

Original Article

**Why can *dl*-Sotalol Prolong the QT Interval In Vivo Despite Its Weak Inhibitory Effect
on hERG K⁺ Channels In Vitro? : Electrophysiological and Pharmacokinetic Analysis
with the Halothane-Anesthetized Guinea-Pig Model**

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Running title: PK/PD analysis of I_{Kr} blockers

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Abstract

In order to bridge the gap of action of *dl*-sotalol between the human ether-a-go-go related gene (hERG) K⁺ channel inhibition in vitro and QT-interval prolongation in vivo, its electropharmacological effect and pharmacokinetic property were simultaneously studied in comparison with those of 10 drugs having potential to prolong the QT interval (positive drugs: bepridil, haloperidol, *dl*-sotalol, terfenadine, thioridazine, moxifloxacin, pimozide, sparfloxacin, diphenhydramine, imipramine and ketoconazole) and 4 drugs lacking such property (negative drugs: enalapril, phenytoin, propranolol or verapamil) with the halothane-anesthetized guinea-pig model. A dose of each drug that caused 10% prolongation of Fridericia-corrected QT interval (QTcF) was calculated, which was compared with respective known hERG K⁺ IC₅₀ value and currently obtained heart/plasma concentration ratio. Each positive drug prolonged the QTcF in a dose-related manner, whereas such effect was not observed by the negative drugs. Drugs with more potent hERG K⁺ channel inhibition showed higher heart/plasma concentration ratio, resulting in more potent QTcF prolongation in vivo. The potency of *dl*-sotalol for QTcF prolongation was flat middle, although its hERG K⁺ channel inhibitory property as well as heart/plasma concentration ratio was the smallest among the positive drugs, which may be partly explained by its lack of binding to plasma protein.

Keywords: QT interval prolongation, Halothane, Guinea pig, Pharmacokinetics, Pharmacodynamics

Introduction

In order to avoid excessive QT-interval prolongation, resulting in the onset of lethal ventricular arrhythmias, almost all new drug candidates have been carefully evaluated according to the guideline ICH S7B for Safety Pharmacology Studies [1]. In vitro electrophysiological studies can provide information concerning the effects of a test substance on action potential duration and/or cardiac ionic currents, whereas in vivo QT assay shows the direct evidence for the drug-induced net repolarization delay [1]. After the guideline became adopted, the number of new compounds with the risk causing torsade de pointes has been substantially reduced [2,3], but there have been some drugs showing contradictory results between the in vitro electrophysiological studies and in vivo QT assays like *dl*-sotalol, which prolongs QTc in vivo much more than that expected from its 50% inhibitory concentration (IC₅₀) for human ether-a-go-go related gene (hERG) K⁺ channel current in vitro [4,5].

In order to bridge such gap of information between the hERG K⁺ channel inhibition and QT-interval prolongation, in this study we simultaneously assessed the electrophysiological effect and pharmacokinetic property of *dl*-sotalol in comparison with those of 14 drugs; namely, 10 drugs with potential to clinically prolong QT interval and 4 drugs without such property [6] by using the halothane-anesthetized guinea-pig model [4,5]. Then, we analyzed the relationship of the QT-prolonging drugs between the IC₅₀ for hERG K⁺ channel, lipophilicity (logP), heart/plasma concentration ratio and extent of QT-interval prolongation, since only drugs that distribute to the heart have the potential to prolong the QT interval via their hERG K⁺ channel inhibition [7]. This is the first report that described why *dl*-sotalol can prolong the QT interval despite its relatively weak inhibitory effect on hERG K⁺ channels.

Materials and Methods

Experiments were performed using male Hartley guinea pigs weighing 280-606 g (n=72) (Japan SLC Inc, Shizuoka, Japan). All experiments were approved by the Animal Research Committee for Animal Experimentation of Toho University (No.11-51-150) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University.

Surgical preparations

The guinea pigs were initially anesthetized with 4% halothane in a closed chamber. After a tracheal cannula was inserted, 1% halothane vaporized with 100% oxygen was inhaled with a rodent ventilator (SN-480-7, Shinano, Tokyo, Japan). The tidal volume and respiratory rate were set at 10 mL/kg and 60 strokes/min, respectively. Body temperature was maintained at 37°C with a heating pad. The right jugular vein was cannulated for drug administration. The surface lead I electrocardiogram was obtained from the limb electrodes. The electrocardiogram was continuously monitored with a polygraph system (MEG-6108, Nihon Kohden, Tokyo, Japan), which was analyzed by using a real time full automatic data analysis system (Notocord-hem 3.4; Notocord Systems, Paris, France).

Experimental protocol

After the assessment of pre-drug basal control values (C), each drug in a low dose was intravenously infused over 30 min with a syringe pump (TE-331S, Terumo, Tokyo, Japan), and the heart rate and QT interval were measured at 5, 15 and 30 min after the start of the drug infusion. Then, each drug in a middle dose was intravenously infused over 30 min, and the heart rate and QT interval were measured at the same points. Finally, each drug in a high dose was intravenously infused over 30 min, and the heart rate and QT interval were

measured at the same points.

The doses of each drug were determined based on the interview form from the manufacturer, previous reports [8-11] and/or our preliminary studies. The following doses were assessed for each drug: bepridil (0.09, 0.3 and 0.9 mg/kg, n=4); haloperidol (0.03, 0.09 and 0.3 mg/kg, n=4); *dl*-sotalol (0.9, 3 and 9 mg/kg, n=4); terfenadine (0.09, 0.3 and 0.9 mg/kg, n=4); thioridazine (0.3, 0.9 and 3 mg/kg, n=4); moxifloxacin (3, 9 and 30 mg/kg, n=4); pimozone (0.003, 0.009 and 0.03 mg/kg, n=4); sparfloxacin (0.9, 3 and 9 mg/kg, n=4); diphenhydramine (0.3, 0.9 and 3 mg/kg, n=4); imipramine (0.9, 3 and 9 mg/kg, n=4); ketoconazole (3, 9 and 30 mg/kg, n=4); enalapril (0.9, 3 and 9 mg/kg, n=4); phenytoin (0.9, 3 and 9 mg/kg, n=4); propranolol (0.9, 3 and 9 mg/kg, n=4); and verapamil (0.03, 0.09 and 0.3 mg/kg, n=4).

Three types of vehicle were used, each of which in a volume of 5 mL/kg/30 min was intravenously infused according to the same protocol for the respective drugs (n=4 for each vehicle). The vehicle for *dl*-sotalol, diphenhydramine, imipramine, ketoconazole, enalapril, propranolol and verapamil was saline, that for haloperidol, terfenadine, thioridazine, moxifloxacin, sparfloxacin and phenytoin was dimethylsulfoxide (DMSO; Kishida Chemical, Co. Ltd., Osaka, Japan)/solubilizing agent (WellsolveTM, Celeste Co., Ltd., Tokyo, Japan)/saline (1/10/89), and that for bepridil and pimozone was WellsolveTM/saline (1/9).

