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Effects of Combination of Sotalol and Verapamil on Initiation, Maintenance and Termination of Ventricular Fibrillation in Swine Hearts

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Abstract

Introduction—Sotalol and verapamil alone reduce reentry incidence during ventricular fibrillation (VF). We tested whether the combination of these two drugs had a synergistic effect on initiation, maintenance and termination of VF.

Methods—Six open-chest pigs received intravenous sotalol (1.5 mg/kg) followed by verapamil (0.136 mg/kg). VF threshold (VFT) was determined by a burst pacing protocol. Two 20 s episodes of VF were recorded from a 21×24 unipolar electrode plaque on the lateral posterior left ventricular epicardium before and after each drug. VF activation patterns were quantified. The duration of long duration VF (LDVF) maintenance was compared to our previous published data.

Results—Sotalol alone and combined with verapamil significantly increased the VFT from 12.3±4.1 to 20.3±7.1 and 26.7±8.6 mA compared with baseline ($P<0.05$). Sotalol decreased the number of wavefronts by 20%, VF activation rate by 17% and conduction velocity 11%, while the addition of verapamil neutralized these effects. Addition of verapamil to sotalol further decreased the fractionation incidence from 14% to 29% and multiplicity from 24% to 31% compared with baseline. The combination of the two drugs increased the VF cycle length, decreased synchronicity, increased regularity index and shortened the duration of LDVF maintenance compared with our previous data of verapamil alone or no drug. Synchronicity index was lower and regularity index was higher in animals in which VF spontaneously terminated earlier than 10 min than in animals in which VF terminated longer than 10 min.

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Conclusion—The combination of sotalol and verapamil increased VFT but accelerated LDVF termination.

Keywords

ventricular fibrillation; sotalol; verapamil

Introduction

Ventricular fibrillation (VF) is the main cause of sudden cardiac death in developed countries.¹ Currently, electrical defibrillation is the only proven effective therapy to terminate VF. In acute ventricular fibrillation, drugs are used after defibrillation attempts to restore normal rhythm. Drugs can also be used on a long-term basis in patients who refuse an implantable cardioverter-defibrillator (ICD) or who are not candidates for an ICD.

Generally, reentry is thought to play an important role in VF initiation and maintenance of short duration of VF.^{2, 3} Separately, we have shown that sotalol⁴ and verapamil⁵ significantly reduce the incidence of reentry by different mechanisms. Sotalol reduces the incidence of reentry by flattening the slope of the restitution curve, decreasing the dispersion of refractoriness and prolongation of refractoriness. Verapamil terminates reentry in the in vivo pig model by both increasing reentrant velocity and decreasing the refractory period, but mainly by increasing the reentrant velocity so that the propagation of reentrant wavefronts is blocked in the reentrant pathway. When sotalol was added to reduce the VF conduction velocity before verapamil was added, the subsequent addition of verapamil did not accelerate the conduction velocity to a degree to further decrease the reentrant incidence.⁵ However, many other antiarrhythmic mechanisms of the combination of both drugs on VF initiation, termination as well as maintenance are unknown. As both drugs flatten the slope of the restitution curve and reduce the dispersion of refractoriness, one hypothesis of the study was that the combination of these two drugs will increase the threshold to initiate VF.

In addition, over the past 20 years, the incidence of out-of-hospital VF has declined compared with asystole as the first recorded rhythm during sudden cardiac arrest (SCA).^{6, 7} Some individuals seen in asystole may have earlier been in VF, but the VF may have degenerated into asystole by the time the first responders arrived. Many patients with a high risk of sudden cardiac death are now under the treatment of an anti-arrhythmia drug and some drugs have been shown to cause a more rapid progression from VF into asystole. This could explain the decline of the incidence of out-of-hospital VF. Since sotalol decreases the conduction velocity and verapamil inhibits the L-type Ca^{++} channels, both effects could work together to terminate late VF. Another hypothesis tested in the study is that the combination of these two drugs causes the termination of VF earlier than with monotherapy alone.

