

QT and RR Intervals in Conscious and Anesthetized Guinea Pigs with Highly Varying RR Intervals and Given QTc-Lengthening Test Articles

Robert L. Hamlin,^{*1} Anusak Kijawornrat,^{*} Bruce W. Keene,^{†‡} and David M. Hamlin[†]

^{*}Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio 43210; [†]QTest Labs, Inc. 6456 Fiesta Drive, Columbus, Ohio 43235; and [‡]Department of Veterinary Clinical Sciences, North Carolina State University, Raleigh, North Carolina 27606

Received on July 17, 2003; accepted on September 16, 2003

A facile system for obtaining electrocardiograms from conscious animals was used to conduct studies on 12 animals studied both conscious and anesthetized, on 4 conscious animals given vehicle (0.5% methylcellulose) and QT-lengthening test articles, and on 6 animals given test articles thought to not lengthen QTc. In 12 animals whose ECGs were monitored via a bipolar transthoracic ECG, heart rates were slowed with 1.0 mg/kg zatebradine, while they were conscious in their slings, and after being anesthetized with ketamine/xylazine. The following regression equations were obtained relating QT to RR: $QT = 44.7 \ln RR - 132.9$, $r^2 = 0.7$, for conscious animals; $QT = 79.4 \ln RR - 287.4$, $r^2 = 0.8$ for anesthetized animals, with RR intervals varying between 150 and 550 ms. The anesthetic increases QT at all RR intervals ($p < 0.001$), but does not change the slope of the relationship between QT and RR when compared with the conscious guinea pig. The Fridericia method was best for correcting QT for RR interval in conscious guinea pigs, but the Bazett method was best for correcting in anesthetized animals. QTc lengthened significantly in all conscious guinea pigs given, orally, cisapride, ketoconazole, and sotalol (positive test articles) and did not change with methylcellulose (the vehicle) or with propranolol, verapamil, or enalapril (negative controls). These techniques and relationships demonstrate that this methodology may be useful in exploring torsadogenic effects of novel pharmacological entities.

Key Words: guinea pig; QT; RR; QTc; anesthesia; electrophysiology.

It is essential to evaluate the potential of a novel pharmacological agent for altering electrophysiology of the heart (Committee for Proprietary Medicinal Products, 1997; International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2002). This includes all electrophysiological properties: chronotropy (measured by heart rate), dromotropy (measured by P, PQ, and QRS durations), QTc, and irritability (spontaneous or provoked ectopia).

¹ To whom correspondence should be address at QTest Labs, Inc., 6456 Fiesta Drive, Columbus, OH 43235. Fax: (614) 760-0900. E-mail: rhamlin@qtestlabs.com.

Guinea pigs are excellent models for investigating electrophysiology because they are inexpensive, they possess specific ion channels (with the exception of I_{To}) similar to man (Bünger *et al.*, 1975; Busch *et al.*, 1994; Carmeliet and Zarman, 1979; Khalifa *et al.*, 1999; Roden *et al.*, 1988), they are tractable and clean, they require only small amounts of test article, they are excellent surrogates for predicting the potential of a test article to retard ventricular repolarization in man, and they develop torsade de pointes in response to pharmacological interventions (accepted by *J. Pharmacol. Toxicol. Methods.*). Because anesthetics are known to alter—either increase or decrease—the sensitivity of a preparation to test articles (Shimizu *et al.*, 1999; Sun *et al.*, 1997), it is highly desirable to study electrophysiology without need for chemical restraint, and furthermore, many forms of physical restraint may inflict discomfort on the animal and produce a sympathetic “storm” which might obfuscate electrophysiological effects of a test article. QT may be corrected for RR interval by a number of methods. Bazett (1920), Fridericia (1920), and Van de Water *et al.* (1989) are three used commonly. There is inadequate information for the guinea pig to determine which method corrects best for RR interval, and whether or not the best method for the conscious guinea pig would also be the best for the anesthetized animal.

For these reasons, this paper reports a methodology of obtaining electrocardiograms in conscious guinea pigs that requires neither manual restraint nor anesthesia. Because the sum (QT) of the durations of ventricular depolarization (QRS) and repolarization (ST - T) is an important electrocardiographic parameter in predicting a liability of a test article for producing the polymorphic tachycardia, torsade de pointes, values of RR interval and QT obtained from healthy, conscious, but quiet guinea pigs are presented. Because QT is known to lengthen as RR interval lengthens (i.e., as heart rate slows), the mathematical relationship between QT and RR for conscious and anesthetized guinea pigs is presented. Three common methods of correcting QT for RR interval are used to determine which is best for the conscious and which is best for the anesthetized.