Measurement of drug concentrations in plasma and heart

After assessing the heart rate and QT interval of the high dose of each drug, a volume of 0.5 mL of arterial blood was drawn from the abdominal aorta, and the heart was excised. The blood was centrifuged at 1,000g for 3 min at 4°C, and the supernatant plasma was stored at -80°C until the drug concentration was measured. The left ventricular free wall was trimmed from the isolated heart, which was minced and homogenized with 3 times of weight of saline. The homogenate was stored at -80°C until the drug concentration was

measured.

Drugs were extracted with acetonitrile from the plasma and heart homogenate. Then, the drug concentrations were measured by LC/MS/MS analysis with HPLC (Agilent 1100 Quaternary system; Agilent Technologies Japan, Ltd., Tokyo, Japan) and ESI-MS/MS (API 4000; Applied Biosystems/MDS SCIEX, Foster City, CA, USA). Chromatographic separation of drugs was achieved on an Intersil Ph-3 column (2.1 mm I.D. x 150 mm, 5 mm; GL Science, Tokyo, Japan) by using 0.1% formic acid/acetonitrile (3/7) as a mobile phase.

Drugs

We examined 11 drugs having potential to prolong the QT interval (positive drugs: bepridil, haloperidol, *dl*-sotalol, terfenadine, thioridazine, moxifloxacin, pimozide, sparfloxacin, diphenhydramine, imipramine and ketoconazole) and 4 drugs lacking such property (negative drugs: enalapril, phenytoin, propranolol and verapamil) [12]. Bepridil, haloperidol, *dl*-sotalol, terfenadine and thioridazine ; moxifloxacin, pimozide and sparfloxacin; and diphenhydramine, imipramine and ketoconazole have been reported to have high, moderate and low risks of drug-induced torsade de pointes, respectively [6].

Bepridil (bepridil hydrochloride), haloperidol, *dl*-sotalol (*dl*-sotalol hydrochloride), terfenadine, thioridazine (thioridazine hydrochloride), pimozide, sparfloxacin, diphenhydramine, imipramine (imipramine hydrochloride), ketoconazole, enalapril (enalapril maleate salt), propranolol ((±)-propranolol hydrochloride) and verapamil (verapamil hydrochloride) were purchased from Sigma-Aldrich corporation (St. Louis, MO, USA), whereas moxifloxacin, phenytoin and halothane were obtained from Toronto Research Chemicals Inc. (Toronto, Canada), Wako Pure Chemical Industries Ltd. (Osaka, Japan) and Takeda Chemical Industries (Osaka, Japan), respectively.

Bepridil was initially dissolved in WellsolveTM in a concentration of 1.8 mg/mL, then diluted with saline containing the same molar of acetic acid to bepridil as a stock solution to a

concentration of 0.18 mg/mL, and finally diluted with WellsolveTM/saline (1/9) to obtain bepridil solution of 0.06 and 0.018 mg/mL. Haloperidol was initially dissolved in DMSO in a concentration of 6 mg/mL, next diluted with WellsolveTM to a concentration of 0.6 mg/mL, then diluted with saline as a stock solution to a concentration of 0.06 mg/mL, and finally diluted with DMSO/WellsolveTM/saline (1/10/89) to obtain haloperidol solution of 0.006 and 0.018 mg/mL. *dl*-Sotalol was dissolved with saline in concentrations of 0.18, 0.6 and 1.8 mg/mL. Terfenadine was initially dissolved in DMSO in a concentration of 18 mg/mL, next diluted with WellsolveTM to a concentration of 1.8 mg/mL, then diluted with saline as a stock solution to a concentration of 0.18 mg/mL, and finally diluted with DMSO/WellsolveTM/saline (1/10/89) to obtain terfenadine solution of 0.018 and 0.06 mg/mL. Thioridazine was initially dissolved in DMSO in a concentration of 60 mg/mL, next diluted with WellsolveTM to a concentration of 6 mg/mL, then diluted with saline to a concentration of 0.6 mg/mL as a stock solution, and finally diluted with DMSO/WellsolveTM/saline (1/10/89) to obtain thioridazine solution of 0.06 and 0.18 mg/mL before each experiment. Moxifloxacin was initially dissolved in DMSO in a concentration of 600 mg/mL, next diluted with WellsolveTM to a concentration of 60 mg/mL, then diluted with saline as a stock solution to a concentration of 6 mg/mL, and finally diluted with DMSO/WellsolveTM/saline (1/10/89) to obtain moxifloxacin solution of 0.6 and 1.8 mg/mL. Pimozide was initially dissolved in WellsolveTM in a concentration of 0.06 mg/mL, then diluted with saline containing the same molar of acetic acid to pimozide as a stock solution to a concentration of 0.006 mg/mL, and finally diluted with WellsolveTM/saline (1/9) to obtain pimozide solution of 0.0006 and 0.0018 mg/mL. Sparfloxacin was initially dissolved in DMSO in a concentration of 180 mg/mL, next diluted with WellsolveTM to a concentration of 18 mg/mL, then diluted with saline containing the same molar of 1 mol/L sodium hydroxide to sparfloxacin as a stock solution to a concentration of 1.8 mg/mL, and finally diluted with DMSO/WellsolveTM/saline (1/10/89) to obtain sparfloxacin solution of 0.18 and 0.6 mg/mL. Diphenhydramine was

dissolved with saline in concentrations of 0.06, 0.18 and 0.6 mg/mL. Imipramine was dissolved with saline in concentrations of 0.18, 0.6 and 1.8 mg/mL. Ketoconazole was initially dissolved in saline containing the same molar of 1 mol/L hydrochloric acid to ketoconazole as a stock solution to a concentration of 6 mg/mL, and finally diluted with saline to obtain ketoconazole solution of 0.6 and 1.8 mg/mL. Enalapril was dissolved with saline in concentrations of 0.18, 0.6 and 1.8 mg/mL. Phenytoin was initially dissolved in DMSO in a concentration of 180 mg/mL, next diluted with WellsolveTM to a concentration of 18 mg/mL, then diluted with saline containing the same molar of 1mol/L sodium hydroxide to phenytoin as a stock solution to a concentration of 1.8 mg/mL, and finally diluted with DMSO/WellsolveTM/saline (1/10/89) to obtain phenytoin solution of 0.18 and 0.6 mg/mL. Propranolol was dissolved with saline in concentrations of 0.18, 0.6 and 1.8 mg/mL. Verapamil was dissolved with saline in concentrations of 0.006, 0.018 and 0.06 mg/mL.

hERG IC₅₀ and LogP data

The IC₅₀ values of each drug for hERG channel current (hERG IC₅₀) were collected from hERG dataset [13], which were obtained with whole cell patch clamp technique at temperature of 35-37°C except that it was at 23°C for sparfloxacin. Median for the hERG IC₅₀ values was calculated when there were multiple data. LogP data was obtained from DrugBank online database [14,15] to estimate the lipophilicity of each drug.

