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Original article

Aging-related changes of QT and RR intervals in conscious guinea pigs

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Abstract

Introduction: Although guinea pigs are suitable for *in vivo* QT assessment of newly discovered drugs at the pre-clinical stage because of the similarity of the ion channels between the guinea pig heart and the human, there is limited data available regarding the characteristics of QT interval in conscious guinea pigs. Aging is one of several factors which have been shown to affect the QT interval in humans and animals. In the present study, we examined the influence of age on QT and RR intervals in conscious guinea pigs. **Methods:** Electrocardiograms were recorded from female Hartley guinea pigs at the age of 6 weeks (young; $n=6$) and 23 months (old; $n=4$) *via* a telemetry system. The QT and RR intervals were measured during daytime and nighttime, and following intravenous bolus injection of E-4031 (0.1 mg/kg) or terfenadine (4 mg/kg). Comparisons were made to determine group differences in: (1) the normal values of the QT and RR intervals, (2) the best-fit QT-correction formula, (3) the circadian rhythm of QT and RR intervals, and (4) drug effects on repolarization. **Results:** The normal values of QT and RR intervals in the old group were significantly longer than those in the young group. The best-fit formula for correcting QT interval was a modified Bazett's formula for both young and old groups. The old group did not show the nocturnal variation of either QT or RR interval. Terfenadine caused significantly greater QTc prolongation in the old group compared to the young. **Discussion:** Aging affects resting QT and RR intervals in conscious female guinea pigs, a factor which should be considered when examining the effects of compounds on cardiac repolarization. Also, the present study suggests a possibility that age can affect QTc prolongation induced by some IKr blockers.

Keywords: ECG; Guinea pig; Aging; QT interval; RR interval; Circadian rhythm; QT-correction formula; QTc prolongation; Telemetry system

1. Introduction

Drug-induced QT interval prolongation has been recognized as a critical issue in drug development for several decades; thus QT assessment in the early stages of drug development is considered by some as indispensable for pre-clinical drug evaluation (De Clerck et al., 2002; Picard & Lacroix, 2003). Guinea pigs have constituted an attractive *in vivo* model to evaluate the effects of newly discovered drugs on the QT interval because of their relatively small size, an ionic current profile during the ventricular action potential similar to that of humans (except for the I_{to} channel), as well as the availability of considerable *in vitro* data on this animal species. We have previously provided detailed methodological information on using conscious free-moving guinea pigs for *in vivo* QT evaluation monitored with an indwelling telemetry electrocardiogram (ECG) system, and validated the feasibility of the telemetry guinea pig model using reference compounds (Shiotani et al., 2005; Shiotani, Harada, Abe, Hamada, & Horii, 2007). However, there seems to be limited data available regarding the characteristics of QT interval in guinea pigs, particularly, the relationships between QT interval and age, circadian rhythm, sex, or autonomic nervous system, which are valuable for researchers who evaluate drug-induced QT prolongation in guinea pigs.

In humans, the QT interval is known to be affected by aging: the normal value of QT interval increases with increasing age (Mangoni, Kinirons, Swift, & Jackson, 2003; Reardon & Malik, 1996; Taneja, Mahnert, Passman, Goldberger, & Kadish, 2001), and circadian modulation of QT interval rate dependence decreases with increasing age (Extramiana et al., 1999). In mice, the appropriate QT-correction formula is different between young and old animals (Chaves et al., 2003). In addition,

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corrected QT intervals (QTc) in humans and dogs have been reported to prolong more apparently in old than in young with QT-prolonging drugs (Kasanuki et al., 1992; Obreztchikova et al., 2003; Yamaguchi & Ito, 1988). On the contrary, to our knowledge, there is no detailed *in vivo* study which has investigated the influences of age on QT interval in conscious guinea pigs. Mittelstadt, Adams, and Spruell (2006) just recently highlighted the effects of age on QT interval in isolated guinea pig hearts, revealing that there seemed to be no difference in the sensitivity of drug-induced QTc prolongation by the IKr blocker, cisapride, between adult (16–17 weeks old) and young hearts (3–4 weeks old). However, a question still remains: Are there integrated effects of aging on resting ECG parameters and/or drug-induced QTc prolongation in conscious guinea pigs?

Therefore, in the present study, we examined the effects of age on QT and RR intervals in conscious guinea pigs. We compared the following parameters between young animals (approximately 6 weeks old) and old animals (approximately 23 months old): the normal values of QT and RR intervals, the best-fitting QT-correction formula, the circadian rhythm (light–dark cycle) of QT and RR intervals, and drug-induced QTc prolongation.

