parameters acquisition and Data analysis**: The day before starting the first experimental session, dogs underwent to a 24 h parameter recording to obtain individual reference values (pre-study Session). On the day of dosing, telemetric signals from each animal were monitored continuously for at least 2 h pre-dose (11:00 am – 1:00 pm; BASAL), and up to 26 h after drugs administration (DRUG ADMINISTRATION). To monitor, recording and analyze the study parameters the above described procedures were applied.

**Results**

**System and method validation**: Twenty-four-h parameters in free-moving, untreated animals (pre-study Session).
No arrhythmias or electrocardiographic abnormalities were observed in any of the recordings, except for the respiratory sinus arrhythmia that is a normal finding in beagles.

Animals showed a nocturnal statistically significant HR reduction, and increase in RR and QT interval duration with respect to day time. Whereas, PQ and QRS did not change (Table 1). Moreover, circadian variations of ventricular depolarization duration were further confirmed by QTbtb and QTc analysis (Fig. 2, 3). Fig. 2 represents also an example of the typical distribution of the QT-RR bins, showing the wide range of RR intervals obtained from 24 h ECG of a given dog. QT/RR showed a non linear relationship, along with a hyperbolic curve due to a flattening of the relation at the longer RR interval (lower HR). It is worth noting that the QT/RR plot exhibits two distinct regions separated by a marked inflexion at an RR interval of approximately 600 ms (Fig. 2); a wide range of QT interval values was also found for the same RR interval. Moreover, although a similar slope, the comparison of QT/RR relationship during Day time and Night demonstrated that, during the night, QT interval duration was systematically longer at any of the RR intervals (Fig. 2). In fact, QT interval duration showed an average Day to Night increase of 5-10 ms at each RRref (Table 3). Thus, QTbtb analysis clearly points out the presence of a rate independent circadian rhythm of cardiac depolarization. Further, QTc analysis confirmed on one side, that QT interval was longer in the Night than in the Day; on the other, the flat QTcVdW curve confirming that it is the less effective formula to correct QT for the wide range of RR intervals present in dogs (Fig. 3) with respect QTcM and QTcReg.

Effect of sotalol: Sotalol (3, 10 and 30 mg kg-1, os), as compared to placebo administration, induced a modest, non dose dependent, but consistent and statistically significant, reduction of HR (p<0.001). Moreover, sotalol administration without affecting QRS interval, increased consistently and dose-dependently RR, PQ and QT intervals duration (Fig. 4). The sotalol-induced increase of uncorrected QT duration (Fig. 4), confirmed by both QTbtb analysis (Table 3, Fig 5) and by rate-corrected QT interval analysis (p<0.01; Fig. 6), which is clearly not rate-related.

Effect of astemizole: Astemizole administration as compared to placebo, induced a relevant and statistically significant increase of HR (p<0.001; Fig. 8) and thus, RR interval duration reduction, only at the higher dose. Whereas, PQ and QRS interval durations unchanged at any dose (Fig. 18). Uncorrect QT interval duration was slightly, but statistically significant increased after administration of 3 and 10 mg/kg (Fig. 8). The not rate-related astemizole induced QT interval prolongation was confirmed by QTbtb analysis (Table 4), and by rate-corrected QT interval analysis with all three correction formulas used (p<0.01; Fig. 9).

“On the road” validation

All parameters recorded during pre-study Sessions were similar in both animal groups (for QT interval see Table 5). Thus, they were averaged and analyzed together. All daily parameter variations previously observed, i.e. nocturnal increase in RR, and QT/QTc, reduction of HR, etc. were substantially confirmed in this second study (data not showed). Remarkably, there was the intra-individual consistency on QT interval duration over time (see Table 2 vs Table 5).

Placebo administration or vehicle infusion did not induce physiologically relevant differences in ECG parameters in the two groups. Thus all control animals were considered as a unique Control Group (n=8).

