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In vivo characterization of rate-dependent impact on the QT interval of *microminipig* assessed by atrial electrical pacing: Development of correction formulae of QT interval

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ABSTRACT. Correction formulae of QT interval were developed for the halothane-anesthetized *microminipigs* by adopting atrial pacing (n=5), which were compared with Bazett's and Fridericia's formulae for humans, and Van de Water's one for dogs. The correction formulae: QTc=QT−0.2072 (RR−750) as linear and QTc=QT/(RR/750)$^{0.4007}$ as non-linear equations, were developed for *microminipigs*. These formulae can better correct the QT interval of the *microminipigs* compared with each of the conventional ones for humans and dogs. Moreover, analysis of the slope constant α values indicates that the rate-dependent change in the ventricular repolarization period of *microminipig* may better mimic that of humans than that of dogs.

KEY WORDS: correction formula, *microminipig*, QTc

The rate-dependent shortening of the QT interval has been explained by the accumulation of slowly activating delayed rectifier K^+ current (I_{Ks}) at higher heart rate [14]. In order to eliminate the heart-rate related influences on the QT interval, several correction formulae have been proposed to better analyze the effects of drugs on the repolarization period. Van de Water's formula: QTc=QT−0.087 (RR−1,000) with the RR interval given in msec was developed for anesthetized dogs [17], whereas Bazett's and Fridericia's formulae: QTc=QT/RR$^{\alpha}$ with the RR interval given in sec, in which α were 1/2 and 1/3, respectively, have been used for humans [2, 4]. The former is a linear equation [17], and the latters are non-linear ones [2, 4].

Microminipig is an extraordinarily small-sized miniature pig, which has been characterized as an alternative *in vivo* experimental model animal to dogs and monkeys for life science research including pharmacological and toxicological studies [1, 3, 5, 7–11, 16, 18]. Although electropharmacological studies have been extensively performed using the halothane-anesthetized *microminipig* [1, 3, 5, 9, 10, 16, 18], it is still unknown how much the conventional correction formulae described above can attenuate the heart rate-dependent impacts on the QT interval of *microminipig* and how much difference is present in the amount of ventricular I_{Ks} current among *microminipigs*, humans and dogs. In order to answer such questions, we tried to develop correction formulae for *microminipigs* by adopting atrial electrical pacing protocol, which were compared with the conventional ones for humans and dogs [13].

Experiments were performed using 5 male *microminipigs* weighing approximately 10 kg, which were obtained from Fuji Micra Inc. (Shizuoka, Japan). All experiments were approved by the Toho University Animal Care and User Committee (No. 18-51-394) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University.

Microminipigs were initially anesthetized by an intramuscular injection of ketamine (16 mg/kg)/xylazine (1.6 mg/kg) and an intravenous injection of propofol (1 mg/kg) through a superficial auricular vein. After intubation with the cuffed endotracheal tube, anesthesia was maintained by inhalation of halothane (1% v/v) vaporized in oxygen with a volume-limited ventilator (SN-

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480-3; Shinano Manufacturing Co., Ltd., Tokyo, Japan). Tidal volume and respiratory rate were set at 10 ml/kg and 15 breaths/min, respectively. Electrocardiogram was obtained from the A-B lead. A standard 4-French quad-polar electrodes catheter (401993; St. Jude Medical Daig Division, Inc., Minnetonka, MN, U.S.A.) was positioned at sinus nodal region of the right atrium via the catheter sheath (RR-A40G07A; Terumo Corporation, Tokyo, Japan) placed at the right femoral vein. The stimulation pulse was set in a rectangular shape, consisting of 2.5 V amplitude (about twice the threshold voltage) and 1 msec duration. The right atrium was electrically paced for approximately 10 sec at each cycle length (CL) of 400, 500, 600, 750, 1,000, 1,200 and 1,500 msec by using a cardiac stimulator (SEC-3102; Nihon Kohden Corporation, Tokyo, Japan) through the distal pair of the electrodes of the catheter. Electrocardiograms were monitored with a polygraph system (RM-6000; Nihon Kohden Corp.), which were analyzed by using a real-time automatic data analysis system (WinVAS3 for Windows ver. 1.1R24; Physio-Tech Co., Ltd., Tokyo, Japan) followed by a manual adjustment. The end of the T-wave was determined using the baseline method. The mean value of the QT interval was calculated using 3 recordings of consecutive electrocardiogram complexes in the latest phase at each pacing CL.