Many cardiovascular studies have been performed on conscious guinea pigs (Akita *et al.*, 2002; Gras *et al.*, 1996; Hey *et al.*

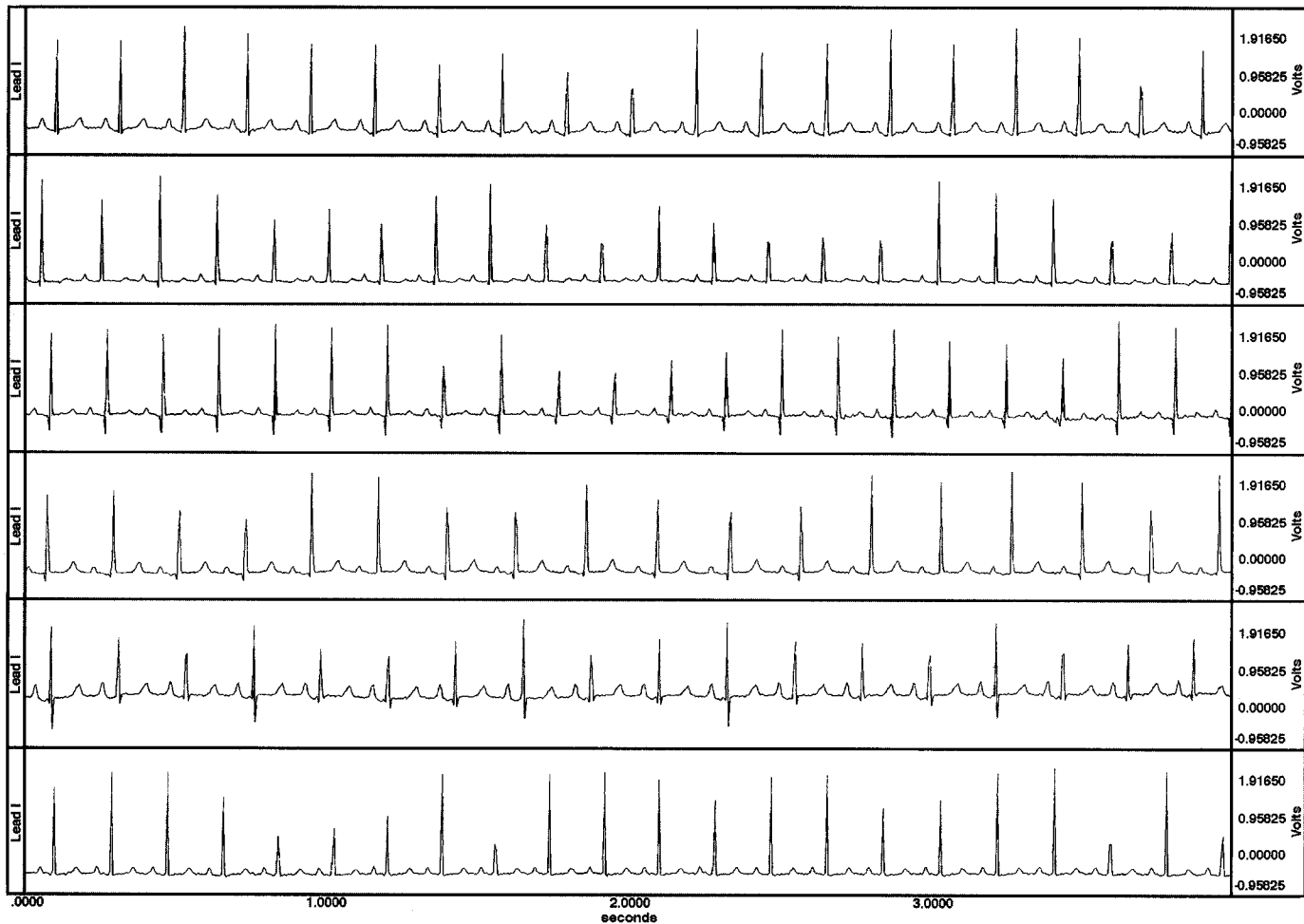


FIG. 1. Bipolar, transthoracic ECGs from 6 of the 12 conscious guinea pigs. Notice the absence of baseline artifacts, the ease of identification of the onset of QRS and the end of T, and the fluctuations in height of R waves as the guinea pigs breath.

al., 1996); however this study presents a new noninvasive technique to obtain ECGs. In addition, this study is the first to compare different QTc correction factors between conscious and anesthetized guinea pigs.