On the contrary, sotalol administration, with respect to Control Group, and consistently with previous validation study, without affecting QRS interval, dose-dependently increased RR, PQ and QT intervals duration. The last finding was confirmed by QTbtb analysis (Table 6), and by rate-corrected QT interval analysis with all three correction formulas used.

Effect of ST1326 in healthy, free-moving dogs

During 24 h infusion of ST1326, HR, RR, PQ and QRS were not affected by treatment in a statistically significant manner at any of the tested doses (data not showed), as well as QT interval. In fact, both QTbtb analysis, as well as the 3 used QT correction formulae, failed to show any significant difference with respect to Control animals (Table 7, Fig. 9).

Discussion

Actually there is not yet agreement about the best method(s) to assess unequivocally drug-induced QT prolongation. In fact, this matter remains object of vivid debate between SF specialist and Regulatory Authority. But the need of simple, efficacious and practical approach to the matter remains the primary objective of the SF Specialist for his daily work. The present report documents our experience on the
possibility, using a commercially available HW/SW system,
1. to equip an efficient CSF Lab
2. to develop and validate a relatively simple protocol
to efficiently test NCEs safety to fulfill the Authority
recommendations.

The efficacy and sensitivity of implemented HW/SW
System are testified from experimental data obtained
during the validation study. In fact, it was sufficiently
sensible to detect the very small circadian variation of
QT interval duration (5-10 ms) and to demonstrate
unambiguously its rate-independency. Moreover,
comparing the circadian pattern of QTc derived by the
three used correction formulae in reference to QTbtb
analysis, we found, as also previously shown in
humans (Smetana et al., 2003), that the extent of
circadian pattern of QTc is substantially influenced by
the correction formula used.

The efficiency of the System to detect drug-induced
QT duration prolongation was challenged with two
well-known QT interval lengthening agents of two
different therapeutic class: sotalol and astemizole.
Administration of both sotalol and astemizole
dose-dependently increased QT interval duration as
immediately evident from QTbtb analysis (RR800= up
to 27 and 10 ms, respectively). The QT interval was
already prolonged dose-dependently at 1 hour and up
to 18 hours. The results, taken as a whole, fully
confirm previous findings with such drugs (Batchvarov
et al., 2002; Batey et al., 2002; Thomsen et al., 2004,
Hashimoto et al. 1998; Snook et al. 1998).

QT correction methods have long been a topic of
much debate. Awareness that classical methods, such
Bazett’s or Fridericia’s, fail to adequately correct the
QT interval on large RR interval spectra, have led to
the application of a number of linear or nonlinear fitting
equations, which force a mathematical function to the
QT/RR relationship with varying degrees of success.
Matsunaga et al (1998) also studied fourteen different
mathematical formulae to fit RR-QT data on
Beat-to-Beat base demonstrating that Carmeliet’s
formula provides one of the best fit. Accordingly, in
our hands, by the means of our experimental setup, to
apply the ECGAuto built in Carmeliet’s fitting formula
to QTbtb, resulted powerful and simple to apply. We
confirmed that QT/RR relationship is curvilinear
(Fossa et al, 2002; Matsunaga et al, 1998) and that
the method is sufficiently sensible to demonstrate
circadian or drug-induced rate independency of QT
interval prolongation. Overall, telemetric long term
ECG recording enables a relatively simple
physiological description of the daily relationship
between RR and QT intervals of undisturbed regularly
housed dogs. Moreover, it adequately describes the
rate independence of drug-induced changes in
repolarization without recourse to a more complicate
mathematical modeling or self made SW. Finally, our
results are in complete agreement with the data
binning technique of Beat-to-Beat analysis described
by Batey and collegues (2002), but with the advantage
that the MSExfel driven data binning was not
necessary. A common criticism to this type of analysis
is that sorting the QT data by RR interval removes the
time-course of a drug’s effect. However, our data
demonstrated that the analytical approach here used,
through the simple division of the 24h records in
different time periods, i.e light and dark, permits to
easily discriminate the circadian HR independency of
QT prolongation. Thus, QT/RR relationship, in the
absence of drug, can be used as a “calibration” to
determine the expected QT value for a given RR at
different day-time. Moreover, the system permits to
integrate the findings with the contemporary
application of individual or population based rate
correction formulae to fully exploit the time course of
the phenomenon.