We developed the QT interval correcting formulae to estimate the QT interval at a CL of 750 msec (QT_{CL750}) from QT/RR relationship under various pacing CLs, since the halothane-anesthetized *microminipig* showed the basal heart rate of approximately 80 bpm [1, 3, 9, 10, 18]. The CL and QT interval values at each pacing CL were plugged into linear and non-linear equations to determine a slope constant "α" as previously described [2, 13, 17].

Linear equation:

$$QT_{CL750}=QT-\alpha(CL-750)$$

$$QT-QT_{CL750}=\alpha(CL-750)$$

Non-linear equation:

$$QT_{CL750}=QT/(CL/750)^{\alpha}$$

$$\log(QT/QT_{CL750})=\alpha\log(CL/750)$$

The α values in linear and non-linear equations were obtained as a slope constant by using linear regression analysis with GraphPad prism 6 (ver. 6.03; GraphPad Software, Inc., La Jolla, CA, U.S.A.). R^2 value of linear regression was calculated with Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, U.S.A.) to confirm the reliability of regression analysis.

Each value of the QT interval was reintroduced into currently developed linear and non-linear formulae in addition to Van de Water's, Bazett's and Fridericia's ones to compare their compatibility [2, 4, 17]. A linear regression analysis between the CL and corrected QT interval was performed for each of the correction formulae. F-test was used to confirm whether the QT interval was corrected appropriately; namely, when a *P*-value was <0.05, the correction was judged to be inadequate.

The QT intervals at pacing CL of 1,200 and 1,500 msec were obtained from only one *microminipig*, since the other 4 animals showed the heart rate of >50 bpm. We did not use the values at pacing CL of 1,200 and 1,500 msec to obtain slope constant α, whereas those values were adopted when comparing each of the correction formulae.

The QT intervals (mean ± S.E.M.) at pacing CL of 400, 500, 600, 750, 1,000, 1,200 and 1,500 msec were 271 ± 6, 299 ± 6, 322 ± 6, 351 ± 11, 394 ± 16, 449 and 467 msec, respectively, as shown in Fig. 1A. The QT interval during the sinus rhythm of each animal is also plotted on Fig. 1A. The QT interval was linearly shortened/prolonged by decreasing/increasing the CL.

The relationship between the CL–750 and $QT–QT_{CL750}$ for linear equation is depicted in Fig. 1B, whereas that between the log(CL/750) and $\log(QT/QT_{CL750})$ for non-linear one is shown in Fig. 1C. The α and R^2 values were 0.2072 and 0.8963 in the linear equation, and 0.4007 and 0.9368 in the non-linear one, respectively. R^2 value was slightly greater in the latter than in the former. The following correction formulae were developed using the slope constant α for the halothane-anesthetized *microminipigs*, in which RR intervals were given in msec.

Linear equation:

$$QTc=QT-0.2072(RR-750)$$

Non-Linear equation:

$$QTc=QT/(RR/750)^{0.4007}$$

The QT interval corrected by the currently developed linear equation formula (QTc) was slightly shortened by increasing the CL, of which slope constant was −0.0155 (Fig. 2A), whereas that by Van de Water's formula was markedly prolonged by increasing the CL, of which slope constant was 0.1052 (Fig. 2B). On the other hand, the QT interval corrected by the currently developed non-linear equation formula (QTc) was modestly prolonged by increasing the CL, of which slope constant was 0.0081 (Fig. 2C), whereas that by Bazett's formula was shortened, of which slope constant was −0.0437 (Fig. 2D), but that by Fridericia's formula was prolonged, of which slope constant was 0.0432 (Fig. 2E). The *P*-value was <0.05 for Van de Water's, Bazett's and Fridericia's formulae, indicating that these corrections were inadequate at least for the *microminipigs* used in this study. Additionally, the QT interval during the sinus rhythm of each animal corrected by each of correction formulae is plotted on Fig. 2.

