The class III antiarrhythmic drugs dofetilide and sotalol prevent AF induction by atrial premature complexes at doses that fail to terminate AF

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Abstract

**Background:** Clinical trials suggest that sotalol and dofetilide are much more effective in preventing atrial fibrillation (AF) than in terminating it. This study evaluated potential mechanisms of discordant sotalol and dofetilide effects on AF termination vs. prevention.

**Methods:** We applied 240-electrode epicardial mapping and programmed stimulation in a vagotonic dog model of AF before and after dofetilide or sotalol.

**Results:** Under control conditions, sustained AF could be induced by single \textit{S}\textsubscript{1} extrastimuli that caused unidirectional block and macroreentry. Sotalol (2 mg/kg) and dofetilide (0.04 mg/kg) failed to terminate AF in any dog, but prevented AF induction by \textit{S}\textsubscript{1} stimuli in 19/22 (86\%) and 4/5 (80\%) of animals, respectively. With sotalol and dofetilide, unidirectional block still occurred, but wavefront reentry failed. The prevention of \textit{S}\textsubscript{1}-induced reentry was related to large increases in the effective refractory period (ERP) at a basic cycle length (BCL) of 1000 ms, leading to ERPs that exceeded the conduction delay following \textit{S}\textsubscript{1}. Reverse use-dependent effects resulted in smaller ERP increases at BCLs closer to the AF cycle length. Although the number of zones of reactivation per cycle during sustained AF were decreased by sotalol and dofetilide, the changes were small and insufficient to terminate AF. **Conclusions:** Sotalol and dofetilide prevent AF initiation by premature depolarizations at doses that fail to terminate vagotonic AF, by increasing ERP at the basic cycle length beyond the associated conduction delay that leads to reentry.

Keywords: Antiarrhythmic agents; Arrhythmia (mechanisms); Supraventricular arrhythmia

1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice and causes a wide range of potential complications [1]. The treatment of AF remains problematic [2]. A better understanding of the mechanisms determining antiarrhythmic drug efficacy would help in improving therapy. With the awareness of the risks of potent class I antiarrhythmic drugs obtained from the Cardiac Arrhythmia Suppression Trial [3] and subsequent analyses [2], antiarrhythmic drug development shifted to class III agents [4]. Clinical trials have shown class III drugs to be relatively ineffective in terminating AF, but effective in preventing AF recurrence [5]. Experimental studies in vagotonic and atrial tachycardia-related models of sustained AF have demonstrated a limited capacity of clinically-relevant doses of rapid delayed rectifier (\textit{I}_{\text{Kr}})-selective blocking class III agents to terminate AF [6–8]. In a previous report from our laboratory, we suggested that reverse use-dependent actions may limit class III efficacy at the rapid rates of AF, but permit them to prevent AF induction by atrial premature complexes (APCs) at slower resting sinus rates [6]. This concept has never been tested experimentally. We therefore designed the present study to (1) determine whether sotalol and dofetilide doses that are ineffective in terminating AF are capable of preventing AF...
induction by APCs and (2) use epicardial mapping to evaluate the mechanism by which AF induction is prevented.

2. Methods

2.1. General preparation

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Adult mongrel dogs (27.2±0.6 kg) were anesthetized with morphine (2 mg/kg i.m., 0.5 mg/kg i.v. every 2 h) and α-chloralose (120 mg/kg i.v., 29.25 mg/kg/h i.v.), and ventilated at 20–25/min. Arterial blood gases were measured every 2 h and kept in the physiological range (SaO₂>90%, pH 7.38–7.44). Catheters were inserted into a femoral artery and both femoral veins. Body temperature was maintained at 37–39°C with a temperature-control system.

The heart was exposed via a sternotomy and a pericardial cradle created. A programmable stimulator (Digital Cardiovascular Instruments, Berkeley, CA) was used to stimulate the right atrium with 2-ms, twice diastolic-threshold pulses. A ventricular demand pacemaker was used to stimulate the ventricles at 80/min when the ventricular rate became excessively slow. The vagus nerves were isolated and divided in the neck. To block cardiac β-adrenergic effects, nadolol was administered at 0.5 mg/kg i.v., followed by 0.25 mg/kg i.v. every 2 h. The sinus node was crushed to permit atrial capture at cycle lengths comparable to resting cycle lengths (800–1000 ms) in man.

