Hemodynamic and electrophysiologic effects of ontazolast in dogs

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Objective—To determine whether QT interval is prolonged or sudden death is caused by ventricular fibrillation resulting from torsades de pointes and to identify hemodynamic effects of ontazolast.

Animals—28 Beagles.

Procedure—Physiologic variables were measured for 2 hours in conscious dogs given ontazolast (0, 1, or 3 mg/kg of body weight, IV) and for 1 hour in anesthetized dogs given cumulative doses of ontazolast (0, 1, 3, 6, or 9 mg/kg, IV).

Results—Ontazolast prolonged QT interval and QT interval corrected for heart rate (QTc) at doses of 6 mg/kg in anesthetized dogs. At 8 mg/kg, both variables remained prolonged but tended to decrease. In conscious dogs, ontazolast increased QT interval and QTc 15 minutes after administration, but both variables returned to reference ranges by 60 minutes. In conscious dogs, ontazolast increased maximum rate of increase of left ventricular pressure and maximal velocity of fiber shortening, indicators of inotropy, and increased tau, indicating a decreased rate of relaxation. One conscious dog receiving 3 mg/kg developed nonfatal torsades de pointes, but another conscious dog developed ventricular fibrillation. Two anesthetized dogs receiving 6 mg/kg developed early afterdepolarizations, and all dogs developed secondary components in their T waves.

Conclusion and Clinical Relevance—Ontazolast possesses potent class-III antiarrhythmic properties and induces prolongation of QTc in a dose-dependent fashion. Because there was a clear dose-dependent prolongation of QT interval in all instances, ontazolast may serve as a positive-control compound for studying other compounds that are believed to prolong the QT interval. (Am J Vet Res 2000;61:1364–1368)

Prolongation of the QT interval of the ECG and sudden death was observed in a number of dogs and monkeys during studies evaluating the safety of ontazolast, a leukotriene B4 inhibitor. We hypothesized that these findings resulted from torsades de pointes terminating in ventricular fibrillation, rather than from acute hemodynamic effects unrelated to electrophysiologic activity. Therefore, the purpose of the study reported here was to investigate electrophysiologic and hemodynamic effects in dogs after administration of graded doses of ontazolast.

Materials and Methods

The study was approved by an institutional laboratory animal care and use committee. Dogs were cared for in accordance with recommendations established in the Guide for the Care and Use of Laboratory Animals. Twenty-eight healthy male and female beagles that weighed between 7 and 11 kg were used in the study. Sixteen were conscious during monitoring, and 12 were anesthetized by administration of morphine (1 mg/kg of body weight, IV) followed by alpha chloralose at 100 mg/kg. IV)

Anesthetized dogs were mechanically ventilated with room air at a rate of 15 breaths/min and a tidal volume of approximately 15 to 20 ml/kg, sustaining Paco2 between 35 and 45 mm Hg, PaO2 > 80 mm Hg, and pH 7.35 to 7.45. Body temperature was maintained between 37.0 and 37.5 C. One day before start of the study, dogs that were monitored while conscious were anesthetized with propofol and 6-F catheter introducers were inserted into a jugular vein and carotid artery. After dogs recovered from anesthesia, miniaturized solid-state pressure transducers were inserted through the introducers and placed into the left ventricle and ascending aorta to measure pulsatile pressures from those positions. Myocardial contractility was estimated by maximum rate of increase of left ventricular pressure (dLVP/dtmax) and maximal velocity of fiber shortening (vmax). Although dP/dtmax is used commonly to estimate contractility, this variable is also dependent on preload. Values for vmax are an estimate of the maximal velocity of fiber shortening at zero load, obtained by extrapolating, to an intraventricular pressure of 0, the graph of dLVP/dtmax vs. P during the period of isovolumetric contraction. The constant (k) for myocardial stiffness is omitted from this equation.