Methods

Animals were managed in accordance with the Guide for the Care and Use of Laboratory Animals,⁸ and the protocol was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

Animal Preparation

We used the same methods as previously reported.^{5, 9} These two previous studies served as comparison for the current study. Six pigs (40±4kg, mean ± SD) were injected intramuscularly with Telazol (4.4mg/kg), xylazine (2.2mg/kg), and atropine (0.04mg/kg) for anesthetic induction. Anesthesia was maintained with isoflurane in 100% oxygen by inhalation. Core body temperature, arterial blood pressure, arterial blood gases, ECG lead II, and serum electrolytes were monitored and maintained within normal ranges throughout the study. The heart was exposed through a median sternotomy and supported in a pericardial sling. A plaque containing 504 electrodes (24×21) with 2-mm space between each electrode was sutured to the posterior lateral LV, with one edge of the plaque adjacent to the posterior descending artery. A stainless steel wire was attached to the right leg as the ground for the unipolar mapping electrodes. The mapping electrodes were made from Teflon-coated silver wires with a diameter of 0.013 inch. The diameter of the tip of each mapping electrode was ~0.2mm. A catheter (model 6942, Sprint, Medtronic) with a 479-mm² surface area electrode in the right ventricle and a 766 mm² surface area electrode in the superior vena cava was inserted for defibrillation.

Measurement of refractory period

The ventricular effective refractory period (VERP) was determined using a decremental S1-S2 pacing protocol from the two middle electrodes of the plaque on the epicardium. Both S1 and S2 stimuli (5 ms duration) were given at twice diastolic threshold. The S1-S1 interval was 300 ms (10 beats), and the initial S1-S2 interval was 280 ms. The S1-S2 interval was decreased in 10 ms decrements until the S2 did not capture the ventricles on the ECG. Then the S1-S2 interval was reset to 10 ms more than this non-capture interval and was decreased in 2 ms decrements until S2 again failed to capture the ventricles. This S1-S2 interval was defined as the VERP.

Determination of ventricular fibrillation threshold (VFT)

VFT was performed by pacing at the same site as measurement of VERP with a primary drive train at a cycle length of 270 ms for 8 beats. After a 50 ms delay, a train of 20 pulses of 5 ms duration each (100 Hz) began for a total duration of 200 ms. The intensity of the train was increased in 2 mA increments until VF developed. The minimum current strength required to induce sustained VF was defined as the VFT.¹⁰

Conduction time (CT) and conduction velocity (V)

The activation time of each channel at each pacing beat was defined as the instant when the most negative 1st order temporal derivative of voltage recording was reached. If the most negative derivative of the recording was not more negative than -0.5 V/s, no activation time was assigned and this electrode recording was excluded from the calculation for this paced beat. We noted that the extracellular recordings could be affected by 60Hz noise artifacts, which could shift the time of the maximum downslope. To reduce the effect of these artifacts, the activation time of each channel was averaged with its 8 neighboring channels to represent the activation time at each electrode. For channels located at the boundary of the plaque the number of neighboring channels was correspondingly reduced. Conduction time

(CT) of each electrode was defined as the interval between the activation time of the pacing electrode and measured electrode.

Activation recovery interval (ARI) measurements

The activation recovery interval (ARI) was determined as an estimate of the action potential duration (APD) and, hence, the refractory period.^{4, 11} A 15-point quadratic differentiating filter was applied to determine the first derivatives of the extracellular potentials and to minimize the interference from noise. Activation time in the unipolar electrograms was defined as the steepest downslope of the QRS complex and recovery time as the fastest upslope of the T wave. The ARI was defined as the interval between the activation time and the recovery time.