Data analysis

Each measurement of the electrocardiogram was the mean of ten consecutive recordings. QT interval was corrected with Fridericia's formula: QTcF=QT/(RR/1000)^{1/3}. Data are presented as mean±SE. The ED₁₀ was defined as a dose (mg/kg) providing a net 10% of QTcF prolongation, which was calculated by using the least squares method with percent changes of QTcF for a drug and its vehicle.

The statistically significant differences in the values between the vehicle- and drug-treated groups were evaluated by two-way, repeated-measures analysis of variance (ANOVA), whereas those among the groups were determined with one-way factorial ANOVA. A correlation between two groups was assessed by using Pearson's correlation coefficient. A P-value of <0.05 was considered to be statistically significant.

Results

Effects on the heart rate

The time courses of changes in the heart rate of each treatment group and of their percent changes from the pre-drug basal value (C) are summarized in Figs. 1 and 2, respectively. The basal value of the heart rate (beats/min) was 175 ± 11 in the saline-treated group, 175 ± 11 in the DMSO /WellsolveTM/saline-treated group, 190 ± 6 in the WellsolveTM/saline-treated group, 172 ± 7 in the bepridil-treated group, 179 ± 5 in the haloperidol-treated group, 193 ± 4 in the *dl*-sotalol-treated group, 181 ± 7 in the terfenadine-treated group, 170 ± 4 in the thioridazine-treated group, 176 ± 4 in the moxifloxacin-treated group, 162 ± 9 in the pimozide-treated group, 192 ± 6 in the sparfloxacin-treated group, 173 ± 3 in the diphenhydramine-treated group, 170 ± 2 in the imipramine-treated group, 171 ± 7 in the ketoconazole-treated group, 186 ± 4 in the enalapril-treated group, 172 ± 3 in the phenytoin-treated group, 181 ± 5 in the propranolol-treated group and 190 ± 3 in the verapamil-treated group. There was no significant difference among these basal values. The absolute values decreased in the thioridazine and moxifloxacin-treated groups compared with their respective vehicle-treated groups, which was not detected in the other groups as shown in Fig. 1. Meanwhile, the relative ones decreased in the *dl*-sotalol, thioridazine, sparfloxacin and propranolol-treated groups compared with their respective vehicle-treated groups, which were not detected in the other groups.

Effects on the QTc

Typical tracings of electrocardiogram showing the effects of *dl*-sotalol and terfenadine are depicted in Fig. 3. The time courses of changes in the QTcF of each treatment group and of their percent changes from the pre-drug basal value (C) are

summarized in Figs. 4 and 5, respectively. The basal value of the QTcF (ms) was 280 ± 3 in the saline-treated group, 273 ± 7 in the DMSO/WellsolveTM/saline-treated group, 271 ± 5 in the WellsolveTM/saline-treated group, 276 ± 4 in the bepridil-treated group, 255 ± 9 in the haloperidol-treated group, 278 ± 4 in the *dl*-sotalol-treated group, 257 ± 4 in the terfenadine-treated group, 282 ± 2 in the thioridazine-treated group, 289 ± 5 in the moxifloxacin-treated group, 296 ± 8 in the pimozide-treated group, 269 ± 8 in the sparfloxacin-treated group, 245 ± 4 in the diphenhydramine-treated group, 278 ± 9 in the imipramine-treated group, 257 ± 5 in the ketoconazole-treated group, 263 ± 5 in the enalapril-treated group, 273 ± 17 in the phenytoin-treated group, 274 ± 10 in the propranolol-treated group and 242 ± 8 in the verapamil-treated group. There was no significant difference among these basal values. The QTcF increased in the *dl*-sotalol, thioridazine, moxifloxacin, pimozide and diphenhydramine-treated groups compared with their respective vehicle-treated groups, which was not detected in the other groups. Meanwhile, the changes in percent increased in the bepridil, haloperidol, *dl*-sotalol, terfenadine, thioridazine, moxifloxacin, pimozide, sparfloxacin, diphenhydramine, imipramine and ketoconazole-treated groups compared with respective vehicle-treated group, which was not detected in the other groups.

A dose that prolonged the QTc by net 10% was calculated for each of the 11 QT-prolonging drugs. The ED₁₀ (mg/kg) was 1.1 in the bepridil-treated group, 0.25 in the haloperidol-treated group, 2.2 in the *dl*-sotalol-treated group, 0.75 in the terfenadine-treated group, 2.4 in the thioridazine-treated group, 18 in the moxifloxacin-treated group, 0.032 in the pimozide-treated group, 4.6 in the sparfloxacin-treated group, 0.81 in the diphenhydramine-treated group, 11 in the imipramine-treated group, and 18 in the ketoconazole-treated group.

Relationships between logP, hERG IC₅₀ value, heart/plasma concentration ratio and ED₁₀

Fig. 6A shows the relationship between logP and hERG IC₅₀ (top), that between logP and heart/plasma concentration ratio (middle), and that between hERG IC₅₀ and heart/plasma concentration ratio (bottom), whereas Fig. 6B indicates that between logP and ED₁₀ (top), that between heart/plasma concentration ratio and ED₁₀ (middle), and that between hERG IC₅₀ and ED₁₀ (bottom). There were linear correlations between logP and hERG IC₅₀, between logP and heart/plasma concentration ratio, between hERG IC₅₀ and heart/plasma concentration ratio, and between hERG IC₅₀ and ED₁₀. Note that greater QTc prolongation was induced by *dl*-sotalol than that expected from its hERG K⁺ IC₅₀ value and heart/plasma concentration ratio.

Discussion

In order to bridge the gap of information between the hERG channel inhibition in vitro and repolarization delay in vivo, pharmacokinetic and pharmacodynamic responses for 11 drugs having potential to prolong the QT interval and for 4 drugs without such property were analyzed in the halothane-anesthetized guinea-pig model [4,5]. We found that a drug with more potent hERG channel inhibitory property tended to more distribute to the myocardium, possibly enhancing the extent of QTc prolongation in vivo.

Effects of drugs on the heart rate

When assessed the values by the percent changes from the basal ones, *dl*-sotalol, thioridazine, sparfloxacin and propranolol decreased the heart rate in a dose-related manner, which was not observed by the other drugs. These results were essentially in accordance with the ones of previous reports in the halothane-anesthetized dogs, which have been shown to reflect the drug-induced electrophysiological responses in humans [9,16-22]. These results together with previously obtained knowledge confirm that the halothane-anesthetized guinea-pig model may be useful for assessing the chronotropic effect of the drugs [4,5].

