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Short communication

# Possibility as an anti-cancer drug of astemizole: Evaluation of arrhythmogenicity by the chronic atrioventricular block canine model

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ABSTRACT

Since astemizole in an oral dose of 50 mg/kg/day was recently reported to exert anti-cancer effect in mice, we evaluated its proarrhythmic potential using the atrioventricular block dogs in order to clarify its cardiac safety profile. An oral dose of 3 mg/kg prolonged the QT interval without affecting the QTc ($n = 4$), whereas that of 30 mg/kg increased the short-term variability of repolarization and induced premature ventricular contractions in each animal, resulting in the onset of torsade de pointes in 1 animal ($n = 4$). Thus, proarrhythmic dose of astemizole would be lower than anti-cancer one, limiting its re-profiling as an anti-cancer drug.



While astemizole was developed as a second-generation histamine type 1 antagonist, it has been shown to block *human ether-á-go-go-related gene* (hERG) $K^+$ channel (1), and to induce the QT-interval prolongation and torsade de pointes in patients, which led the drug to withdraw from the commercial market in 1999 (1). Recently, *ether à-go-go-1* (Eag1) as well as hERG $K^+$ channels have been reported to be highly expressed in various cancer cells, and blockade of either channel was shown to inhibit proliferation of the cancer (2). Since astemizole was found to inhibit Eag1 in addition to hERG $K^+$ channels, it has gained enormous interest again as a new anti-cancer drug, (1,3). By now, astemizole has been shown to suppress cervical cancer cell proliferation by increasing apoptosis at concentrations of 2 and 5 μM in vitro (3); moreover, to inhibit human breast cancer cell growth in an oral dose of 50 mg/kg/day for 3 weeks in mice (4). Since experimental evidence showing a direct causal link between astemizole administration and onset of torsade de pointes remains still limited (5), we precisely evaluated the extent of its proarrhythmic potential in order to begin to re-profile astemizole as an anti-cancer drug. For this purpose, we adopted the chronic atrioventricular block canine model, which has been widely used for quantifying the torsadogenic potential of many drugs (6).

All experiments were approved by the Animal Research Committee for Animal Experimentation of Toho University (No. 15-55-152) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University. Atrioventricular block model was prepared using 4 beagle dogs of either sex weighing approximately 10 kg. The animals were obtained through Kitayama Labes Co., Ltd. (Nagano). The catheter ablation was used for producing complete atrioventricular block with an onset of stable idioventricular escaped rhythm as previously described (6).

Experiments were performed ≥4 weeks after the induction of complete atrioventricular block (6). A Holter recording and analysis system (QR2100 and HS1000, Fukuda M-E Kogyo Co., Ltd., Tokyo) was used to record and analyze electrocardiogram over 24 h. The effects of astemizole on the idioventricular rate, QT interval and corrected QT calculated with the Fridericia's formula (QTcF): $QTcF = QT/(RR/1000)^{1/3}$ in addition to its proarrhythmic events were assessed without anesthesia. The idioventricular rate, QT interval and QTcF were expressed as the mean of 10 consecutive complexes. In this study, torsade de pointes was defined as a polymorphic ventricular tachycardia associated with QT-interval prolongation, consisting of 5 beats or more twisting QRS complexes around the baseline (7). On the first day, 2 h after the start of Holter electrocardiogram recording, 3 mg/kg of astemizole (Sigma–Aldrich, St. Louis, MO, USA) was orally administered at 10:00 am with gelatin capsules (J.P No.00, Kobayashi capsule, Hyogo). On the second day, 30 mg/kg of the drug was orally administered in the

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same manner. The electrocardiogram variables were analyzed 0.5–1 h before, and 1, 2, 3, 4, 6, 8, 12 and 21 h after the start of the administration.

Poincaré plots with $QT_n$ versus $QT_{n+1}$ were prepared for each of two analysis time points; namely, electrocardiogram of 51 consecutive beats under the stable idioventricular rhythm without ectopic activity was adopted before and about 2 h after the administration of 30 mg/kg of astemizole. The mean orthogonal distance from the diagonal to the points of the Poincaré plot was determined as short-term variability ($=\Sigma|QT_{n+1} - QT_n|/[50\sqrt{2}]$), whereas the mean distance to the mean of the parameter parallel to the diagonal of the Poincaré plot was done as long-term variability ($=\Sigma|QT_{n+1} + QT_n - 2QT_{mean}|/[50\sqrt{2}]$) (8).

Data are presented as the mean ± S.E.M. The statistical analysis was performed by using a software GraphPad Prizm 6 (ver6.03, GraphPad Software, Inc., La Jolla, CA, USA). The statistical significances within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by a post-hoc test for mean values comparison. A $p$-value <0.05 was considered to be statistically significant.