VF induction and termination

Any VF induced during the VFT determination and S1-S1 pacing protocol was used for VF analysis. If no VF was induced during S1-S1 pacing protocol, it was induced by a 9 V battery applied briefly to the right ventricle. Each VF episode was mapped for 20 s before it was halted by a 400–600V biphasic shock (6/4 msec) delivered from a defibrillator (Ventritex, HVSO2, St. Jude Medical Inc, CA, USA) via the catheter electrodes. The last VF episode of each animal after the combination of sotalol and verapamil was continually recorded without resuscitation. Spontaneous VF termination was defined as the absence of activations for 10 s. The duration of VF was compared to that in our previous long duration VF (LDVF) studies in pigs without any anti-arrhythmic drugs⁹ and with verapamil alone in pigs.⁵

Sotalol and verapamil administration

After the baseline measurements of VERP, restitution, VFT and the electrical induction of 2 episodes of VF, sotalol was infused intravenously at a dose of 1.5 mg/kg to achieve class III anti-arrhythmic action. The same measurements and 2 more episodes of VF induction were repeated 15 min post-injection. After the sotalol measurements, a dose of verapamil at 0.136 mg/kg was injected over 5 min and all the same measurements, except for the restitution measurement, were repeated 15 min post-verapamil-injection.

Quantitative analysis of VF activation

Quantitative analysis of VF activation patterns was performed on a Linux system computer using algorithms discussed in detail elsewhere.^{12, 13} Specifically, from the recordings made during the first 20 s of each VF episode, we defined the effects of sotalol separately and in combination with verapamil on the 5 quantitative VF parameters as follows: (1) number of wavefronts; (2) fractionation incidence; (3) block incidence (4) multiplicity (5) activation rate.

To investigate the LDVF, the 10 minutes of VF were divided into 300 2-second episodes. For each episode, activation was identified as the 9-point dV/dt ¹⁴ of the recordings that reached a minimum of more than -0.3 mV/ms. To quantify the organization level of the LDVF, we defined a synchronicity index (syn-idx) and a regularity index (reg-idx) for each 2-second episode.¹⁵ Syn-idx is an inverse measure of the synchronicity of activation time. When the

activations in all channels are simultaneous, syn-idx is 0. With less synchronous activation time, syn-idx becomes larger. Reg-idx is an inverse measure of the regularity of activation times. When the cycle length is constant in each channel, reg-idx is 0. With less regular activation times, reg-idx becomes larger.

Statistical Analysis

Results are expressed as mean $\pm$ SD. All VF variables were compared before and after sotalol administration and after the combination of sotalol and verapamil using multivariate analysis of variance (MANOVA) with repeated measures to test for differences between baseline and treatments. When differences were found, individual differences were determined with the Tukey test. Dispersion of refractoriness and CV were quantified using the relative dispersion (the SD divided by the mean of the ARIs and CV, in percent) of the ARIs and CV of the plaque electrodes. The χ^2 test was used to compare the duration of VF data from the current combined drug study and the two previous studies of verapamil and sotalol separately.^{5, 9} For all analyses, $p < 0.05$ was considered statistically significant.

Results

VERP

Compared to baseline, sotalol significantly prolonged the VERP by 17% (200 ± 17 ms, 233 ± 23 ms, $P < 0.001$). The combination of sotalol with verapamil slightly shortened VERP compared to sotalol alone (220 ± 21 ms vs 233 ± 23 ms, $P = \text{NS}$). Also, the VERP after the combination of sotalol and verapamil was not significantly longer than VERP at baseline ($P = \text{NS}$).

VFT

Sotalol significantly increased the VFT from 12.3 ± 4.1 mA at baseline to 20.3 ± 7.1 mA ($P = 0.02$). The combination of sotalol and verapamil further increased the VFT to 26.7 ± 8.6 mA ($P = 0.003$) compared to baseline.