Myocardial lusitropy, a term used to describe ease of relaxation, was estimated as the reciprocal of tau. Tau is the duration required for intraventricular pressure to decrease 63% from the time of dLVP/dtmax to a pressure of 10 mm Hg greater than left ventricular end-diastolic pressure. It is believed to relate inversely with the rate of resequstration of calcium from troponin-C to the sarcoplasmic reticulum. Whereas Weiss et al used a value of –1 divided by the slope of the decay in pressure measured immediately after dP/dtmax, we calculated tau directly from the decay in pressure up to an end-diastolic pressure of 10 mm Hg above the end-diastolic pressure, a method used commonly in clinical cardiology. Tau, an estimate of stiffness, relates inversely with lusitropy.

A flow-directed fluid-filled thermistor-tipped catheter was advanced so that a port, which could be used for recording pressure or injection of room-temperature saline (0.9% NaCl solution), was positioned in the right atria, and the thermistor bead for measuring thermodilution cardiac output and a second port for recording pressure were placed in the pulmonary trunk. Systemic vascular resistance was estimated as follows: (mean aortic pressure – mean right atrial pressure)/cardiac output. Aortic impedance (Zao) was estimated as follows: pulsatile aortic pressure/stroke volume. Stroke volume was estimated as follows: cardiac output/heart rate. The ECG leads I, aVF, and V3 were recorded. The QT corrected for heart rate (QTc) was calculated by use of the method of Fridericia, as follows: QTc = QT/√RR. Conscious

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dogs received IV injections of vehicle (4 dogs) or ontazolast (1 mg/kg, 6 dogs; 3 mg/kg, 6 dogs). All physiologic signals were digitized on-line at a frequency of 1 kHz, and values of variables from the digitized signals were measured during a control period and 15, 30, 60, and 120 minutes after administration, using a commercially available system.

In anesthetized dogs, a multielectrode catheter was placed in the right ventricle to search for a tendency toward arrhythmia by use of programmed electrical stimulation (PES). The PES was performed, using a 2-millisecond application of current at twice the middiastolic threshold. The basic cycle length for S1 was 300 milliseconds, whereas S2 was detected 15 milliseconds after the effective refractory period (RP) of the terminal S1, and S3 was detected 15 milliseconds after the effective refractory period of S2. Increased irritability caused by triggered activity or reentry would have been considered when 3 unprovoked ventricular depolarizations were detected or when an unprovoked ventricular depolarization was detected following PES.

In the dogs anesthetized with alpha chloralose, in addition to ECG leads I, aVF, and V3, a bipolar electrogram was obtained with one pole impinging on the right ventricular endocardium and the second pole, which was 5 mm distant, positioned in the right ventricular lumen and not touching the myocardium. This electrogram has been used to approximate the time course of, but not the absolute voltage for, a monophasic action potential (MAP). The MAP was measured conventionally to determine duration of action potential at 50% repolarization (APD50) and 90% repolarization (APD90) and for early afterdepolarizations (ie, depolarizations detected during the descending limb [phase 3] of, and triggered by, the preceding action potential). Six dogs received single boluses IV resulting in cumulative doses of 0 (control, vehicle-PEG 400), 1, 3, 6, and 8 mg of ontazolast/kg. Six dogs were used as time-control dogs for the study (ie, given the same amount and volume of PEG). Recordings of all physiologic variables were made approximately 15 minutes after the end of each dose.

We are not aware of pharmacokinetic data for ontazolast in dogs; however, plasma concentration of ontazolast in rats decreased in an apparent triexponential manner, with an extremely rapid early exponential (distribution) phase, a middle (clinically relevant) exponential phase with a half-life of more than 3 hours, and a terminal phase with a half-life that was impossible to estimate, because the concentrations were so low. Because all measurements in the study reported here were made within 1 hour after initial administration, negligible amounts of ontazolast were excreted, and we assumed that the cumulative dose was the sum of all doses administered.

Data for each group (ie, time-control dogs and principals) were compared by use of a 2-way ANOVA with repeated-measures design among and within groups. When a significant F-statistic was obtained, specific means were compared by use of the Schelle posthoc analysis among groups and contrast within groups. A value of P < 0.05 was considered to be significant. Graphs were made of the percentage
change of each variable, compared with the measurement obtained before dose administration.