The linear and non-linear correction formulae were developed for *microminipig* for the first time to better correct the QT interval against various heart rate. As clearly shown in Fig. 2A and 2B, the new linear equation for *microminipig* could better correct the QT interval than Van de Water's formula. On the other hand, as shown in Fig. 2C–E, the new non-linear equation for *microminipig* could better correct the QT interval than Bazett's or Fridericia's formula. In addition, as shown in Fig. 1B and 1C, the R^2 value was 0.8963 for linear equation, which was 0.9368 for non-linear one, suggesting that the latter might have potential to better correct the QT interval.

The extent of frequency-dependent shortening of the QT interval is known to depend on the *in vivo* net function of I_{Ks} [6]. In order to better characterize such profile of *microminipig*, we calculated the slope constant α value of *microminipigs* to estimate the QT interval at a CL of 1,000 msec (60 bpm), and directly compared it with those in Framingham Heart Study and of

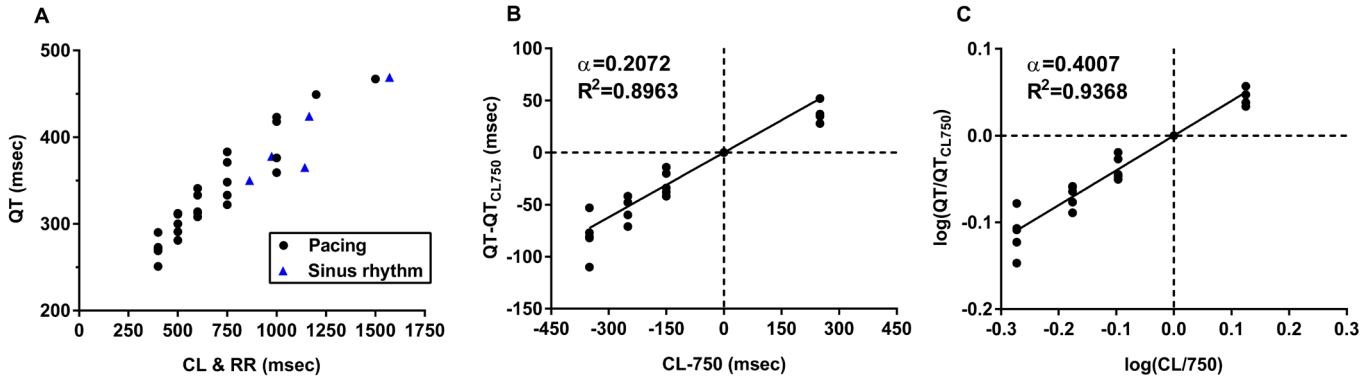

Fig. 1. Development of correction formulae of QT interval against various cycle length (CL), which were obtained from the halothane-anesthetized *microminipigs* with an atrial electrical pacing (n=5). Relationship between the CL and QT interval (A, black circles); that between the CL–750 and QT–QT_{CL750} for linear equation (B); and that between log(CL/750) and log(QT/QT_{CL750}) for non-linear one (C). The slope constant α and R^2 values were obtained by linear regression analysis. The QT interval during the sinus rhythm of each animal was also plotted for reference (A, blue triangles).