2.2. AF model

Experiments were performed in a well-characterized model of vagotonic AF [6,7]. This model was chosen over the atrial-tachycardia model [8,9] because repeated inductions of AF were needed to characterize susceptibility to AF induction by APCs. Repeated episodes of sustained AF would require repeated electrical cardioversions in the atrial-tachycardia model. Sustained vagotonic AF can be terminated by stopping vagal stimulation, without requiring electrical cardioversion, and permitting the subsequent resumption of vagotonic conditions by re-instituting vagal stimulation during sinus rhythm.

Bipolar hook electrodes (stainless steel insulated with Teflon except for the distal 1–2 cm) were inserted within and parallel to each vagus nerve. Bipolar stimulation (0.1-ms square-wave pulses, 10 Hz, 60% of voltage for asystole) was applied (Grass DS-9F stimulator) continuously during AF, the measurement of ERP and conduction velocity (CV), and AF induction.

2.3. Measurement of electrophysiological variables

Activation maps for CV measurements were obtained after 45 s of pacing at the right atrial appendage at basic cycle lengths (BCLs) of 200, 300, 400 and (whenever possible)1000 ms. Fifteen basic (S₁) stimuli were followed by a premature (S₂) stimulus at an S₁S₂ interval that was reduced by 10-ms decrements from the BCL, with the ERP defined as the longest S₁S₂ interval failing to produce a response. AF was then induced with single S₁s at a BCL of 1000 ms (or the longest cycle length maintaining 1:1 capture if the spontaneous cycle length was <1000 ms), beginning 100 ms beyond the ERP and decrementing by 10-ms intervals. Each coupling interval was studied twice before concluding that AF could not be induced. AF was defined as a rapid (>450/min), irregular atrial rhythm with varying atrial electrogram morphology and as sustained if >20 min without spontaneous termination. A 2-s window of activation data was acquired during AF to analyze activation patterns and measure AF cycle length (AFCL). AF was considered inducible if induced reproducibly on at least two consecutive occasions at a given S₁S₂ coupling interval.

The AFCL was calculated at each of 50 widely-distributed sites by counting cycles over a 2-s activation window. The results at all sites were averaged to obtain the overall mean AFCL. The activation pattern during AF was studied by constructing sequential activation maps. Zones of reactivation were defined as discrete areas of early activation adjacent to zones of late activation in a cycle, that were reactivated at the onset of the next cycle. The number of reactivation zones was determined for three successive AF cycles in each dog under each condition.

CV was determined by analyzing activation during continuous longitudinal propagation at five electrode sites in Bachmann’s bundle (Fig. 1). Distance from the proximal site was plotted against activation time, and CV was determined from the slope of the best-fit regression line (Fig. 1C). The same sites were used for all conduction velocity measurements for each experiment. The wavelength was calculated as CV×ERP.

2.4. Activation mapping

Five silicon plaques containing 240 bipolar electrodes were attached to the epicardial surface of both atria [8,10]. Electrodes consisted of Teflon-coated stainless steel wires (270 µm diameter) with interpolar distances of 1 mm, inter-electrode distances 3–6 mm. A decapolar ring-electrode catheter (10 electrodes, 2.5 mm inter-polar and 10 mm inter-electrode distance) was inserted via the femoral vein and positioned fluoroscopically adjacent to the septum to record five bipolar septal electrograms. Electrogram signals were filtered (10–450 Hz), digitized (12-bit resolution and 1-kHz sampling rate), and transmitted into a Silicon Graphics computer. Activation times were ana-
prepared on the day of each experiment and (for dofetilide) protected from light. Vagal stimulation parameters were defined as described above, and maintenance of AF during 20 min of vagal stimulation under control conditions was verified. AF was then re-initiated and maintained for 20 min, to confirm AF stability. AF was then terminated and AERP and CV were measured at four BCLs (200, 300, 400 and 1000 ms) during vagal stimulation. If AF was induced by single Ss at a BCL of 1000 ms, an activation window was obtained for subsequent mapping and AF was terminated. Following a 5-min rest period, vagal stimulation was re-initiated and AF induction re-tested at the same coupling interval. The window of vulnerability was defined as the range of coupling intervals over which AF was consistently induced by single extrastimuli.

AF was then re-induced during vagal stimulation, and a drug given after 5 min of AF. If AF was not stopped 20 min after drug administration, activation data were obtained and vagal stimulation was discontinued to return to sinus rhythm. Electrophysiologic measurements were then repeated during vagal stimulation, and induction of AF attempted. If sustained AF could not be induced, an S was introduced at the shortest S–S interval maintaining capture to define the ERP of the APC initiated by S, activation data were obtained and the experiment was terminated.