Effects on the QTcF

When assessed by the percent changes from the basal values, bepridil, haloperidol, *dl*-sotalol, terfenadine, thioridazine, moxifloxacin, pimozide, sparfloxacin, diphenhydramine, imipramine and ketoconazole prolonged QTcF in a dose-related manner, which was not observed by the other drugs. These results were in good accordance with the ones of previous clinical reports [12] as well as those in halothane-anesthetized dogs [9], confirming that the halothane-anesthetized guinea-pig model can be useful for assessing the drug-induced QT-interval prolongation [4,5].

Analysis of the relationships between logP and hERG IC₅₀; between logP and heart/plasma concentration ratio; and between hERG IC₅₀ and heart/plasma concentration ratio

Since drugs with clinical risk of long QT syndrome have been known to prolong QTc by >10% in dogs or monkeys [23-25], we used the ED₁₀ value as a marker of potency of drug-induced repolarization delay. There was a good correlation between logP and hERG IC₅₀ (Fig.6A top), supporting previous reports that more positively charged lipophilic compounds can inhibit hERG current more potently [26,27]. Furthermore, there was a good correlation between logP and heart/plasma concentration ratio (Fig.6A middle), supporting a previous report of 5 types of antipsychotic drugs that the extent of myocardial distribution could depend on that of lipophilicity expressed as logP [28]. Thus, logP may be one of the common determinants of hERG IC₅₀ and heart/plasma concentration ratio. Importantly, there was a good correlation between hERG IC₅₀ and heart/plasma concentration ratio (Fig.6A bottom), indicating that more potent hERG channel blockers can more accumulate in the myocardium in vivo, which may have further increased the proarrhythmic risk of potent hERG channel blockers.

Analysis of the relationships between logP and ED₁₀; between heart/plasma concentration ratio and ED₁₀; and between hERG IC₅₀ and ED₁₀

The correlations coefficient 'r²' were 0.1566 between logP and ED₁₀ (Fig.6B top); 0.2724 between heart/plasma concentration ratio and ED₁₀ (Fig.6B middle); and 0.4918 between hERG IC₅₀ and ED₁₀ (Fig.6B bottom) in non-linear algorithm, suggesting that the extent of QT-interval prolongation may be more dependent on the hERG IC₅₀ compared with the others. It should be noted that the most potent hERG channel inhibitor pimozone showed smaller ED₁₀ compared with that expected from the regression formula, which may support

1 the hypothesis; more potent hERG channel inhibitor may more distribute to the myocardium,
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3 enhancing the extent of QTc prolongation in vivo.
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7 ***Potential explanation for the contradictory results of *dl*-sotalol between hERG IC₅₀ and***
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9 ***ED₁₀***
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11 The hERG inhibitory effect and distribution to the myocardium of *dl*-sotalol were
12 the smallest among the positive drugs (Fig. 6A bottom). Contrary to our expectation based
13 on these results, the ED₁₀ of *dl*-sotalol was the flat middle in the whole positive drugs (Fig.
14 6B). The protein-binding ratio of *dl*-sotalol in plasma has been shown to be zero, whereas
15 those were 45 to 99% for the other drugs [29]. Thus, total concentration of *dl*-sotalol equals
16 to its pharmacologically active one unlike the other drugs, which may partly explain the
17 greater QT-interval prolongation of *dl*-sotalol than that expected from its hERG IC₅₀.
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Conclusion

The halothane-anesthetized guinea-pig model may be useful for assessing the drug-induced chronotropic effect and QT-interval prolongation; moreover, ED₁₀ for QT-interval prolongation in this model may become a reliable guide to predict the drug-induced repolarization delay in clinical. More potent hERG channel blockers can more distribute to the myocardium, resulting in more potent QT-interval prolongation in vivo. Also, lack of plasma protein binding of *dl*-sotalol may partly explain its relatively potent QT-interval prolongation.

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Conflict of interest: The authors declare no conflicts of interest.

References

1. The ICH Steering Committee. The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals (S7B), The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), The Guideline was recommended for adoption at Step 5 of the ICH process in May 2005. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S7B/Step4/S7B_Guideline.pdf. Accessed December 21, 2014.
2. Darpo, B. (2010). The thorough QT study four years after the implementation of the ICH E14 guidance. *Br. J. Pharmacol.* 159: 49-57.
3. Sugiyama, A., Hashimoto, H., Nakamura, Y., Fujita, T., Kumagai, Y. (2014). QT/QTc study conducted in Japanese adult healthy subjects: a novel xanthine oxidase inhibitor topiroxostat was not associated with QT prolongation. *J. Clin. Pharmacol.* 54: 446-452.
4. Sakaguchi, Y., Sugiyama, A., Takao, S., Akie, Y., Takahara, A., Hashimoto, K. (2005). Halothane sensitizes the guinea-pig heart to pharmacological I_{Kr} blockade: comparison with urethane anesthesia. *J. Pharmacol. Sci.* 99: 185-190.
5. Sakaguchi, Y., Takahara, A., Nakamura, Y., Akie, Y., 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.
6. Behr, E.R., Roden, D. (2013). Drug-induced arrhythmia: pharmacogenomic prescribing? *Eur. Heart. J.* 34: 89-95.
7. Minematsu, T., Ohtani, H., Yamada, Y., Sawada, Y., Sato, H., Iga, T. (2001). Quantitative relationship between myocardial concentration of tacrolimus and QT

- prolongation in guinea pigs: pharmacokinetic/pharmacodynamic model incorporating a site of adverse effect. *J. Pharmacokinet. Pharmacodyn.* 28: 533-554.
8. Deneer, V.H., Lie-A-Huen, L., Kingma, J.H., Proost, J.H., Kelder, J.C., Brouwers, J.R. (1998). Absorption kinetics of oral sotalol combined with cisapride and sublingual sotalol in healthy subjects. *Br. J. Clin. Pharmacol.* 45: 485-490.
 9. Sugiyama, A. (2008). Sensitive and reliable proarrhythmia in vivo animal models for predicting drug-induced torsades de pointes in patients with remodelled hearts. *Br. J. Pharmacol.* 154: 1528-1537.
 10. Montay, G., Bruno, R., Vergniol, J.C., Ebmeier, M., Le Roux, Y., Guimart, C., Frydman, A., Chassard, D., Thebault, J.J. (1994). Pharmacokinetics of sparfloxacin in humans after single oral administration at doses of 200, 400, 600, and 800 mg. *J. Clin. Pharmacol.* 34: 1071-1076.
 11. Baxter, J.G., Brass, C., Schentag, J.J., Slaughter, R.L. (1986). Pharmacokinetics of ketoconazole administered intravenously to dogs and orally as tablet and solution to humans and dogs. *J. Pharm. Sci.* 75: 443-447.
 12. CredibleMeds[®]. Available from: www.crediblemeds.org. Accessed December 21, 2014.
 13. Polak, S., Wiśniowska, B., Brandys, J. (2009). Collation, assessment and analysis of literature in vitro data on hERG receptor blocking potency for subsequent modeling of drugs' cardiotoxic properties. *J. Appl. Toxicol.* 29: 183-206.
 14. Law, V., Knox, C., Djoumbou, Y., Jewison, T., Guo, A.C., Liu, Y., Maciejewski, A., Arndt, D., Wilson, M., Neveu, V., Tang, A., Gabriel, G., Ly, C., Adamjee, S., Dame, Z.T., Han, B., Zhou, Y., Wishart, D.S. (2014). DrugBank 4.0: shedding new light on drug metabolism. *Nucleic Acids Res.* 42(Database issue):D1091-1097.
 15. DrugBank Version 4.1. Available from: www.drugbank.ca. Accessed December 21, 2014.