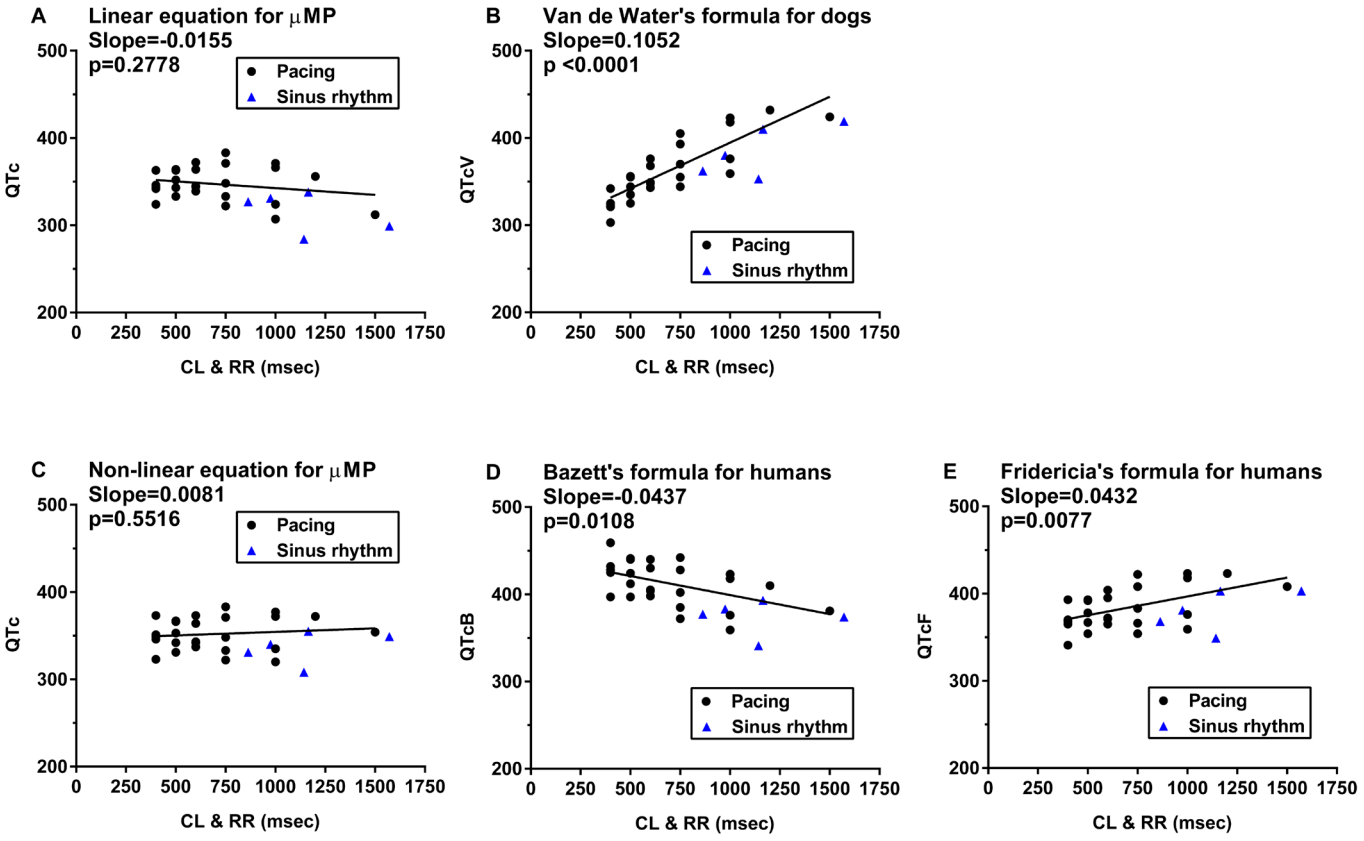

Fig. 2. Assessment of correction formulae of QT interval by introducing each value of the QT interval at various cycle length (CL). Linear regression analyses between the pacing CL and corrected QT interval (QTc) were performed. The QTc was calculated by using the currently developed linear equation formula for *microminipig* (*μ*MP) (A, black circles) and Van de Water's formula for dogs (QTcV) (B, black circles); and the currently developed non-linear equation formula for *μ*MP (C, black circles), Bazett's formula for humans (QTcB) (D, black circles) and Fridericia's formula for humans (QTcF) (E, black circles). The slope constant (slope) was obtained by linear regression analysis. When a *P*-value was <0.05, the correction was considered to be inadequate. Its reverse would mean the QT interval was appropriately corrected. The QTc calculated by the correction formulae for each animal during the sinus rhythm is also plotted for reference (blue triangles).

Bazett's and Fridericia's formulae for humans [2, 4, 12], and of Van de Water's formula for dogs [17], as depicted in Table 1. In addition, we calculated the slope constant α of the non-linear formula for dogs by using our previous experimental results with

Table 1. The slope constant α values in linear and non-linear correction formulae for *microminipigs,* humans and dogs

	Linear correction formula	Non-linear correction formula
Microminipigs	α=0.2072 (CL=750 msec)	α=0.4007 (CL=750 msec)
	α=0.1861 (CL=1,000 msec)	α=0.3841 (CL=1,000 msec)
Humans	α=0.1540 (Framingham Heart study)	α=0.3333 (Fridericia's formula)
		α=0.5000 (Bazett's formula)
Dogs	α=0.0870 (Van de Water's formula)	α=0.1301 (CL=1,000 msec)

The slope constant α value in non-linear correction formula for dogs was obtained by reanalyzing the relationship between the ventricular monophonic action potential duration and pacing cycle length (CL) in the acute atrioventricular block dogs [15]. The conventional correction formulae for humans and dogs have been widely used to estimate the QT interval at a CL of 1,000 msec (60 bpm).