The advantage of chronically telemeter-implanted
dogs as test system is supported, today, by a large
bulk of evidence (Batey et al., 2002; Fossa et al., 2005;
Holzgrefe et al., 2006) and it is also fully confirmed by
our results. In fact, by employing this technology, a
small cohort of animals was sufficient to provide
meaningfull information using cross-over study
following a Latin Square design. Although the number
of data points obtained from a 24 h recording in this
study is very large, results are manageable by a
standard commercially available HW/SW combination.
Further, the built in QTbtb analysis as well as some
formulae for QTc interval analysis, permits to combine
the classical population-based QT evaluation methods
to the new potent individual-based methods emerging
in the international literature (Malik et al., 2002; Fossa
et al., 2005; Holzgrefe et al., 2006) without sensible
time cost increases.

Effectiveness of different corrections: When safety
studies are planned, it is important, to avoid over- or
under-estimation of the real effect on QT exerted by
NCE. Time course plot of daily trend of RR and QT
interval showed a variation of parameters along the
day. Circadian rate independency of QT interval, as
well as drug-induced QT prolongation, was properly
detected by applying the Regression and Matsunaga
rate-correction equations. On the other hand, the Van de Water equation gave unclear results and did not adequately represent the circadian or drug induced, QT/RR relation changes. Overall the impression was that the Van de Water equation over-corrects at high HR and under-corrects at low HR. Consequently, it is not useful and thus anymore used.

Conclusion

We have described an experimental set-up, combining dog as animal model, telemetric technology, and validated HW/SW System that permits contemporary QTbtt and QT interval rate-correction analytical approaches. This set-up is easily usable, allows the generation of huge databases of ECG complexes and the correct identification of drug-induced QT changes. The results strongly support the use of uncorrected QT at comparable RR intervals, as they are provided by long term recording, to properly generate unambiguous information on ventricular repolarization using QTc as complementary analysis. Moreover, for pre-development safety assessment, it is sufficient to apply, for its immediacy and efficacy, the Matsunaga QTc formula. Whereas it is advisable for a more complete analysis to add QTcreg formula, that requires more time to implement, only for GLP Regulatory studies.

We hope that our experience with this set-up should help colleagues during implementation of own Cardiovascular Safety Pharmacology Labs, and that the described protocol might be taken into consideration as one of the possible standard procedures in assessing the cardiovascular safety of new compounds under development.

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References


Illustrations

Illustration 1

Figure 1 Results of the automatic ECG analysis by the means of the defined library. Markers on the waves are in the upper part of the panel. Values of parameters measured for each cycle are reported at the bottom of the panel.
Illustration 2

Figure 2 Example of a typical plot of the QT/RR relationship obtained from an untreated, free moving dog. The 24 hours QT and RR bins values were divided in two time series: light (green point) and night (dark blue point).
Illustration 3

Figure 3 Time course of the QT and QTc (QTcM= Matsunaga; QTcVW= Van der Water; QTcReg= Regression) during the day. Mean ± sem of the 6 untreated free-moving dogs. Gray box defines the dark period.
Illustration 4

Figure 4 Effect of vehicle and sotalol 3, 10 and 30 mg kg\(^{-1}\) on ECG intervals. RR, PQ and QT were significantly increased by sotalol. Two way ANOVA for repeated measure p<0.001.
Illustration 5

Figure 5 Example of a typical plot obtained from QT/RR relationship fitted with Carmeliet’s equation of a sotalol treated (30 mg kg⁻¹) dog. It is evident the huge increase produced by the drug treatment (red point) of QT interval duration for each RR interval.
Illustration 6

Figure 6 Effect of vehicle and sotalol 3, 10 and 30 mg kg\(^{-1}\) on QT and QTc (see legend of fig. 3) intervals. QT/QTc was dose-dependently increased by sotalol. Two way ANOVA for repeated measure p$<0.0001$. 