16. Ishizaka, T., Takahara, A., Iwasaki, H., Mitsumori, Y., Kise, H., Nakamura, Y., Sugiyama, A. (2008). Comparison of electropharmacological effects of bepridil and sotalol in halothane-anesthetized dogs. *Circ. J.* 72: 1003-1011.
17. Sugiyama, A., Satoh, Y., Hashimoto, K. (2001). In vivo canine model comparison of cardiohemodynamic and electrophysiological effects of a new antipsychotic drug aripiprazole (OPC-14597) to haloperidol. *Toxicol. Appl. Pharmacol.* 173: 120-128.
18. Chiba, K., Sugiyama, A., Hagiwara, T., Takahashi, S., Takasuna, K., Hashimoto, K. (2004). In vivo experimental approach for the risk assessment of fluoroquinolone antibacterial agents-induced long QT syndrome. *Eur. J. Pharmacol.* 486: 189-200.
19. Chiba, K., Sugiyama, A., Satoh, Y., Shiina, H., Hashimoto, K. (2000). Proarrhythmic effects of fluoroquinolone antibacterial agents: in vivo effects as physiologic substrate for torsades. *Toxicol. Appl. Pharmacol.* 169: 8-16.
20. Mitsumori, Y., Nakamura, Y., Hoshiai, K., Nagayama, Y., Adachi-Akahane, S., Koizumi, S., Matsumoto, M., Sugiyama, A. (2010). In vivo canine model comparison of cardiovascular effects of antidepressants milnacipran and imipramine. *Cardiovasc. Toxicol.* 10: 275-282.
21. Shiina, H., Sugiyama, A., Takahara, A., Satoh, Y., Hashimoto, K. (2000). Comparison of the electropharmacological effects of verapamil and propranolol in the halothane-anesthetized in vivo canine model under monophasic action potential monitoring. *Jpn. Circ. J.* 64: 777-782.
22. Takahara, A., Sugiyama, A., Satoh, Y., Wang, K., Honsho, S., Hashimoto, K. (2005). Halothane sensitizes the canine heart to pharmacological I_{Kr} blockade. *Eur. J. Pharmacol.* 507: 169-177.
23. Tashibu, H., Miyazaki, H., Aoki, K., Akie, Y., Yamamoto, K. (2005). QT PRODACT: in vivo QT assay in anesthetized dog for detecting the potential for QT interval prolongation by human pharmaceuticals. *J. Pharmacol. Sci.* 99: 473-486.

- 1 24. Toyoshima, S., Kanno, A., Kitayama, T., Sekiya, K., Nakai, K., Haruna, M., Mino, T.,
2 Miyazaki, H., Yano, K., Yamamoto, K. (2005). QT PRODACT: in vivo QT assay in
3 the conscious dog for assessing the potential for QT interval prolongation by human
4 pharmaceuticals. *J. Pharmacol. Sci.* 99: 459-471.
5
6
7
8
9
10 25. Ando, K., Hombo, T., Kanno, A., Ikeda, H., Imaizumi, M., Shimizu, N., Sakamoto, K.,
11 Kitani, S., Yamamoto, Y., Hizume, S., Nakai, K., Kitayama, T., Yamamoto, K. (2005).
12 QT PRODACT: in vivo QT assay with a conscious monkey for assessment of the
13 potential for drug-induced QT interval prolongation. *J. Pharmacol. Sci.* 99: 487-500.
14
15
16
17
18
19 26. Young, R.J., Green, D.V., Luscombe, C.N., Hill, A.P. (2011). Getting physical in drug
20 discovery II: the impact of chromatographic hydrophobicity measurements and
21 aromaticity. *Drug. Discov. Today.* 16: 822-830.
22
23
24
25
26 27. Waring, M.J. and Johnstone, C. (2007). A quantitative assessment of hERG liability as
27 a function of lipophilicity. *Bioorg. Med. Chem. Lett.* 17: 1759–1764.
28
29
30
31 28. Titier, K., Canal, M., D  ridet, E., Abouelfath, A., Gromb, S., Molimard, M., Moore, N.
32 (2004). Determination of myocardium to plasma concentration ratios of five
33 antipsychotic drugs: comparison with their ability to induce arrhythmia and sudden
34 death in clinical practice. *Toxicol. Appl. Pharmacol.* 199: 52-60.
35
36
37
38
39
40 29. Redfern, W.S., Carlsson, L., Davis, A.S., Lynch, W.G., MacKenzie, I., Palethorpe, S.,
41 Siegl, P.K., Strang, I., Sullivan, A.T., Wallis, R., Camm, A.J., Hammond, T.G. (2003).
42 Relationships between preclinical cardiac electrophysiology, clinical QT interval
43 prolongation and torsade de pointes for a broad range of drugs: evidence for a
44 provisional safety margin in drug development. *Cardiovasc. Res.* 58: 32-45.
45
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Figure legends

Fig.1

Time courses of the effects of 15 drugs on the absolute values (beats/min) of the heart rate (HR). Data are presented as the mean \pm SE (n=4 for each treatment group). Three escalating i.v. doses of each drug (circles) were cumulatively infused over 30 min, respectively. P<0.01 and P<0.05 indicate statistically significant difference in the values between the vehicle (open circles)- and drug (closed circles)-treated groups. N.S means that there is no statistically significant difference in the values between the vehicle- and drug-treated groups.

Fig.2

Time courses of the effects of 15 drugs on the percent changes (%) of the heart rate (HR). Data are presented as the mean \pm SE (n=4 for each treatment group). Three escalating i.v. doses of each drug (circles) were cumulatively infused over 30 min, respectively. P<0.01 and P<0.05 indicate statistically significant difference in the values between the vehicle (open circles)- and drug (closed circles)-treated groups. N.S means that there is no statistically significant difference in the values between the vehicle- and drug-treated groups.

Fig.3

Typical tracings of the electrocardiogram showing the effects of *dl*-sotalol and terfenadine.

(A) Electrocardiogram at pre-drug control (Control) and 30 min after the start of 9 mg/kg of *dl*-sotalol infusion. (B) Electrocardiogram at pre-drug control (Control) and 30 min after the start of 0.9 mg/kg of terfenadine infusion.