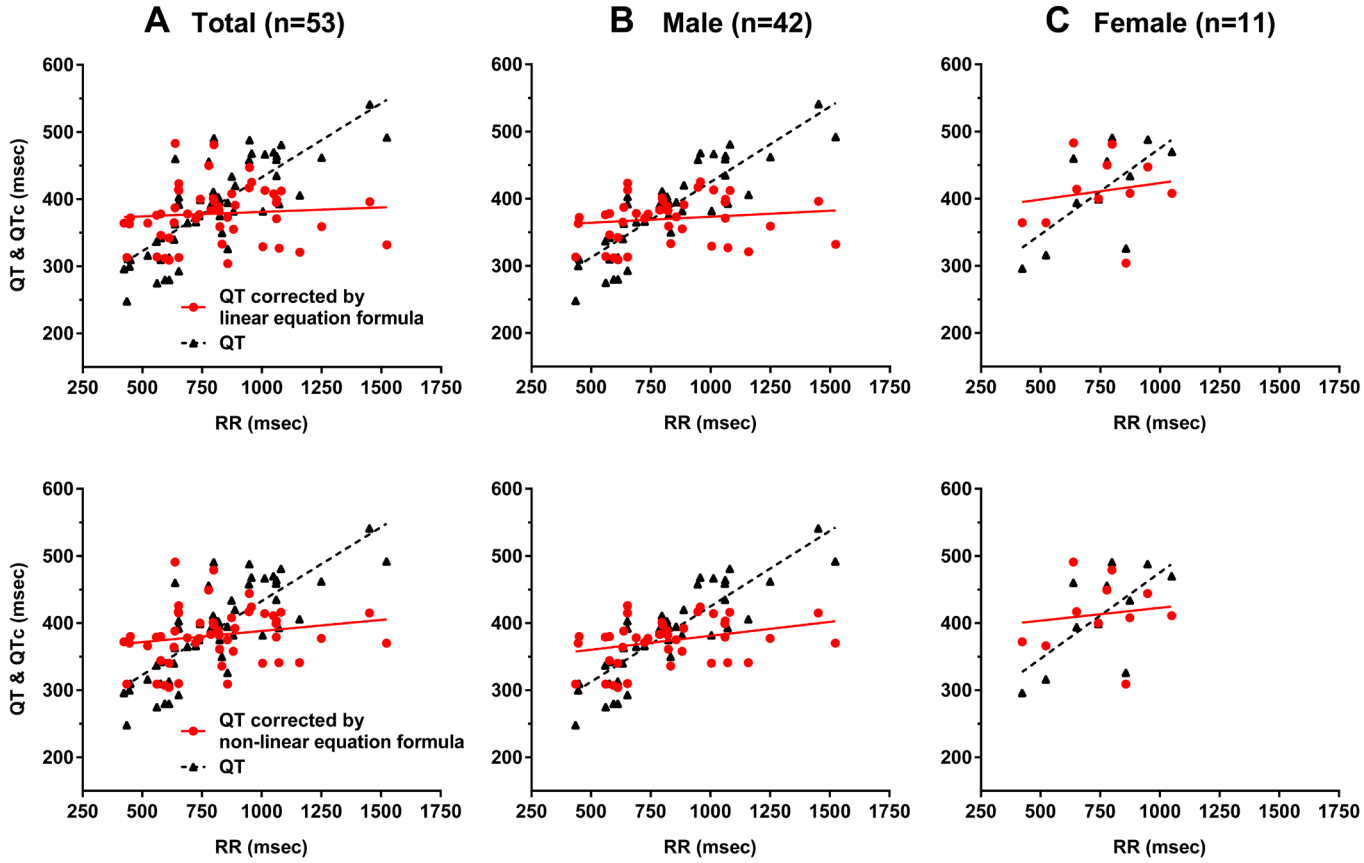

Fig. 3. Evaluation of correction formulae by using the QT interval and RR interval obtained from our previous studies using 42 male and 11 female *micominipigs* [1, 3, 5, 8, 9, 15, 17]. Linear regression analyses between the RR interval and QT interval (black triangles), and those between the RR interval and corrected QT interval (red circles) were performed for total (A), male (B) and female (C) animals. The QT interval was corrected by using the currently developed linear equation formula (upper) and non-linear equation formula (lower) for *microminipigs*.