MATERIALS AND METHODS

This study was approved by the ILACUC of QTest Labs, Inc. A total of 22 guinea pigs were used. All weighed between 350 and 450 grams; half were males and half were females. Six males and six females were used to compare conscious to anesthetized guinea pigs. Ten additional guinea pigs received test articles: four were given test articles known to prolong QTc, and six were given test article known to not change QTc. First they were placed, without chemical interventions, into a comfortable, padded sling, in ventral recumbency. The sling is fitted with copper plates, which sandwich the cranial aspect of the thorax so a bipolar transthoracic electrocardiogram between points rV2 and V2 can be obtained. The electrodes have a hole in the middle so that electrode paste can be injected, without disturbing the guinea pig, through the electrodes and minimize impedance between the electrode and skin. The right and left arm electrodes are attached to the right and left hemithoraces, the electrocardiogram is switched to limb lead I, and a bipolar transthoracic ECG

is obtained on a Biopac MP100 Data Acquisition Unit (Biopac Systems, Inc., Santa Barbara, CA). The high pass filter was set at 0.01 Hz, and the low pass filter at 1 kHz, and signals were sampled at 2 kHz. Tracings were obtained for 15 to 60 seconds while the guinea pigs were conscious and quiet. Next, guinea pigs were given, ip, 1.0 mg/kg of zatebradine (Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT), a funny channel blocker, which retards heart rate with no direct effect on any other electrophysiological parameter (Goethals *et al.*, 1993; Hamlin *et al.*, 2003), and tracings were obtained at numerous RR intervals generated in response to zatebradine. This was used to generate curves of QT versus RR interval, since RR interval could be lengthened by zatebradine. RR and QT durations were measured from the ECG at all heart rates, and QT was corrected for RR interval by dividing QT by the cube root of the RR interval. Plots were made of QT versus RR interval, and regression lines with r^2 and p values were calculated using Sigma Plot (SPSS Inc., Chicago, IL). QT was corrected for RR interval by dividing QT by the square root (Bazett, 1920) and cube root (Fridericia, 1920) of RR, and by subtracting from QT, $0.087(RR - 1000)$ (Van de Water *et al.*, 1989). Plots were made to determine which method(s) for the awake and for the anesthetized guinea pigs produced the least dependence of QT on RR. One week later, the entire sequence was repeated, but after the guinea pigs had been anesthetized, ip, with ketamine (33 mg/kg)/xylazine (3.3 mg/kg).

In separate study, four guinea pigs, while conscious and in a sling, were

given, orally, 150 mg/kg cisapride, 100 mg/kg ketoconazole, and 100 mg/kg sotalol, in 0.5 ml of 0.5% methylcellulose (the vehicle). Six additional guinea pigs (negative controls) were given, orally, propranolol (10 mg/kg), verapamil (2.5 mg/kg), and enalapril (5 mg/kg) in 0.5 ml of methylcellulose. All doses were selected using information from the literature (Cushman *et al.*, 1989; Gras *et al.*, 1996; Hey *et al.*, 1996; Kii *et al.*, 2001; Levy, 1976; Meuldermans *et al.*, 1998; Saitoh *et al.*, 2002; Yaoita *et al.*, 2002). There was no randomization of exposure to test articles, because at least 3 days (> 8 half lives) occurred between dosing. Bipolar transthoracic ECGs were obtained before dosing, and every 30 minutes postdosing for 2 hours. All test articles are known to achieve T_{max} before 2 hours. Recordings were made for between 15 and 30 seconds, which included >70 beats. Measurements were made of at least 10 consecutive cardiac cycles, and the average was used. Plots of mean values versus time were made, differences between each test article and vehicle were calculated, and differences of significance between each test article and vehicle were sought by a one-way, ANOVA with repeated measures design on time. When indicated by a significant *F* statistic, specific means were compared by the Tukey post-hoc test.