Fig.4

Time courses of the effects of 15 drugs on the absolute values (ms) of QTcF. Data are presented as the mean \pm SE (n=4 for each treatment group). Three escalating i.v. doses of each drug (circles) were cumulatively infused over 30 min, respectively. $P<0.01$ and $P<0.05$ indicate statistically significant difference in the values between the vehicle (open circles)- and drug (closed circles)-treated groups. N.S means that there is no statistically significant difference in the values between the vehicle- and drug-treated groups.

Fig.5

Time courses of the effects of 15 drugs on the percent changes (%) of QTcF. Data are presented as the mean \pm SE (n=4 for each treatment group). Three escalating i.v. doses of each drug (circles) were cumulatively infused over 30 min, respectively. $P<0.01$ and $P<0.05$ indicate statistically significant difference in the values between the vehicle (open circles)- and drug (closed circles)-treated groups. N.S means that there is no statistically significant difference in the values between the vehicle- and drug-treated groups.

Fig.6

Relationship between logP and hERG IC₅₀ (6A top); that between logP and heart/plasma concentration ratio (6A middle); that between hERG IC₅₀ and heart/plasma concentration ratio (6A bottom); that between logP and ED₁₀ (6B top); that between heart/plasma concentration ratio and ED₁₀. (6B middle); and that between hERG IC₅₀ and ED₁₀. (6B bottom). Terfenadine (Ter), bepridil (Bep), haloperidol (Hal), thioridazine (Thi) and *dl*-sotalol (Sot) (red circles); pimoziide (Pim), moxifloxacin (Mox) and sparfloxacin (Spa) (yellow circles); and imipramine (Imi), diphenhydramine (Dip), ketoconazole (Ket) (blue circles) have been reported to have high, moderate and low risks of drug-induced torsade de pointes, respectively (6). hERG IC₅₀: 50% inhibitory concentration for human ether-a-go-go related gene channel current.

Fig1

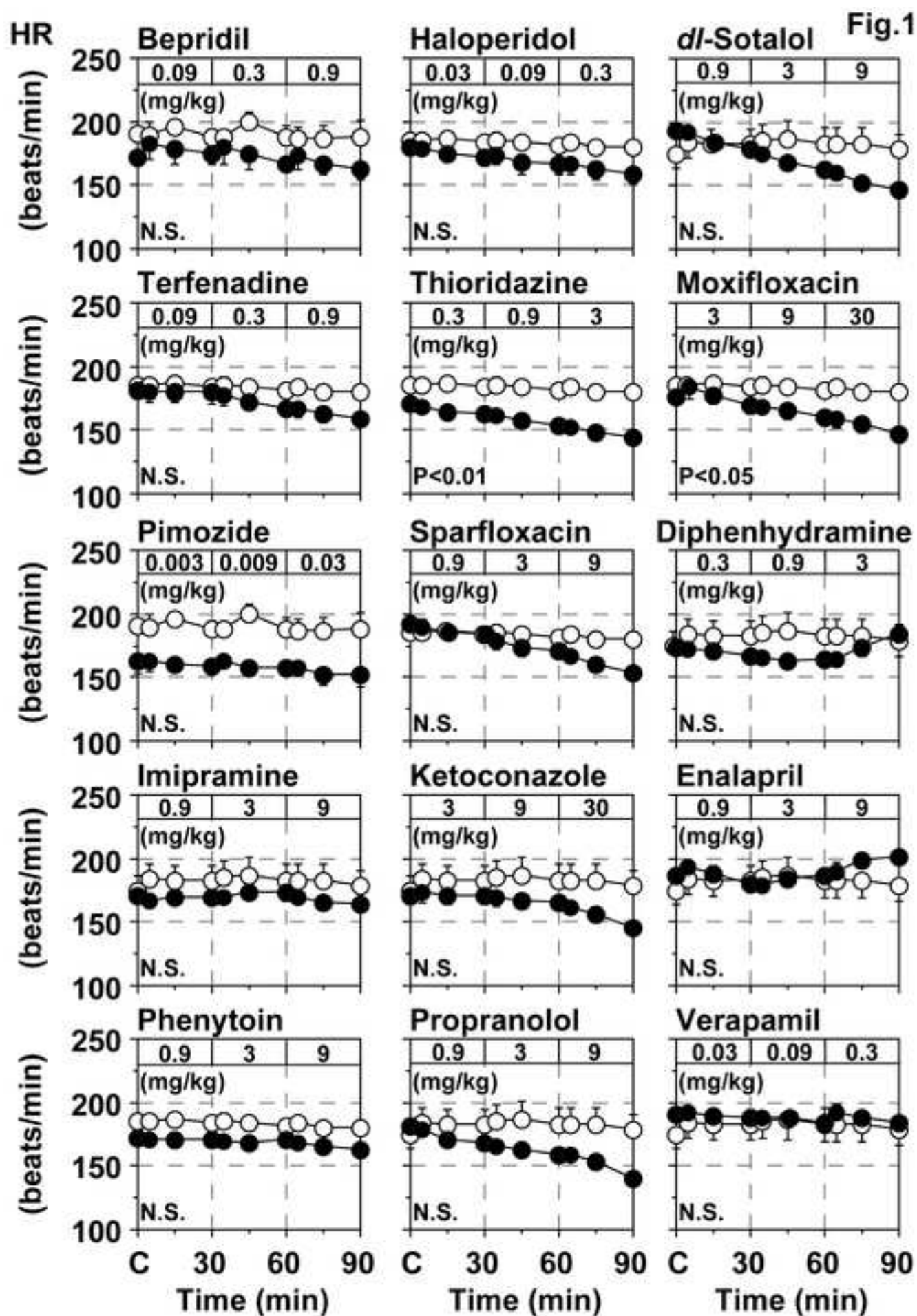
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Fig2