the acute atrioventricular block dogs [15], which was also described in Table 1. The slope constant α of the non-linear equation for *microminipigs* was between those of Bazett's and Fridericia's formulae for humans, and much greater than that for dogs. Meanwhile, the slope constant α of the linear equation for *microminipigs* was greater than those in Framingham Heart Study for humans or Van de Water's formula for dogs; and importantly, the slope constant α for *microminipigs* was closer to that for humans than that for dogs. Similar results were described previously for minipigs [6], suggesting that *microminipigs* may have similar I_{Ks} function to minipigs. Thus, the amount of net ventricular I_{Ks} channel in *microminipigs* can be considered to be comparable to that in humans and much higher than that in dogs.

The current correction formulae were developed by using the data obtained from 5 male *microminipigs*, the number of which might be too small to apply these formulae to the global population of *microminipigs*. Accordingly, we assessed whether the new correction formulae could properly correct the QT interval by using previously obtained experimental results of the heart rate and QT interval from 42 male and 11 female *micominipigs* [1, 3, 5, 9, 10, 16, 18]. The new correction formulae made the slope of regression lines for the QT interval of total, male and female *micominipigs* more flat, as shown in Fig. 3. These results suggest that

the new correction formulae can apply to the global population of *micominipigs*.

In conclusion, the currently developed formulae can better correct the QT interval of the halothane-anesthetized *microminipig* compared with each of the conventional correction formulae for humans and dogs. These new formulae can be used for re-analyzing previously obtained electrocardiographic data of *microminipig* with the halothane anesthesia. Moreover, analysis of the slope constant α values indicates that the rate-dependent change in the ventricular repolarization period of *microminipig* may better mimic that of humans than that of dogs.