RESULTS

Most guinea pigs fell asleep or were extremely quiet within 1 minute after being placed in the sling; they awakened briefly when the zatebradine was given, but then they either fell asleep or remained quiet until being removed from the sling. Electrocardiograms of excellent quality for interpretation were obtained from all conscious (Fig. 1) and anesthetized guinea pigs. Plots of QT duration versus RR duration for conscious and for anesthetized guinea pigs are shown (Fig. 2). The curvilinear relationships are expressed by the equations; both r^2 and *p* values are shown. The r^2 demonstrates that more than 70% of the variability of QT for the awake guinea pigs and more than 80% of r^2 for the anesthetized guinea pigs are determined by RR interval, and that the relationships are highly significant ($p < 0.001$) for both. It can be observed that the anesthetic displaces the curve upward (i.e., longer QTs for anesthetized

than for conscious) compared to the curve from the naturally sleeping or quiet guinea pigs, but that the slope does not change significantly. Plots (Figs 3A and 3B) of QTc, using all three methods of correction for RR interval, were made against RR. Slope for the equations regressing QTc with RR intervals was smallest for the Fridericia ($r^2 = 0.029$) method for the awake guinea pigs, but was smallest for the Bazett method ($r^2 = 0.058$) for the anesthetized guinea pigs.

QTc(F) lengthened in all guinea pigs given any of the test articles known to lengthen QTc and failed to lengthen when given methylcellulose or test articles thought to not lengthen QTc. Figure 4 shows the mean values and SEM of QTc, both before and after dosing, and Figure 5 shows the differences between QTc for each test article and methylcellulose. The levels of significance in the difference are labeled by asterisks. None of the guinea pigs developed arrhythmias coincident with QT lengthening.

DISCUSSION

The purposes of this study, to demonstrate that ECGs of high quality could be obtained, to compare QT intervals from conscious and anesthetized guinea pigs, and to establish a correction for QT based upon RR interval, were all achieved. Furthermore, it was demonstrated that QTc lengthening occurred in response to all three test articles thought to lengthen QTc, and failed to lengthen in response to all three test articles thought to not lengthen QTc. This study demonstrates that a single bipolar, transthoracic ECG, from which RR and QT may be measured easily, can be obtained from guinea pigs placed in a comfortable sling, that a relationship between QT and RR can be modeled by a logarithmic relationship, and that anesthesia

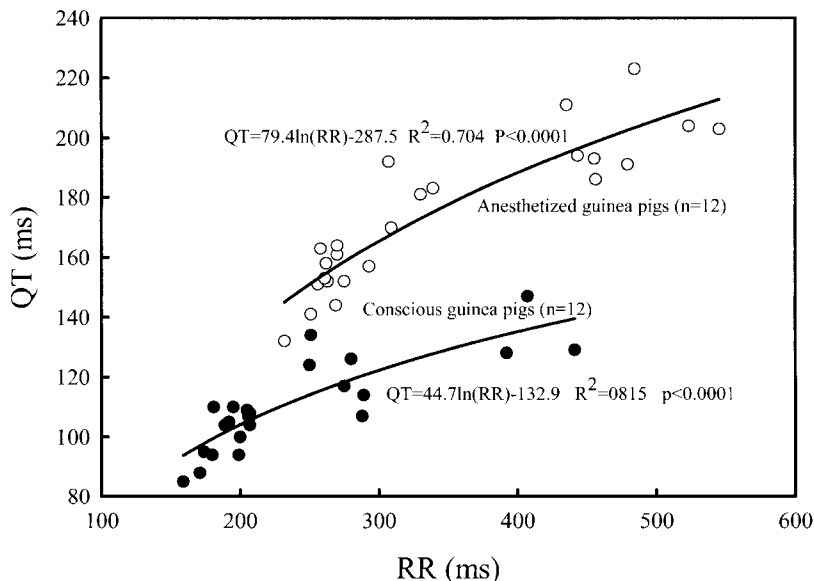


FIG. 2. Plots of QT versus RR interval for all guinea pigs, both conscious (closed circle) and anesthetized (opened circle). Notice that the RR intervals varied from approximately 150 ms (a heart rate of 400/minute) to 550 ms (a heart rate of 109/minute). Each data point is an average of 10 consecutive cardiac cycles from a different guinea pig.