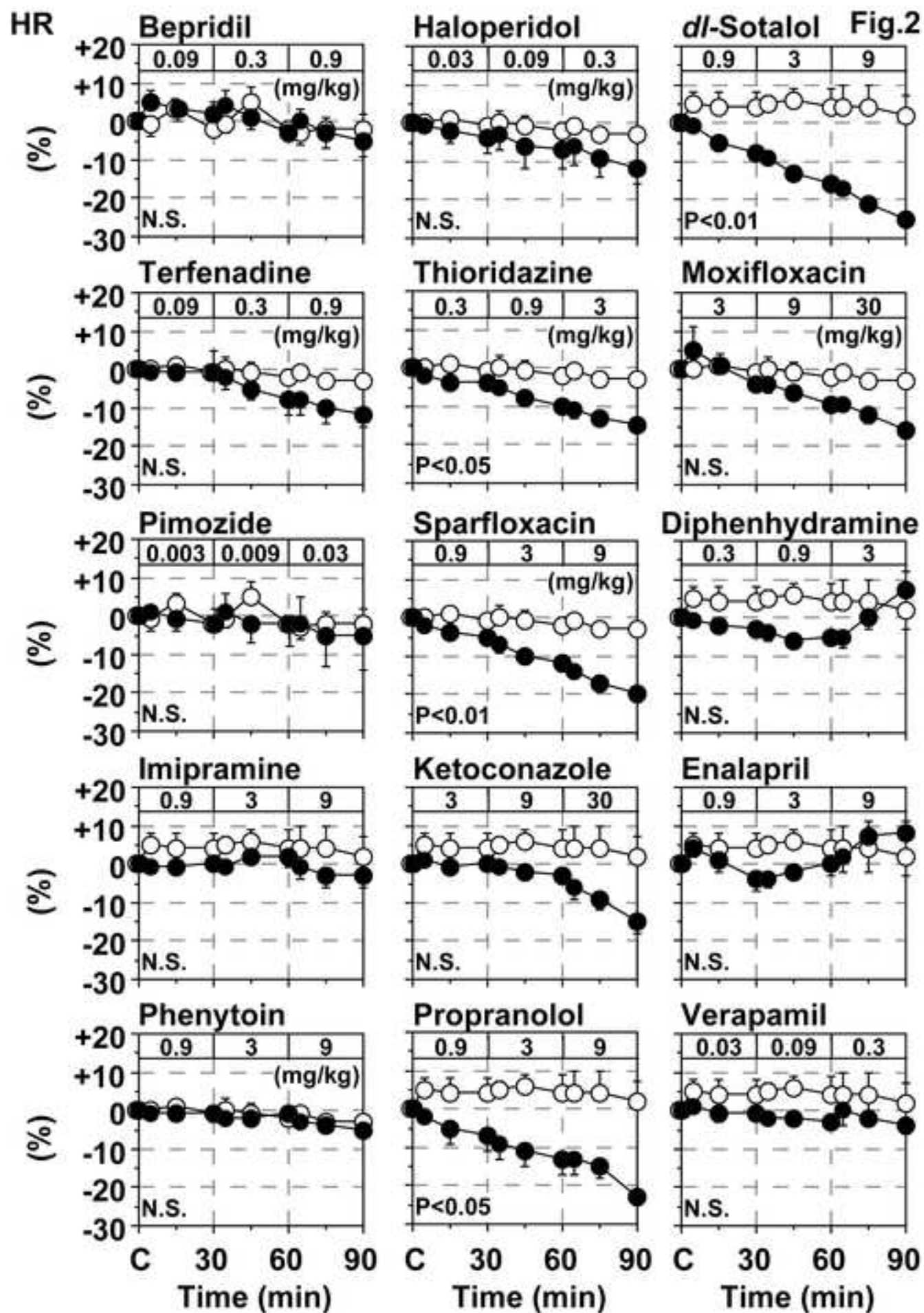
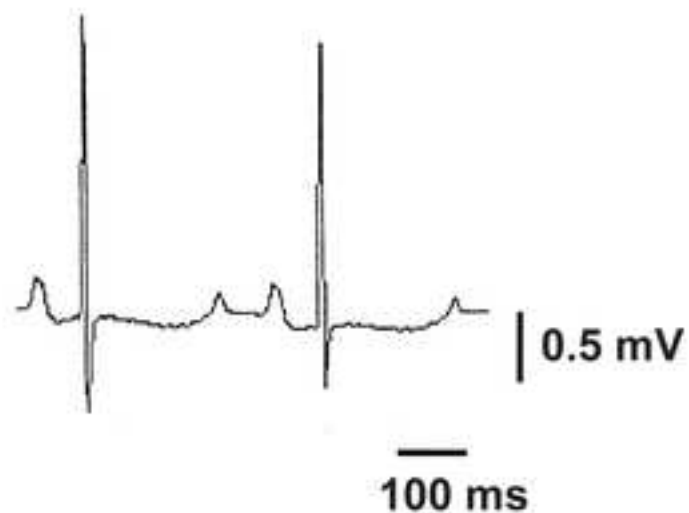
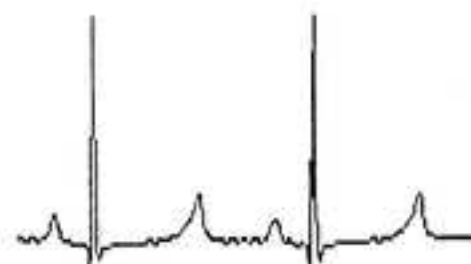
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Fig.3