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REFERENCES

1. Ando, K., Takahara, A., Nakamura, Y., Wada, T., Chiba, K., Goto, A., Lubna, N. J., Hagiwara-Nagasawa, M., Izumi-Nakaseko, H., Hoshiai, K., Akie, Y., Naito, A. T. and Sugiyama, A. 2018. Changes of electrocardiogram and hemodynamics in response to dipyridamole: *In vivo* comparative analyses using anesthetized beagle dogs and microminipigs. *J. Pharmacol. Sci.* **136**: 86–92. [Medline] [CrossRef]
2. Bazett, H. C. 1920. An analysis of the time-relations of electrocardiograms. *Heart* **7**: 353–370.
3. Cao, X., Wada, T., Nakamura, Y., Matsukura, S., Izumi-Nakaseko, H., Ando, K., Naito, A. T. and Sugiyama, A. 2017. Sensitivity and reliability of halothaneanesthetized microminipigs to assess risk for drug-induced long QT syndrome. *Basic Clin. Pharmacol. Toxicol.* **121**: 465–470. [Medline] [CrossRef]
4. Fridericia, L. S. 2003. The duration of systole in an electrocardiogram in normal humans and in patients with heart disease. 1920. *Ann. Noninvasive Electrocardiol.* **8**: 343–351. [Medline] [CrossRef]
5. Goto, A., Hagiwara-Nagasawa, M., Izumi-Nakaseko, H., Kitta, K., Hoshiai, K., Chiba, K., Ando, K., Akie, Y., Naito, A. T. and Sugiyama, A. 2018. Use of microminipigs for unveiling unknown mechanisms of azithromycin-induced cardiovascular death. *J. Pharmacol. Sci.* **138**: 198–202. [Medline] [CrossRef]
6. Holzgrefe, H., Ferber, G., Champeroux, P., Gill, M., Honda, M., Greiter-Wilke, A., Baird, T., Meyer, O. and Saulnier, M. 2014. Preclinical QT safety assessment: cross-species comparisons and human translation from an industry consortium. *J. Pharmacol. Toxicol. Methods* **69**: 61–101. [Medline] [CrossRef]
7. Iwatsuki-Horimoto, K., Nakajima, N., Shibata, M., Takahashi, K., Sato, Y., Kiso, M., Yamayoshi, S., Ito, M., Enya, S., Otake, M., Kangawa, A., da Silva Lopes, T. J., Ito, H., Hasegawa, H. and Kawaoka, Y. 2017. The microminipig as an animal model for influenza a virus infection. *J. Virol.* **91**: e01716–e16. [Medline] [CrossRef]
8. Kawaguchi, H., Miyoshi, N., Miura, N., Fujiki, M., Horiuchi, M., Izumi, Y., Miyajima, H., Nagata, R., Misumi, K., Takeuchi, T., Tanimoto, A. and Yoshida, H. 2011. Microminipig, a non-rodent experimental animal optimized for life science research:novel atherosclerosis model induced by high fat and cholesterol diet. *J. Pharmacol. Sci.* **115**: 115–121. [Medline] [CrossRef]
9. Lubna, N. J., Nakamura, Y., Hagiwara-Nagasawa, M., Goto, A., Chiba, K., Kitta, K., Izumi-Nakaseko, H., Ando, K., Naito, A. T., Akie, Y. and Sugiyama, A. 2018. Electropharmacological characterization of microminipigs as a laboratory animal using anti-influenza virus drug oseltamivir. *J. Toxicol. Sci.* **43**: 507–512. [Medline] [CrossRef]
10. Matsukura, S., Nakamura, Y., Cao, X., Wada, T., Izumi-Nakaseko, H., Ando, K., Yamazaki, H. and Sugiyama, A. 2017. Characterization of microminipigs as an *in vivo* experimental model for cardiac safety pharmacology. *J. Pharmacol. Sci.* **133**: 103–109. [Medline] [CrossRef]
11. Mogi, M., Toda, A., Iwasaki, K., Kusumoto, S., Takehara, H., Shimizu, M., Murayama, N., Izumi, H., Utoh, M. and Yamazaki, H. 2012. Simultaneous pharmacokinetics assessment of caffeine, warfarin, omeprazole, metoprolol, and midazolam intravenously or orally administered to Microminipigs. *J. Toxicol. Sci.* **37**: 1157–1164. [Medline] [CrossRef]
12. Sagie, A., Larson, M. G., Goldberg, R. J., Bengtson, J. R. and Levy, D. 1992. An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). *Am. J. Cardiol.* **70**: 797–801. [Medline] [CrossRef]
13. Sakaguchi, Y., Takahara, A., Nakamura, Y., Akie, Y. and Sugiyama, A. 2009. Halothane-anaesthetized, closed-chest, guinea-pig model for assessment of drug-induced QT-interval prolongation. *Basic Clin. Pharmacol. Toxicol.* **104**: 43–48. [Medline] [CrossRef]
14. Stengl, M., Volders, P. G., Thomsen, M. B., Spätjens, R. L., Sipido, K. R. and Vos, M. A. 2003. Accumulation of slowly activating delayed rectifier potassium current (I_{Ks}) in canine ventricular myocytes. *J. Physiol.* **551**: 777–786. [Medline] [CrossRef]
15. Sugiyama, A., Ishida, Y., Satoh, Y., Aoki, S., Hori, M., Akie, Y., Kobayashi, Y. and Hashimoto, K. 2002. Electrophysiological, anatomical and histological remodeling of the heart to AV block enhances susceptibility to arrhythmogenic effects of QT-prolonging drugs. *Jpn. J. Pharmacol.* **88**: 341–350. [Medline] [CrossRef]
16. Tanikawa, Y., Hagiwara-Nagasawa, M., Kambayashi, R., Goto, A., Chiba, K., Kitta, K., Hoshiai, K., Izumi-Nakaseko, H., Naito, A. T. and Sugiyama, A. 2019. Characterization of microminipig as a laboratory animal for safety pharmacology study by analyzing fluvoxamine-induced cardiovascular and dermatological adverse reactions. *Cardiovasc. Toxicol.* **19**: 412–421. [Medline] [CrossRef]
17. 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. [Medline] [CrossRef]
18. Yokoyama, H., Nakamura, Y., Saito, H., Nagayama, Y., Hoshiai, K., Wada, T., Izumi-Nakaseko, H., Ando, K., Akie, Y. and Sugiyama, A. 2017. Pharmacological characterization of microminipig as a model to assess the drug-induced cardiovascular responses for non-clinical toxicity and/or safety pharmacology studies. *J. Toxicol. Sci.* **42**: 93–101. [Medline] [CrossRef]