![Graph showing effect of vehicle and sotalol on QT and QTc intervals.](image-url)
Illustration 7

Figure 7 Effect of vehicle and astemizole 1, 3 and 10 mg kg\(^{-1}\) on ECG intervals. RR interval was only significantly decreased by astemizole 10 mg kg\(^{-1}\) (p<0.01). Whereas QT interval was significantly prolonged by 3 and 10 mg kg\(^{-1}\) (two way ANOVA for repeated measure p<0.01).
Illustration 8

Figure 8 Effect of vehicle and astemizole 1, 3 and 10 mg kg\(^{-1}\) on QT and QTc (see legend of fig. 3) intervals. Astemizole increased significantly and dose-dependently QT/QTc (two way ANOVA for repeated measure p<0.001).
Illustration 9

Figure 9 ST1326 infusion (up to 6 mg kg-1 24h-1) did non modify significantly QTc interval as compared to Vehicle treated animals. Positive effect of Sotalol (10 mg kg-1) is reported for comparison, (two way ANOVA p<0.001).
Illustration 10

Table 1. Diurnal range of ECG intervals by telemetric recording of free-moving, untreated dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>24 h mean ±SD</th>
<th>Light mean ±SD</th>
<th>Dark mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>HR</td>
<td>78 ± 8.5</td>
<td>88 ± 5.8</td>
<td>74 ± 6.1*</td>
</tr>
<tr>
<td>Normal</td>
<td>RR</td>
<td>833 ± 115</td>
<td>719 ± 55</td>
<td>872 ± 99*#</td>
</tr>
<tr>
<td>Normal</td>
<td>PQ</td>
<td>111 ± 3.4</td>
<td>111 ± 3.5</td>
<td>110 ± 2.9</td>
</tr>
<tr>
<td>Normal</td>
<td>QRS</td>
<td>40.9 ± 0.90</td>
<td>40.5 ± 0.86</td>
<td>40.5 ± 0.82</td>
</tr>
<tr>
<td>Normal</td>
<td>QT</td>
<td>231 ± 0.5</td>
<td>227 ± 5.1</td>
<td>235±6.6*</td>
</tr>
</tbody>
</table>

* p< 0.01 vs light; # p< 0.01 vs 24h (paired student t test)
Illustration 11

Table 2. Daily QT interval duration at 3 different cycle lengths. Data derived from individual Beat-to-Beat analysis in untreated telemetered dogs.

<table>
<thead>
<tr>
<th>Dog</th>
<th>QT_{RR600}</th>
<th>QT_{RR800}</th>
<th>QT_{RR1000}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day ms</td>
<td>Night ms</td>
<td>Day ms</td>
</tr>
<tr>
<td>1099</td>
<td>236</td>
<td>246</td>
<td>251</td>
</tr>
<tr>
<td>1520</td>
<td>251</td>
<td>262</td>
<td>258</td>
</tr>
<tr>
<td>7050</td>
<td>236</td>
<td>249</td>
<td>249</td>
</tr>
<tr>
<td>7258</td>
<td>219</td>
<td>223</td>
<td>225</td>
</tr>
<tr>
<td>Partial Mean</td>
<td>236</td>
<td>245***</td>
<td>246</td>
</tr>
</tbody>
</table>

±SD (n=4) 13 16 14 18 15 18

<table>
<thead>
<tr>
<th>Dog</th>
<th>QT_{RR600}</th>
<th>QT_{RR800}</th>
<th>QT_{RR1000}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day ms</td>
<td>Night ms</td>
<td>Day ms</td>
</tr>
<tr>
<td>1948</td>
<td>217</td>
<td>223</td>
<td>225</td>
</tr>
<tr>
<td>7148</td>
<td>212</td>
<td>217</td>
<td>215</td>
</tr>
<tr>
<td>7468</td>
<td>216</td>
<td>226</td>
<td>223</td>
</tr>
<tr>
<td>Total</td>
<td>Mean</td>
<td>227</td>
<td>235**</td>
</tr>
<tr>
<td></td>
<td>±SD (n=7)</td>
<td>14</td>
<td>17</td>
</tr>
</tbody>
</table>
Paired student t test *=p<0.05; **=0.01, ***0.001 vs day
Illustration 12

Table 3. QT interval duration at 3 different cycle lengths in placebo or sotalol treated healthy dogs (mean ±SD, n=7).