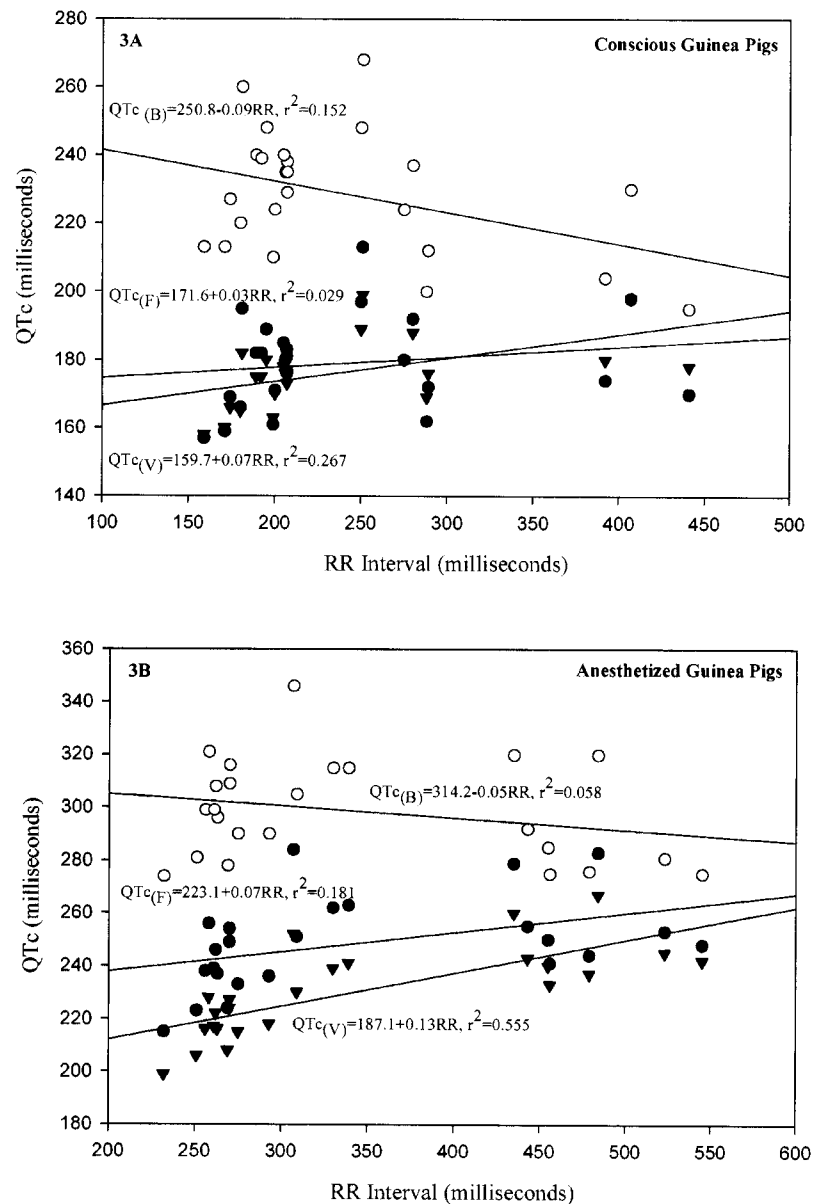


FIG. 3. Plots of QTc corrected by three methods versus RR interval for all guinea pigs, either (A) conscious or (B) anesthetized. Notice that the Bazett (opened circle) correction is best for the anesthetized guinea pigs, but that the Fridericia (closed circle) correction is best for the conscious guinea pigs, while the Van de Water formula (closed triangle) failed to remove the influence of heart rate in both conscious and anesthetized guinea pigs. Each data point is an average of 10 consecutive cardiac cycles from a different guinea pig.

with ketamine/xylazine displaces upward the curvilinear relationship.

The study reports that a single lead is adequate for measuring intervals important to identify a liability of a test article for lengthening ventricular repolarization. If the guinea pig heart may be modeled as an equivalent dipole as for the dog and man (Hamlin *et al.*, 1968), three mutually orthogonal leads should provide over 85% of the electrocardiographic information available from a greater number of leads, but a single lead—as used in this study—provides the dominant portion of dipolar information. There is no evidence, from toxicological studies or from studies in safety pharmacology, that test articles force the model to become more than dipolar; therefore information from the transthoracic bipolar ECG reported here is adequate

for identifying the most common manifestations of toxicity (e.g., changes in heart rate, production of arrhythmia, lengthening of QT). Also, a single bipolar transthoracic lead with electrodes close to the heart produces high voltages and is less likely to produce ECGs that are not obfuscated by 60 Hz or muscle tremor artifacts.

This study shows that the method of Fridericia for correcting QT for RR interval of conscious guinea pigs is the best, but that the method of Bazett is best for the anesthetized guinea pigs, consistent with the observations of Hayes *et al.* (1994) and De Clerck *et al.* (2002). The “best” relationship is defined as the one with a slope for the regression equation closest to 0. Of course there are many other methods for correcting, but with more than 70% of the variability of QT depending upon RR,

QTcs calculated in this study are useful for identifying a QTc liability independent of heart rate.