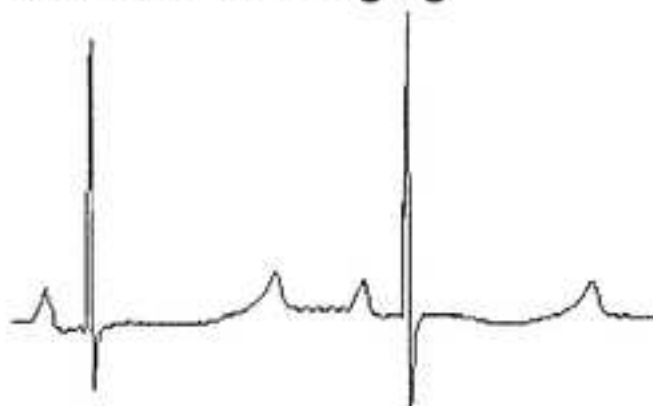
A. *dl*-Sotalol
Control



B. Terfenadine
Control



30 min after 9 mg/kg



30 min after 0.9 mg/kg

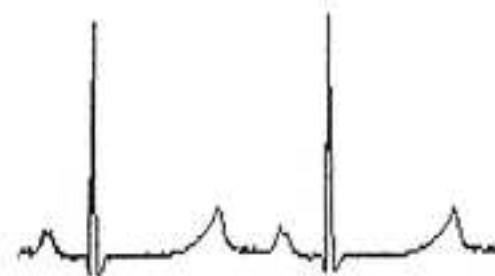


Fig4
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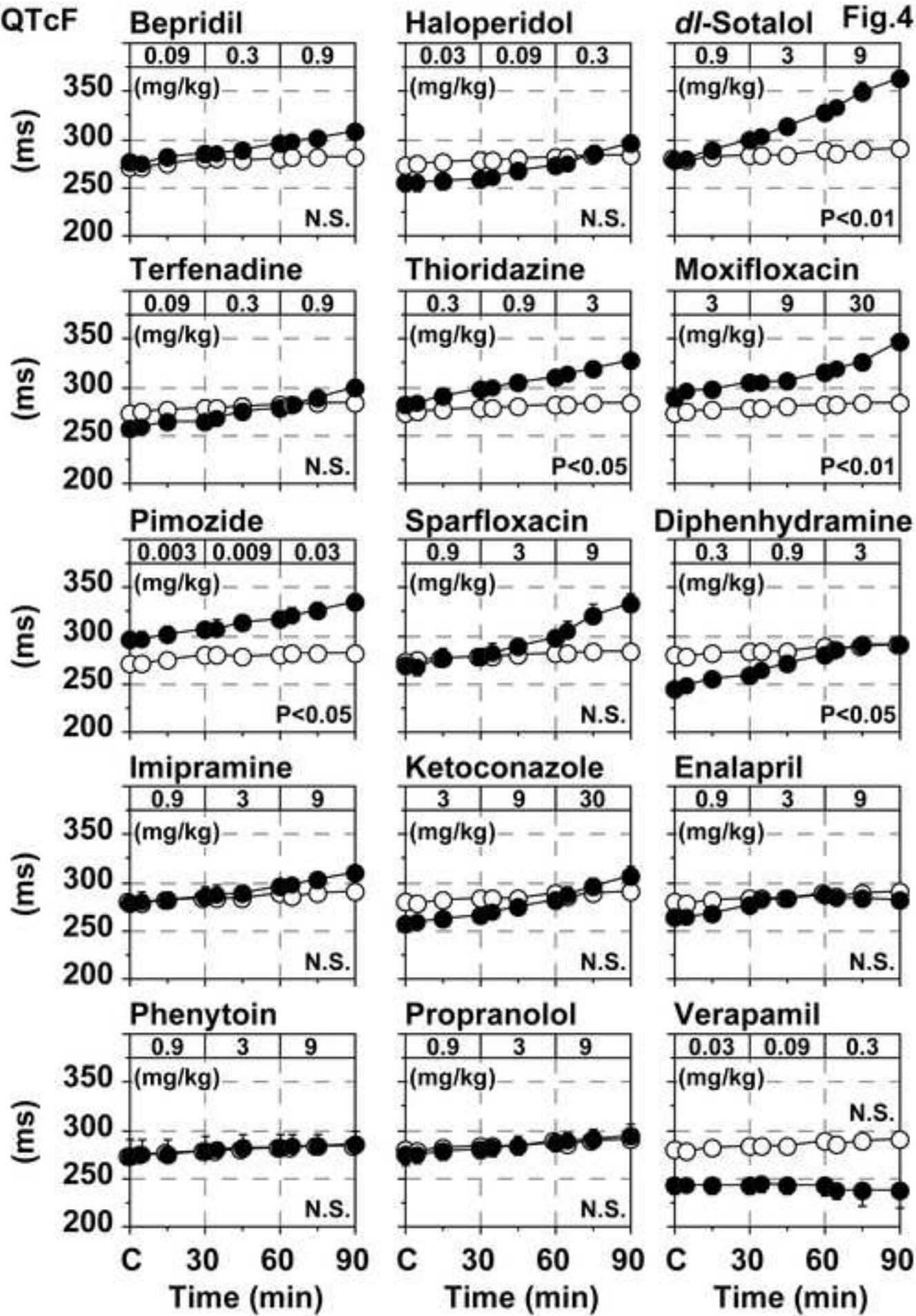


Fig5

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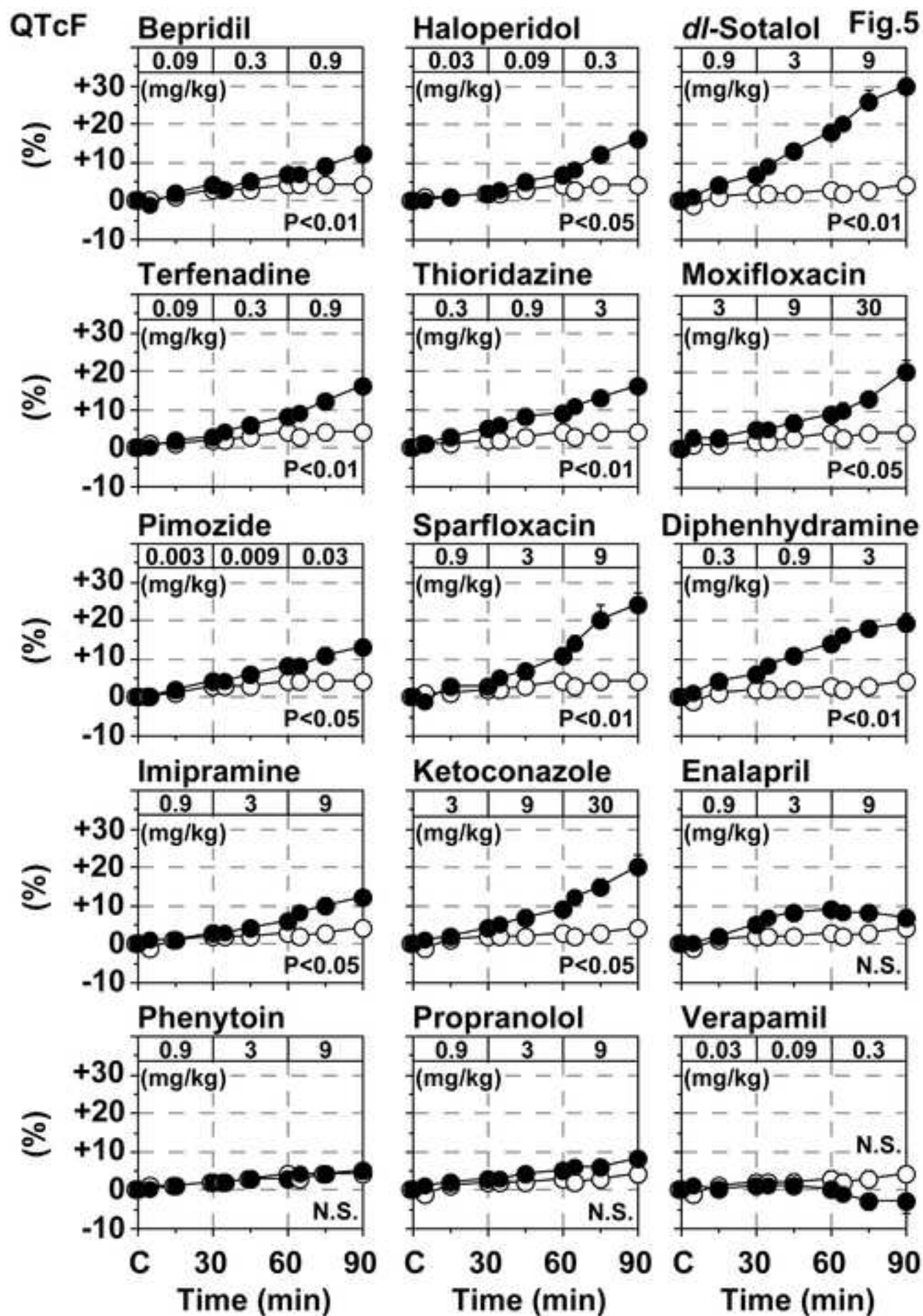


Fig.6