2. Methods

2.1. Animals

Ten female guinea pigs (Crj:Hartley) at the age of 6 weeks (400–500 g, $n=6$) and 23 months (850–1000 g, $n=4$) were purchased from Charles River Japan Inc. (Kanagawa, Japan) to represent young and old animals, respectively (Jones, Lancaster, & Boyett, 2004); the number of the 23-month old animal was limited due to unavailability of the aged animals. In the present study, we used female animals because female-elderly patients were reported to have predominance of showing drug-induced QTc prolongation/torsades de pointes (Manouvrier et al., 1986), and also, the availability of female-aged animals was another factor in selection. The 6-week animals, considered to be sexually mature (Larsen & Regal, 2002; Suzuki & Japanese Society for Laboratory Animal Resources, 1988), were regarded as the young group, and the 23-month animals were regarded as the old group. Animals were maintained on a 12-hour light/dark cycle (lights on at 7:00 and lights off at 19:00) and kept in individual cages floored with wood shavings. The temperature of the animal room was regulated at 23±2 °C, and the relative humidity at 55±15%. Standard guinea pig pellets (RC4; Oriental Yeast Co., Tokyo, Japan) and water were supplied *ad libitum*: the animals were given access to food and water freely during or preceding ECG recording periods.

All procedures involving animals were approved by the animal care and use of committee of Pfizer Global Research & Development, Nagoya Laboratories.

2.2. Transmitter implantation

An ECG transmitter (TA11CA-F40, 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 induced with 1.5–3.0% isoflurane. A bipolar lead for ECG was implanted with the negative pole between the scapulas and the positive pole close to the sternum. After implantation, all animals were subcutaneously administered a single dose of antibiotic (Mycillin solution, 10 mg/kg, Meiji Seika Kaisha, Ltd., Tokyo, Japan). Animals were allowed to recover for 7 days before recording ECG data.

2.3. Analysis of QT and RR intervals

ECG signals were collected using the Dataquest ART data acquisition system (Data Sciences International Inc., St. Paul, MN), and automatically analyzed using the HEM version 3.4 (NOTOCORD SYSTEMS, Croissy-sur-Seine, France). Fiducial marks were visually reviewed for accuracy. Signals were sampled at 1 kHz. The band-pass filter of the ECG signal was 1–200 Hz. The QT interval and the preceding RR interval were calculated as the average value from ECGs recorded for 1 minute (min); if fiducial markers were not acceptable for any waves, the numeric values of the waves were deleted, and then the 1-min averages were calculated.

2.4. QT-correction formulae

Values of QT and RR intervals (20–24 data points of 1-min averages) were collected from each animal during both light and dark periods. The QT interval, combined 1-min average for all animals in each age group together, was corrected for the RR interval by dividing the QT by the RR, RR square root (Bazett, 1920), cubic root (Fridericia, 1920), fourth root (Kawataki, Kashima, Toda, & Tanaka, 1984) or logarithm (Matsunaga et al., 1997). To decide the best QT-correction formula, a QT-correcting method for dogs was used (Matsunaga et al., 1997):

One-parameter regression formulae were defined as

$$\mathrm{QT} = af(\mathrm{RR}),$$

Where the constant value a provided a direct corrected QT interval (QTc) for an RR interval of X:

$$a = \mathrm{QTc}/f(X);\ \text{therefore},\mathrm{QTc} = f(X) \times \mathrm{QT}/f(\mathrm{RR}).$$

In the present study, f(RR) meant RR, $\mathrm{RR}^{1/2}$, $\mathrm{RR}^{1/3}$, $\mathrm{RR}^{1/4}$ or logRR, and $f(X)$ was considered to be averages of RR, $\mathrm{RR}^{1/2}$, $\mathrm{RR}^{1/3}$, $\mathrm{RR}^{1/4}$ or logRR (RR in ms). The regression between QTc and RR interval was plotted graphically, and the slope for each regression line was calculated using the SAS analysis package (version 8.0, SAS Institute Inc., Cary, NC). The correction formula resulting in a slope closest to zero was determined to be most appropriate for each young and old group (Hamlin, Kijtawornrat, Keene, & Hamlin, 2003; Shiotani et al., 2007).

2.5. Light–dark cycle of QT and RR intervals

The mean values of QT intervals during the light period (at 7:00–18:59) and dark period (at 19:00–6:59) were compared to examine the disappearance/appearance of the circadian rhythm of QT interval. The changes of RR interval and QTc during the