Ketamine/xylazine can be used as a “universal” anesthetic (Flecknell, 1996), since all animals can be safely and effectively anesthetized with it. The slope of the relationship between QT and RR is not changed by the anesthetic, but the curve is displaced upward. With a greater number of guinea pigs, it is possible that the slopes between conscious and anesthetized may differ. There is no data available to demonstrate if either the displacement or slope would hold true for other anesthetic regimen. Nonetheless, the fact that ketamine/xylazine produced a significant displacement of the QT versus RR relationship establishes the possibility that other anesthetics may affect the displacement or the slope even more than ketamine/xylazine. In addition, anesthetics may alter renal and hepatic blood flows and rates of metabolism and/or excretion. Finally, all anesthetics—even morphine/chloralose—affect autonomic tone, which may alter electrophysiological effects of test articles. Thus if at all possible, conducting studies on conscious subjects should be preferred.

This study also showed that this model is sensitive for detecting lengthening of QTc for three compounds known to lengthen QTc, while showing no lengthening due to vehicle or to other test articles known not to lengthen QTc. Blood levels of the test articles were not measured, but the doses of test articles given were close to those given orally to other experimental animals (guinea pig and rat) (Cushman *et al.*, 1989; Gras *et al.*, 1996; Hey *et al.*, 1996; Kii *et al.*, 2001; Levy, 1976;

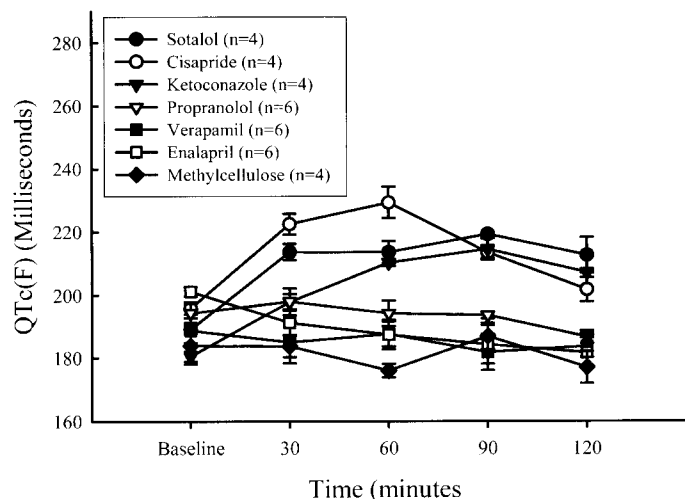


FIG. 4. Plots of means and SEM of QTc(F) versus time after dosing of three test articles known to lengthen QTc(F), three test articles known not to lengthen QTc(F), and for the methylcellulose vehicle. Each data point is the average of 10 consecutive cardiac cycles. Notice the lengthening of QTc(F) for the three test article but not for the vehicle. Each mean is the mean of four guinea pigs receiving positive test articles, six guinea pigs receiving negative test articles, and four guinea pigs receiving vehicle (methylcellulose). Doses of test articles were in mg/kg: sotalol (100), cisapride (150), ketoconazole (100), propranolol (10), verapamil (2.5), enalapril (5), methylcellulose (0.5%).

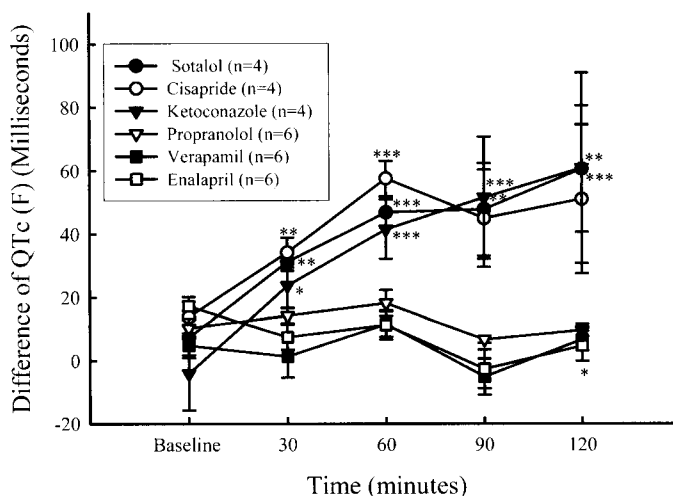


FIG. 5. Plots of means and SEM of the difference between QTc (in ms) for each test article and the vehicle. Each data point is the average of 10 consecutive cardiac cycles. An asterisk indicates when a difference changed with statistical significance from baseline. One asterisk indicates a $p < 0.05$, two asterisks indicate a $p < 0.01$, and three asterisks indicate a $p < 0.001$. Each mean is the mean of four guinea pigs receiving positive test articles, and six guinea pigs receiving negative test articles. Doses of test articles were in mg/kg: sotalol (100), cisapride (150), ketoconazole (100), propranolol (10), verapamil (2.5), enalapril (5).