<table>
<thead>
<tr>
<th>RR interval</th>
<th>Placebo Pre (ms)</th>
<th>Placebo Post (ms)</th>
<th>Sotalol 3 mg kg⁻¹ Pre (ms)</th>
<th>Sotalol 3 mg kg⁻¹ Post (ms)</th>
<th>Sotalol 10 mg kg⁻¹ Pre (ms)</th>
<th>Sotalol 10 mg kg⁻¹ Post (ms)</th>
<th>Sotalol 30 mg kg⁻¹ Pre (ms)</th>
<th>Sotalol 30 mg kg⁻¹ Post (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>224±5.5</td>
<td>223±5.2</td>
<td>224±3.9</td>
<td>233±3.6*</td>
<td>224±5.3</td>
<td>237±7.7*</td>
<td>225±6.8</td>
<td>249±4.0*</td>
</tr>
<tr>
<td>800</td>
<td>232±6.8</td>
<td>230±6.3</td>
<td>231±4.4</td>
<td>240±5.4*</td>
<td>233±6.8</td>
<td>248±9.4*</td>
<td>232±6.2</td>
<td>259±8.5*</td>
</tr>
<tr>
<td>1000</td>
<td>234±8.0</td>
<td>233±7.4</td>
<td>235±5.4</td>
<td>244±6.3*</td>
<td>236±8.4</td>
<td>253±10.7*</td>
<td>236±6.6</td>
<td>264±9.2*</td>
</tr>
</tbody>
</table>

Statistical analysis (Dunnett’s multiple comparison test) of effects on QT/RR interval relationship. Within treatments (p<0.01 pre vs postdose,* and across treatment (p<0.0001 sotalol vs placebo,°)
Illustration 13

Table 4. QT interval duration at 3 different cycle lengths in placebo or astemizole treated healthy dogs (mean &plusmn;SD, n=7).

<table>
<thead>
<tr>
<th>RR interval</th>
<th>Placebo Pre ms</th>
<th>Placebo Post ms</th>
<th>Atemizole 1 mg kg⁻¹ Pre ms</th>
<th>Atemizole 1 mg kg⁻¹ Post ms</th>
<th>Atemizole 3 mg kg⁻¹ Pre ms</th>
<th>Atemizole 3 mg kg⁻¹ Post ms</th>
<th>Atemizole 10 mg kg⁻¹ Pre ms</th>
<th>Atemizole 10 mg kg⁻¹ Post ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>224±5.5</td>
<td>223±5.2</td>
<td>227±6.1</td>
<td>229±5.9</td>
<td>229±7.7</td>
<td>234±3.9°</td>
<td>226±5.1</td>
<td>239±7.9°</td>
</tr>
<tr>
<td>800</td>
<td>232±6.8</td>
<td>230±6.3</td>
<td>236±4.8</td>
<td>236±5.0</td>
<td>237±6.7</td>
<td>242±2.9°</td>
<td>233±4.6</td>
<td>243±5.5°</td>
</tr>
<tr>
<td>1000</td>
<td>234±8.0</td>
<td>233±7.4</td>
<td>239±4.2</td>
<td>239±4.3</td>
<td>239±6.4</td>
<td>244±3.5°</td>
<td>236±4.6</td>
<td>244±5.0°</td>
</tr>
</tbody>
</table>

Statistical analysis (Dunnett’s multiple comparison test) of effects on QT/RR interval relationship. Within treatments (p<0.01 pre vs postdose,*) and across treatment (p<0.0001 astemizole vs placebo,°)
Illustration 14

Table 5. Daily QT interval duration at 3 different cycle lengths. Data derived from individual Beat-to-Beat analysis in untreated telemetered dogs.