2.6. Statistical analysis

Data are expressed as mean±S.E. Statistical comparisons were made with ANOVA followed a range test (Bonferroni-adjusted t for two-way ANOVA and Tukey’s test for one-way ANOVA).

3. Results

3.1. Electrophysiologic effects during atrial pacing

Neither sotalol nor dofetilide altered CV at any BCL (Fig. 2, left), nor was CV altered for the APC induced at the smallest S–S producing capture (Fig. 2, right). Both drugs significantly prolonged atrial ERP, with changes increasing with increasing BCL (reverse use-dependence, Fig. 3). Changes in wavelength also showed reverse use-dependence (Fig. 4).

3.2. Effects on AF

Neither sotalol nor dofetilide terminated AF in any dog, but both drugs prevented AF induction by single extrastimuli. Under control conditions, single extrastimuli induced sustained AF in all dogs, with a vulnerable window from 97±5 to 111±7 ms in the sotalol group and 83±4 to 96±8 ms in the dofetilide group. Sotalol prevented AF
induction by extrastimuli in 19/22 dogs (86%) and dofetilide prevented AF induction in 4/5 dogs (80%).

To understand the mechanisms by which sotalol and dofetilide prevented AF induction, we analyzed activation maps during AF induction under control conditions and then after drug during application of the most closely-coupled extrastimuli with capture. Fig. 5A shows the induction of AF by a single $S_2$ during stimulation at the right atrial appendage under control conditions. Activation maps are shown, along with selected electrograms at the left. The impulse resulting from the last pulse of the basic train ($S_1$) spreads down the right atrial free wall towards the AV ring, and across Bachmann’s bundle to the left atrium. The upper end of the septum is activated at 71 ms, and the lower end of the septum is activated with a conduction delay (defined as conduction time from initial activation near the stimulation site to activation of the distal septum) of 97 ms. The ERP of the complex resulting from $S_1$ was 100 ms, and an $S_2$ was applied 110 ms after $S_1$ (a coupling interval 10 ms greater than the ERP), resulting in activation shown in the middle panel. This premature complex showed slowed activation, with a conduction delay to the distal septum of 126 ms. This delay is greater than the ERP at the stimulation site, permitting reactivation in the right atrial appendage and resulting in the unstimulated reentrant activation (B3) at the right.

Fig. 5B shows the results of programmed stimulation at the same site in the same dog after sotalol administration. Activation during the last basic complex (left) was not significantly changed by sotalol. Sotalol substantially increased the ERP of basic beats, from 100 (under control) to 220 ms. The middle panel shows the activation resulting from an $S_2$ delivered 230 ms (10 ms greater than the ERP) following $S_1$. The conduction delay to the distal septum was 106 ms and no reentry followed. The right panel shows activation resulting from the earliest complex that could be initiated by an $S_1$ at the same stimulation site, with an $S_2-S_1$ interval of 140 ms, which also failed to induce reentry. The ERP of the complex resulting from $S_2$ was thus 130 ms, longer than the septal conduction delay and potentially accounting for the failure of reentry from the distal septum to the right atrium for the complex initiated by $S_2$. It should be noted that the conduction delay to the distal septum for the complex initiated by $S_1$ was 140 ms, which was likely just greater than the ERP at the

Fig. 2. Left: Conduction velocity measured at cycle lengths of 200, 300, 400 and 1000 ms, during vagal stimulation under control conditions and after sotalol or dofetilide infusion. Right: Conduction velocity of the activation initiated by $S_3$ at the shortest $S_3-S_2$ interval achievable. Values are mean±S.E.
Fig. 3. Effects of sotalol and dofetilide on atrial effective refractory period (ERP). The atrial ERP was measured at basic cycle lengths (BCL) of 200, 300, 400 and 1000 ms. Values are mean ± S.E. *** P<0.001 for frequency-dependence of drug action. Explicit P values are shown vs. control at each BCL.

RA appendage. The lack of reentry with S2 must then be due to block at a site in the reentrant pathway with an ERP longer than the ERP at the RA appendage, or to reentry actually involving a region more proximal in the septum than the distal recording site.

Fig. 6A shows the induction of reentry under control conditions in a dog from the dofetilide group. The septal conduction delay resulting from the earliest S1 with capture (middle panel) was 101 ms (of the same order as the basic ERP at the right atrial appendage), activating the distal septum at 228 ms. This delay was sufficient to activate the right atrial free wall 24 ms later (at 252 ms), resulting in the reentrant response shown at the right. Fig. 6B shows failure of reentry for the earliest-coupled S1 and S2 with capture in the presence of dofetilide. Once again, the septal conduction delay associated with S2 was substantially less than the ERP of the activation caused by S1 (140 ms), accounting for the failure of reentry from the septum to the right atrium.