**Results**

Conscious dogs—When comparing values at the same time periods for dogs that received 1 or 3 mg of ontazolast/kg with those for dogs that received vehicle (time-controls), we did not detect changes in heart rate, PQ interval, QRS duration, cardiac output, stroke volume, systemic vascular resistance, Zao, left ventricular end-diastolic or right atrial pressures, tau, or aortic pressure; however, QT interval, QTc, dLVP/dt max, and vmax all changed in dogs given 3 mg/kg but did not change in dogs given 1 mg/kg (Fig 1 and 2). Tau increased significantly for dogs given doses of 1 and 3 mg/kg, compared with values obtained before administration. Significant changes in QT interval and QTc were greatest 15 minutes after administration and returned to values not significantly different from values obtained before administration by 60 minutes. Following the recording made 15 minutes after administration, 1 dog that received a dose of 3 mg/kg died of ventricular fibrillation, and another dog that received the same dose developed torsades de pointes (Fig 3), but it survived. Both arrhythmias developed spontaneously. None of the dogs had an arrhythmia provoked by use of PES.

Anesthetized dogs—Heart rate slowed in dogs given a dose of 1 mg of ontazolast/kg (Fig 4), but mean systemic arterial pressure did not change significantly. However, QT interval and QTc were prolonged for dogs given doses of 3 to 8 mg/kg, compared with values for time-control dogs, but these variables were prolonged the greatest for dogs given a dose of 6 mg/kg and actually tended to be less prolonged for dogs given a dose of 8 mg/kg. There was significant prolongation of RP, APD50, and APD90; APD50, APD90, and RP all had similar peaks in dogs given a dose of 6 mg/kg and a tendency to reverse in dogs given a dose of 8 mg/kg.

![Figure 3](image-url)

*Figure 3—Representative graph of the effect of ontazolast on the electrocardiogram (lead V3) in a recording obtained 15 minutes after IV administration of 3 mg of ontazolast/kg in a conscious dog. Torsades de pointes is evident during the indicated period.*

![Figure 4](image-url)

*Figure 4—Effects of ontazolast on heart rate (A), QT interval (B), QTc (C), effective refractory period (D), action potential duration at 50% of repolarization (APD50; E), and action potential duration at 90% of repolarization (APD90; F) after administration in anesthetized dogs. ● = Ontazolast (1, 3, 6, and 8 mg/kg, IV; 6) ○ = Vehicle (6). See Fig 1 for remainder of key.*
During recording of APD₅₀ and APD₉₀ following administration of the dose at a rate of 6 mg/kg, 2 dogs developed early afterdepolarizations (Fig 5). Similar to the conscious dogs, an arrhythmia was not provoked in any anesthetized dogs by use of PES.

Conscious and anesthetized dogs—in both groups of dogs receiving ontazolast, T-waves in leads aVF and V₃ increased in duration and became greatly negative (Fig 5) or developed a second peak (Fig 6), which was possibly a U-wave or was triggered after depolarization.

**Discussion**

At the same time periods, a dose of 1 mg of ontazolast/kg in conscious dogs did not cause significant changes in any variable measured or calculated, compared with values of dogs receiving vehicle. However, QT interval and QTc of dogs given 1 mg/kg appeared to be greater 15 minutes after administration than in the values obtained before administration. These measures of duration of electrical systole (ie, depolarization and repolarization) peaked at 15 minutes after administration, with QT interval prolonged a mean of 77 milliseconds and QTc prolonged a mean of 62 milliseconds over values obtained before administration.

Federal drug regulatory agencies have great interest in drugs that could potentially prolong repolarization (ie, increase QT interval), because delayed repolarization may lead to sudden death attributable to polymorphic ventricular tachycardia (torsades de pointes) and ventricular fibrillation. In fact, any compound intended for use in humans that prolongs QT interval must be analyzed for its potential to alter specific membrane potassium-ion channels.