<table>
<thead>
<tr>
<th>Dog</th>
<th>QT&lt;sub&gt;RR600&lt;/sub&gt;</th>
<th>QT&lt;sub&gt;RR800&lt;/sub&gt;</th>
<th>QT&lt;sub&gt;RR1000&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day ms</td>
<td>Night ms</td>
<td>Day ms</td>
</tr>
<tr>
<td>Group 1</td>
<td>1099</td>
<td>235</td>
<td>246</td>
</tr>
<tr>
<td></td>
<td>1520</td>
<td>253</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>7050</td>
<td>236</td>
<td>249</td>
</tr>
<tr>
<td></td>
<td>7258</td>
<td>221</td>
<td>226</td>
</tr>
<tr>
<td>Partial Mean</td>
<td>236</td>
<td>245*</td>
<td>246</td>
</tr>
<tr>
<td>±SD</td>
<td>13.1</td>
<td>14.2</td>
<td>14.6</td>
</tr>
<tr>
<td>Group 2</td>
<td>1066</td>
<td>237</td>
<td>251</td>
</tr>
<tr>
<td></td>
<td>0157</td>
<td>234</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>1520</td>
<td>241</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>1845</td>
<td>220</td>
<td>238</td>
</tr>
<tr>
<td>Partial Mean</td>
<td>233</td>
<td>244*</td>
<td>239</td>
</tr>
<tr>
<td>±SD</td>
<td>9.1</td>
<td>7.8</td>
<td>9.2</td>
</tr>
<tr>
<td>Total Mean</td>
<td>235</td>
<td>245*</td>
<td>243</td>
</tr>
<tr>
<td>±SD</td>
<td>10.6</td>
<td>10.6</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Paired student t test *=p<0.01 vs day.
Illustration 15

Table 6. QT interval duration at 3 different cycle lengths in placebo (n=8) or sotalol treated dogs (mean ±SD, n=4).

<table>
<thead>
<tr>
<th>RR interval</th>
<th>Placebo</th>
<th>Sotalol 3 mg kg⁻¹</th>
<th>Sotalol 10 mg kg⁻¹</th>
<th>Sotalol 30 mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>600 ms</td>
<td>239±7.3</td>
<td>246±6.8</td>
<td>240±8.7</td>
<td>246±12</td>
</tr>
<tr>
<td>800 ms</td>
<td>247±9.0</td>
<td>253±6.2</td>
<td>251±10</td>
<td>253±13</td>
</tr>
<tr>
<td>1000 ms</td>
<td>250±9.5</td>
<td>256±5.9</td>
<td>255±9.9</td>
<td>256±13</td>
</tr>
</tbody>
</table>

Statistical analysis (Dunnett’s multiple comparison test) of effects on QT/RR interval relationship. Within treatments (p<0.01 pre vs postdose,*) and across treatment (p<0.0001 sotalol vs placebo,°)
Illustration 16

Table 7. QT interval duration at 3 different cycle lengths in placebo (mean ±SD, n=8) or ST1326 treated dogs (n=4).

<table>
<thead>
<tr>
<th>RR interval</th>
<th>Placebo</th>
<th>ST1326 1 mg kg⁻¹ 24 h⁻¹</th>
<th>ST1326 3 mg kg⁻¹ 24 h⁻¹</th>
<th>ST1326 6 mg kg⁻¹ 24 h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>600</td>
<td>239±7.3</td>
<td>246±6.8</td>
<td>233±7.5</td>
<td>238±7.4</td>
</tr>
<tr>
<td>800</td>
<td>247±9.0</td>
<td>253±6.2</td>
<td>242±8.5</td>
<td>247±7.9</td>
</tr>
<tr>
<td>1000</td>
<td>250±9.5</td>
<td>256±5.9</td>
<td>245±9.2</td>
<td>249±8.54</td>
</tr>
</tbody>
</table>
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