Under control conditions, premature stimulation at the right atrial appendage generally appeared to cause reactivation of the right atrium via the septum. Fig. 7 shows mean data from dogs in which sotalol and dofetilide prevented AF induction by single electrically-induced APCs. Under control conditions, the conduction delay to the distal septum slightly exceeded the ERP of the activation caused by S1, permitting reentry. Both dofetilide and sotalol substantially increased the ERP, so that it exceeded the distal septal conduction delay and reentry was prevented.

Fig. 8A shows consecutive cycles (defined as the shortest interval with activation at all sites) during AF before and after sotalol. Zones of reactivation are indicated by the stars. Sotalol decreased slightly the number of zones of reactivation and prolonged the cycle length, consistent with an increase in the ERP; however, multiple reactivation zones are present both before and after the drug. Fig. 8B shows the same type of data for a dog in the dofetilide group, illustrating qualitatively similar phenomena to those seen with sotalol. Fig. 9 shows a quantitative analysis of the AFCL (panel A) and number of reactivation zones (panel B) before and after each drug. Both drugs increased AFCL and decreased the number of reactivation zones per
Fig. 5. Induction of atrial reentry by programmed electrical stimulation under control conditions (panel A) and during sotalol infusion (panel B). Selected electrograms (left) are followed by activation maps of three successive cycles corresponding to the activation windows delimited by the vertical lines superimposed on the electrograms. The premature beat (S₂) was delivered at a coupling interval just beyond the atrial ERP. Asterisks on the activation maps indicate stimulation sites. S₁, S₂, S₃=activations induced by S₁, S₂, and S₃, respectively. B₃=spontaneous cycle following S₃ beat. CD=conduction time from stimulation site to distal septum; ERP=effective refractory period at stimulation site. Arrows indicate direction of wavefront propagation. Double line is site of failure of reentry, presumably due to persistent right atrial refractoriness. Isochrone lines (20-ms isochrones) were generated automatically by a validated computer algorithm. Note that septal activation times (shown in boxes) were out of the epicardial plane and were intentionally excluded from epicardial isochrone calculation.

4.1. Efficacy of class III drugs in preventing AF compared to their ability to terminate the arrhythmia

Numerous studies attest to the greater efficacy of class III drugs, and more specifically sotalol and dofetilide, in preventing AF compared to their ability to terminate the arrhythmia. In a controlled trial, Hohnloser et al. [5] found that sotalol converted significantly fewer AF patients than quinidine to sinus rhythm, but that their efficacy in AF prevention is equivalent. Two other studies found sotalol to be inferior to quinidine for AF cardioversion [11,12], and an additional study found sotalol to be significantly less effective than flecainide [13]. In contrast, sotalol has been found to be as effective as quinidine [14,15] and propafenone [16,17] and more effective than placebo [18] in preventing AF recurrence.

Two small studies failed to demonstrate efficacy of
dofetilide in AF conversion [19,20]. Subsequent larger studies showed that dofetilide is more effective than placebo in terminating AF and atrial flutter; however, dofetilide conversion rates for AF are relatively low: 14.5% in one study [21] and 24% in the other [22]. In contrast, dofetilide is clearly effective in preventing AF recurrence [23]. Thus, the clinical evidence suggests that sotalol and dofetilide are more effective in preventing AF than in terminating it.

4.2. Novel findings and relationship to previous literature

Our study is the first of which we are aware to demonstrate, in an experimental model of AF, differential efficacy of class III drugs for AF termination vs. prevention that parallels clinical observations. In addition, we gained insights into the mechanism by which these drugs prevented AF initiation by APCs with the use of epicardial mapping. Efficacy in preventing AF initiation was associated with substantial ERP prolongation at rates comparable to sinus rhythm in man, such that the ERP exceeded the conduction delay that produced reentry under control conditions. Epicardial mapping also provided insights into the failure of sotalol and dofetilide to terminate AF, in that despite increasing the AFCL these drugs failed to reduce the number of reactivation zones per AF cycle in any important way. Alesse et al. [24] have previously shown that vagal AF is maintained by an average of four to six functional reentry wavefronts. Although both dofetilide and sotalol reduced the number of reactivation zones during AF significantly, the number of such zones decreased from just over six to just over five per cycle, an effect insufficient to terminate AF. The differential efficacy of these agents in AF prevention vs. termination is therefore likely related to their reverse use-dependent actions on ERP, such that ERP is more prolonged at slower rates like those of sinus rhythm than during AF. This possibility was suggested by an earlier study in our laboratory [6], but has never before been tested experimentally.