Meuldermans *et al.*, 1998; Saitoh *et al.*, 2002; Yaoita *et al.*, 2002).

The greatest features of this preparation are the speed, ease, and lack of psychological stimulation with which ECGs may be obtained in extremely quiet or naturally sleeping guinea pigs, a species whose heart possesses great similarities to man in terms of specific ion channels (except for I_{T0}). This study adds credibility to the use of guinea pigs in toxicology and safety pharmacology and provides data against which future studies may be compared; in particular the study showed that QTc prolongation can be identified in conscious guinea pigs dosed orally. Because the guinea pigs became extremely quiet or asleep within 60 seconds of placing them in the sling, this method permits following the time-course of changes in QTc without need for chronic instrumentation. Because of how quickly the guinea pigs fall asleep once in the sling, it would be possible to measure drug effects every 1 minute, without those effects being obfuscated by psychological influences. This would be nearly impossible in many other species, which either literally never “settle down” or require a prolonged period to obfuscate psychological factors.

ACKNOWLEDGMENTS

COI: This study was conducted in a company, QTest Labs, for which the senior author is a paid consultant, and the last author is a partial owner.

REFERENCES

- Akita, M., Ishii, K., Kuwahara, M., and Tsubone, H. (2002). Power spectral analysis of heart rate variability for assessment of diurnal variation of autonomic nervous activity in guinea pigs. *Exp. Anim.* **51**, 1–7.
- Bazett, H. C. (1920). An analysis of the time-relations of electrocardiograms. *Heart* **7**, 353–370.
- Bünger, R., Haddy, F., Queregässer, A., and Gerlach, E. (1975). An isolated guinea pig heart preparation with *in vivo* like features. *Pflugers Arch.* **353**, 317–326.
- Busch, A. E., Malloy, K., Groh, W. J., Varnum, M. D., Adelman, J. P., and Maylie, J. (1994). The novel class III antiarrhythmics NE-10064 and NE-10133 inhibit I_{Ks} channels expressed in *Xenopus* oocytes and I_{Ks} in guinea pigs cardiac myocytes. *Biochem. Biophys. Res. Commun.* **202**, 265–270.
- Carmeliet, E., and Zarman, M. Y. (1979). Comparative effects of lignocaine and lorcaïnide on conduction in the Langendorff perfused guinea pig heart. *Cardiovasc. Res.* **13**, 439–449.
- Committee for Proprietary Medicinal Products. (1997). Points to Consider. The assessments of the potential for QT interval prolongation by non-cardiovascular medicinal products. The European agency for the evaluation of medicinal products.
- Cushman, D. W., Wang, F. L., Fung, W. C., Grover, G. J., Harvey, C. M., Scalese, R. J., Mitch, S. L., and DeForrest, J. M. (1989). Comparisons *in vitro*, *ex vivo*, and *in vivo* of the actions of several structurally diverse inhibitors of angiotensin converting enzyme (ACE). *Br. J. Clin. Pharmacol.* **28**, 115s–131s.
- De Clerck, F., Van de Water, A., D'Aubioul, J., Lu, H. R., van Rossem, K., Hermans, A., and Van Ammel, K. (2002). *In vivo* measurement of QT prolongation, dispersion and arrhythmogenesis: Application to the preclinical cardiovascular safety pharmacology of a new chemical entity. *Fundam. Clin. Pharmacol.* **16**, 125–140.
- Flecknell, P. A. (1996). Anesthesia of common laboratory species. In: *Laboratory Animal Anaesthesia*. 2nd ed., pp.178–182. Academic Press, London.
- Fridericia, L. S. (1920). Die systolendauer in elektrokardiogramm bei normalen menschen und bei herzfranken. *Acta. Med. Scand.* **53**, 469–486.
- Goethals, M., Raes, A., Van Bogaret, P. P. (1993). Use-dependent block of the pacemaker current I_f in rabbit sinoatrial node cells by zatebradine (US-FS 49): On the mode of action of sinus node inhibitors. *Circulation* **88**, 2389–2401.