Effects of sotalol on ARI and CV

Compared to baseline, sotalol significantly prolonged ARI by 12% and decreased CV by 11% at PCL of 300 ms (Table 1). Sotalol alone significantly reduced the relative dispersion of ARI by 38%, however, it did not significantly alter the dispersion of CV. Adding verapamil to sotalol decreased the ARI by 19% compared with sotalol alone, the drug combination also further reduced the relative dispersion of ARI by 8%. Adding verapamil to sotalol significantly reduced the dispersion of CV by 18%.

Quantitative Analysis of short duration VF Activation Patterns

Quantitative data from all 1 s intervals of VF were pooled in Table 2. Examples of VF activation sequences at baseline, after sotalol and after the combination of sotalol and verapamil are shown in Figure 1.

Both sotalol and verapamil decreased fractionation incidence and multiplicity during VF, thus the combination of the two drugs further decreased fractionation incidence and

multiplicity (Table 2). Sotalol decreased the number of wavefront, activation rate and conduction velocity, while verapamil increased those parameters, net effects of the combination of these two drugs is toward to decrease number of wavefront, activation rate and conduction velocity (Table 2).

Long duration of ventricular fibrillation

VF cycle length and duration of VF from induction to spontaneous termination

—When compared with previous control and verapamil alone animals, the combination of verapamil and sotalol significantly increased the VF cycle length during the majority of the entire duration of the VF episode (Figure 2A). No VF spontaneously terminated within 10 min in the previous control group without any antiarrhythmia drug administrated.⁹ There was one animal in the previous study with verapamil in which VF spontaneously terminated at 8 min.⁵

Since there were 4 of 6 animals in the drug combination group in which VF spontaneously terminated before the end of the 10 min recording period, we only statistically analyzed the significance of the beginning 300 s of VF data when compared with control and verapamil alone group. The syn-idx was slightly but significantly lower during the first 300 sec of VF in the drug combination group (Figure 2B) when compared with control and verapamil alone groups. From 300 s to the end of analysis at 10 min of LDVF, the syn-idx in the drug combination group was markedly lower than the other two groups (Figure 4B). The reg-idx in the drug combination group was significantly higher during the first 300 sec of VF when compared with the control group and verapamil alone group (Figure 2C).

VF spontaneously terminated within 10 min in 4 of the 6 pigs with the combination of sotalol and verapamil. The mean VF duration in these 4 pigs was 6.4 ± 2.3 min (Figure 3, Table 3). Compared with our previous studies, VF spontaneously terminated within 10 min in one of the 6 pigs with verapamil only and no VF spontaneously terminated within 10 min in 6 pigs without anti-arrhythmic drug (Table 3).

In the sotalol and verapamil combination group, the 2 animals with VF duration of 10 min had the shortest VF cycle length (Figure 4A). The shorter the duration of VF was, the longer the VF cycle length was. The synchronicity index was lower in animals in which the VF terminated before 10 min (Figure 4B). Immediately before each early VF terminated, we observed that there was a substantial increase in the regularity index (Figure 4C).

Discussion

The main findings of this study are: (1) Both sotalol alone and in combination with verapamil significantly increased VFT; (2) The synergic effects in reduction of fractionation and multiplicity by combination of the two drugs resulted in VF more organization; (3) However, the combination of these two drugs could accelerate the progression of VF from onset to spontaneous termination with asystole in swine.