Previous studies have shown that clinically relevant doses of sotalol [6,25] and dofetilide [8] are relatively ineffective in terminating vagotonic AF. Our finding of an important role of the septum in AF initiation are compatible with previous observations in the sterile pericarditis model [26]. In contrast to our findings, Wijffels et al. [27] reported that a variety of antiarrhythmic drugs (hydro-
Fig. 7. Comparison of the effects of sotalol and dofetilide on atrial effective refractory period (AERP) vs. conduction delay to distal septum. Under control conditions, the conduction delay was slightly greater than the AERP, allowing the occurrence of atrial reentry. Sotalol and dofetilide did not alter conduction delay, but greatly increased AERP, thereby preventing AF induction. Values are mean±S.E.

Fig. 8. Activation maps of two consecutive cycles during sustained AF before and after drug infusion in representative dogs. Isochronal lines are drawn at 20-ms intervals. Asterisks indicate reactivation zones, defined as zones activated early in one cycle (adjacent to late-activated zones) which are then re-activated early in the next cycle. During sotalol and dofetilide infusion, there was a modest reduction in the number of zones of reactivation and an increase in AF cycle length.

4.3. Limitations

The model we used was vagotonic AF. We chose this model primarily because sustained AF can readily be terminated by stopping vagal nerve stimulation, without having to resort to electrical cardioversion. This is an important advantage for a study evaluating vulnerability to AF initiation by premature atrial extrastimuli in a milieu that supports sustained AF, because AF is induced repeatedly over the course of the study. A requirement for repeated electrical cardioversion would introduce a substantial additional variable to the study (electrophysiological effects of repeated cardioversion), and the waiting times necessary for restabilization after each cardioversion would greatly lengthen the study, decreasing feasibility and increasing the risk of systematic time-dependent changes. Although enhanced vagal tone may be important in many clinical cases of AF [29], a prominent role of vagal tone is not observed in the majority of cases. Nonetheless, the responses of vagotonic AF to antiarrhythmic drugs resemble those for chemical cardioversion of recent-onset clinical AF [30], and some of the primary electrophysiological features of vagotonic AF (decreased ERP, decreased ERP rate-adaptation and increased ERP heterogeneity) resemble those observed in tachycardia-induced atrial remodeling [9,31]. The lack of sotalol...
useful drug as a primary agent [32,33] or accessory compound [35] in the termination of AF. Ibutilide has been shown superior in AF termination compared to sotalol [33] and procainamide [34]. This discrepancy may be due to an additional mechanism of ibutilide’s class III action (enhancement of a plateau Na⁺ current) [36] in addition to I[Kr] blockage [37].

The limitations of our mapping methods also need to be kept in mind. It appeared in many cases that reactivation involved the atrial septum and was directed towards the region of the initiating extrastimulus. However, endocardial activation (other than at the septum) is not accessed by the epicardial electrode arrays we used, and the resolution of all mapping systems is limited. Consequently, a degree of uncertainty remains regarding the precise pathways of reactivation and the underlying determinants.

Finally, we cannot completely exclude an interaction between sotalol and vagal effects. Sotalol has been shown to inhibit cholinesterase activity [38], and to reduce I[K(Ach)] [39]. However, these actions are seen at concentrations (>300 and 100 μM, respectively) that are >6-fold the average concentrations (~15 μM) achieved by the doses used in the present study [6]. Furthermore, in previous studies we have shown that neither sotalol nor dofetilide at the doses we used alter the vagal frequency-heart rate change relationship [6,7].

5. Conclusions

We found that sotalol and dofetilide are more effective in preventing AF initiation by APCs than in terminating sustained AF in a vagotonic model. The efficacy in preventing AF induction appeared to be due to strong increases in the AERP at slow rates, such that the maximum conduction delay was insufficient to allow for reentry. At the rapid rates of AF, alterations in atrial reactivation were relatively small, presumably because of reverse use-dependent effects on ERP, and insufficient to terminate AF. These results provide potential insights into the mechanisms by which sotalol and dofetilide prevent AF occurrence more effectively than they terminate the arrhythmia.

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