Similarly, a dose of 3 mg/kg did not cause changes in measured variables, except for QT interval, QTc, dLVP/dt_max, and v_max, compared with values for the vehicle group. At 15 minutes after administration of a dose of 3 mg/kg, QT interval (mean, 199 milliseconds; \( P = 0.008 \)) and QTc (mean, 195 milliseconds; \( P < 0.001 \)) were significantly prolonged, compared with values obtained before administration (QT interval, 77 milliseconds; QTc, 62 milliseconds). It was expected that QTc would have been prolonged less than QT interval, because heart rate slowed, and a portion of the QT interval prolongation undoubtedly was attributable to the reduction in heart rate. The electrophysiologic mechanism for such prolongation is not known, but it may involve depression of potassium-ion channels (\( I_{Kp}, I_{Kr}, I_{TO} \)) or an increase in conductance over \( I_{Ca} \). Such prolongation of the QT interval and QTc may serve as the electrophysiologic substrate for torsades de pointes,\(^{12,14}\) which we observed in 1 dog but which also has been observed in other studies in numerous conscious dogs and monkeys. We recorded early afterdepolarizations in 2 dogs, but in all dogs at all doses of ontazolast, the T waves for the aVF and V₃ leads went from positive to negative or developed secondary peaks that probably represented early afterdepolarizations, similar to those recorded in the 2 dogs with the nonsuction-type endocardial electrode. These changes in the T wave undoubtedly reflected alterations in conductances of specific membrane ion channels, most likely potassium channels.
The fact that arrhythmias could not be induced by use of PES in either group of dogs may indicate that the stimulation protocol was not sufficiently robust or that the mechanism for arrhythmia was not amenable to study by use of this method. Arrhythmias resulting from increased automaticity usually are not provoked by programmed stimulation, whereas arrhythmias induced by reentry are most amenable to study by stimulation, and those induced by triggered activity may or may not be provoked.11 The fact that none of the anesthetized dogs developed arrhythmias after administration of ontazolast is consistent with known antiarrhythmic properties of morphine and alpha chloralose.

It appears that ontazolast possesses potent class-III antiarrhythmic properties in that it prolongs repolarization in a dose-dependent manner but does not have an effect on depolarization (ie, QRS duration was not prolonged). Furthermore, because of its dose-response association, it may be an excellent compound to serve as a positive-control compound for investigation of effects of other compounds on repolarization. Unlike other compounds that prolong QT interval, use of ontazolast induced a 100% yield of prolongation in a dose-dependent manner.

Future studies must be directed at establishing the precise mechanisms by which ontazolast prolongs repolarization, especially the effects on specific ion channels. We do not understand the reason that anesthetized dogs had a peak prolongation of repolarization (APD50 and APD90) when given 3 to 6 mg/kg, whereas administration of 8 mg/kg caused less prolongation. Azimilide, a class-III antiarrhythmic, causes similar effects in canine ventricular myocytes, because it prolongs QT interval by blocking potassium channels when used at a concentration of 1 µM and then shortens QT interval by blocking calcium channels when used at a concentration of 5 µM.12 It also is clear that in conscious dogs receiving 3 mg/kg, inotropy significantly (P < 0.001) increased (ie, dLVP/dtmax) increased from a mean value prior to administration of 4.543 mm Hg/s to a mean 30 minutes after administration of 6.114 mm Hg/s). Tau, a variable that relates inversely to lusitropy, increased significantly (P = 0.002) from a mean value of 19.9 milliseconds prior to administration to a mean value of 33.2 milliseconds at 15 minutes after administration. This apparent increase in inotropy and decrease in lusitropy could be explained by increased calcium ions entering a cell or by a decreased rate of sequestration of calcium by the sarcoplasmic reticulum.13 Thus, it appears that ontazolast may affect potassium and calcium channels.

Analysis of results of the study reported here supported our hypothesis on the cause of sudden death in animals after administration of ontazolast. We believe that sudden death in response to ontazolast was not caused by hemodynamic consequences but was most likely caused by torsades de pointes deteriorating into ventricular fibrillation.

References