- Gras, J., Llenas, J., Palacios, J. M., and Roberts, D. J. (1996). The role of ketoconazole in the QTc interval prolonging effects of H_1 -antihistamines in a guinea-pig model of arrhythmogenicity. *Brit. J. Pharmacol.* **119**, 187–188.
- Hamlin, R. L., Nakayama, T., Nakayama, H., Carnes, C. A. (2003). Effects of changing heart rate on electrophysiological and hemodynamic function in the dog. *Life Sci.* **72**, 1919–1930.
- Hamlin, R. L., Pipers, F. S., Smith, C. R. (1968). Computer methods for analysis of dipolar characteristics of the electrocardiogram. *Am. J. Vet. Res.* **29**, 1867–1881.
- Hayes, E., Pugsley, M. K., Penz, W. P., Adaikan, G., and Walker, M. J. (1994). Relationship between QT and RR intervals in rats, guinea pigs, rabbits, and primates. *J. Pharmacol. Toxicol. Methods* **32**, 201–207.
- Hey, J. A., del Prado, M., Sherwood, J., Kreutner, W., and Egan, R. W. (1996). Comparative analysis of the cardiotoxicity proclivities of second generation antihistamines in an experimental model predictive of adverse clinical ECG effects. *Drug Res.* **46**, 153–158.
- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. (2002). Draft Concensus Step 2 Guidelines. Safety pharmacology for assessing the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals.
- Khalifa, M., Drolet, B., Daleau, P., Lefez, C., Gilbert, M., Plante, S., O'Hara, G. E., Gleeton, O., Hamelin, B. A., and Turgeon, J. (1999). Block of potassium currents in guinea pig ventricular myocytes and lengthening of cardiac repolarization in man by the histamine H_1 receptor antagonist diphenhydramine. *J. Pharmacol. Exp. Ther.* **288**, 858–865.
- Kii, Y., Nakatsuji, K., Nose, I., Yabuuchi, M., Yasuyuki, Y., and Ito, T. (2001). Effects of 5-HT₄ receptor agonists, cisapride and mosapride citrate on electrocardiogram in anaesthetized rats and guinea pigs and conscious cats. *Pharmacol. Toxicol.* **89**, 96–103.
- Levy, J. V. (1976). Beta-adrenergic receptor blocking drugs in spontaneous hypertension. *Am. J. Med.* **61**, 779–789.
- Meuldermans, W., Hendrickx, J., Lauwers, W., Hurkmans, R., Mostmans, E., Swysen, E., Bracke, J., Knaeps, A., and Heykants, J. (1998). Excretion and biotransformation of cisapride in rats after oral administration. *Drug Metab. Dispos.* **16**, 410–419.
- Roden, D. M., Bennett, P. B., Snyders, D. J., Balser, J. R., and Hondeghem, L. M. (1988). Quinidine delays I_K activation in guinea pigs ventricular myocytes. *Circ. Res.* **62**, 1055–1058.
- Saitoh, M., Sugiyama, A., Nakazawa, T., and Hashimoto, K. (2002). Cardiovascular effects of orally administered HNS-32, an originally synthesized anulene-1-carboxamide derivative, assessed in the *in vivo* rat model. *Jpn. J. Pharmacol.* **89**, 316–319.
- Shimizu, W., McMahon, B., and Antzelevitch, C. (1999). Sodium pentobarbital reduces transmural dispersion of repolarization and prevents torsade de pointes in models of acquired and congenital long QT syndromes. *J. Cardiovasc. Electrophysiol.* **10**, 156–164.
- Sun, Z. Q., Eddlestone, G. T., and Antzelevitch, C. (1997). Ionic mechanisms underlying the effects of sodium pentobarbital to diminish transmural dispersion of repolarization. [abstr.] *PACE* **20**, 11–1116.
- Van de Water, A., Verheyen, J., Xhonneux, R., and Reneman, R. S. (1989). An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. *J. Pharmacol. Methods* **22**, 207–217.
- Yaoita, H., Sakabe, A., Maehara, K., and Maruyama, Y. (2002). Different effects of carvedilol, metoprolol, and propranolol on left ventricular remodeling after coronary stenosis or after permanent coronary occlusion in rats. *Circulation.* **105**, 975–980.