The time courses of the changes in the electrocardiogram variables and the number of surviving animals after the administrations of astemizole are summarized in Fig. 1 (n = 4 for each dose). The pre-drug control values (C) of the idioventricular rate, QT interval and QTcF were 31 ± 2 beats/min, 319 ± 18 ms and 256 ± 13 for the low dose of 3 mg/kg, and those were 31 ± 1 beats/min, 331 ± 17 ms and 270 ± 16 for the high dose of 30 mg/kg, respectively. The low dose prolonged the QT interval for 6–12 h, whereas no significant change was detected in the other variables. The high dose hardly affected any of these variables.

Typical tracings of the electrocardiogram showing premature ventricular contractions by the high dose are depicted in Fig. 2A, whereas its results of onset of premature ventricular contractions and torsade de pointes, and of mortality are summarized in Fig. 2B. The low dose did not induce premature ventricular contraction or torsade de pointes. The high dose frequently induced premature ventricular contractions in each animal, but it did not change the focus of idioventricular rhythm during the observation period as shown in Fig. 2A. Moreover, the high dose induced torsade de pointes in 1 out of 4 animals at 2.6 h degenerating into the ventricular fibrillation, which resulted in the animal's death (#3). Two animals (#2 and #4) were found to be dead 1 and 2 days after the cessation of the Holter electrocardiogram recording, respectively.

Poincaré plots for each animal were depicted in Fig. 2C, indicating that short-term variability increased in each animal irrespective of the QT-interval changes. The short-term variability of repolarization increased, whereas no significant change was detected in the long-term variability as summarized in Table 1.

Since the chronic atrioventricular block model was found to simulate a patient who was the most sensitive to blockers of rapidly activating delayed rectifier $K^+$ current, we have proposed the following risk stratification of proarrhythmia (6). When torsade de pointes was induced by 1–3 times of the therapeutic dose, the drug can be considered to have high risk of proarrhythmia. If torsade de

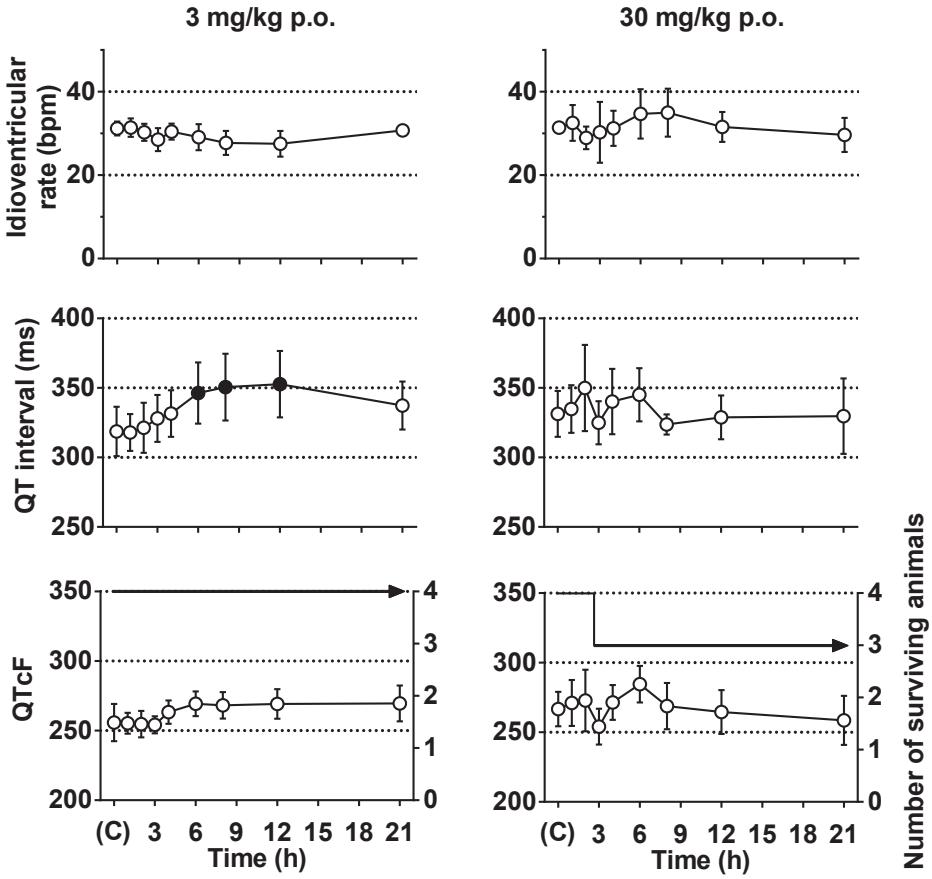


**Fig. 1.** The time courses of the changes in the idioventricular rate (top), QT interval (middle), and QTcF and the number of surviving animals (bottom) before and after the oral administration of astemizole of 3 (left) and 30 (right) mg/kg (n = 4 for each dose). Larger variability of the QT interval together with decrease of the number of surviving animals after the high dose administration would have made the statistical power smaller to detect the QT-interval prolongation. Data are presented as mean ± S.E.M. Closed symbols represent significant changes from the respective pre-drug control values (C) by $p$-value <0.05.