The effects of sotalol and verapamil on the electrical induction of VF

Since the first demonstration of VF induction by a single electrical shock delivered late in systole to the frog's ventricle,¹⁶ many studies have been performed to quantify VF inducibility. An electrical stimulus within a certain range of strengths delivered to the myocardium during the vulnerable period of the cardiac cycle in normal sinus or paced rhythm will induce VF.¹⁷ The lowest stimulation strength that can induce VF is known as the VF threshold (VFT), which has been used as an index for the efficacy of anti-fibrillation drugs.¹⁰ Reentry is the main mechanism for the initiation of VF. The heterogeneities of refractoriness and conduction are the most critical for electrical initiation of the reentrant circle. Impulses from a stimulation electrode travel in certain directions through the heart, but not in all directions. Because of the heterogeneous properties of cardiac tissue, certain impulses travel for a short distance until they reach refractory areas of the heart and then are blocked. Other impulses pass between the refractory areas and continue to travel in the excitable tissue and those impulses may travel back to a previously refractory area to form the reentrant circles. Researchers have traditionally emphasized the importance of pre-existing electrophysiological and anatomical heterogeneities.^{18, 19} But recently, researchers are considering that dynamic heterogeneity, which arises from cardiac restitution properties, may be more critical.^{20–22} The slope of the APD restitution curve, in which APD is plotted against the preceding DI, is the main determinant of wave break. We have previously demonstrated that treatment with either sotalol or verapamil causes a significant flattening of the steep restitution curve^{4, 5, 10} and a reduction of the fractionation incidence of wavefronts^{4, 5}. The synergistic effect of verapamil and sotalol together in reducing the fractionation of wavefronts is consistent with their increase of VFT.

Sotalol and verapamil were also demonstrated to have a synergistic effect in reducing the dispersion of ARI and conduction velocity measured during pacing in this study, which put the heart tissue into a more homogenous state, decreased the vulnerability of the heart to convert from VT, and thus increased the VFT.

The effects of sotalol with verapamil on the progression of LDVF

While VF is the most commonly witnessed arrhythmia at the time of SCD,²³ in the last two decades the incidence of out-of-hospital VF has declined compared with asystole as the initially recorded rhythm.^{6, 7} Multiple factors, such as increased age of the victims, severe underlying disease, anti-arrhythmia drug use, cardiac pump failure without an arrhythmic event, and an increase in implantable-cardioverter defibrillator use to prevent VF in high risk patients, might all be related to this relative decrease in the incidence of finding patients in VF.

Recently, Wang et al described that the drugs (beta-blockers and angiotensin converting enzyme inhibitors) widely used in primary and secondary coronary artery disease prevention strategies shortened the duration of VF, which resulted in the reduced incidence of VF as the presenting rhythm in SCA.²⁴ In this study, we found that the combined anti-arrhythmic drugs accelerated the progression from VF to asystole (Figure 2). The duration (6.4 ± 2.3 min) of VF degeneration into asystole in swine after receiving both of the drugs was in the range of 4–10 min, which is the mean time from collapse until arrival of a first responder and

electrical defibrillation in the pre-hospital setting where most SCAs occur.^{25,26} The *d,L*-sotalol applied in the present study has β -adrenergic blocking activity that has been reported to decrease the duration of VF. The depolarization from the Ca^{++} slow inward current can be inhibited by verapamil and is mandatory for the maintenance of LDVF as VF progresses. Therefore, sotalol in combination with verapamil significantly decreased the progression of LDVF after onset of cardiac arrest more than either drug alone. In support of this explanation, the activation interval in the combination group was longer than in the control or verapamil alone groups.

We defined the syn-idx and reg-idx in our previous endocardial mapping study to quantitatively describe canine endocardial electrical synchrony during LDVF.¹⁵ We demonstrated that during the endocardial electrical synchrony of LDVF, the endocardium was excited almost simultaneously and that Purkinje activation played an important role in this endocardial electrical synchrony. Purkinje activation was registered in 6% of electrodes in the porcine epicardial layer, which differentiated the porcine epicardial activation patterns from canine.²⁷ We employed the syn-idx and reg-idx on pig epicardial mapping data to measure the spatial-temporal complexity of activation waveforms during LDVF. The lower syn-idx in the drug combination group indicates that the epicardium activated more synchronously than with verapamil alone or without any drug. It is interesting that in the drug combination group, the four animals that spontaneously terminated VF before 10 min also showed a lower syn-idx than the other 2 animals in which VF lasted 10 min. Reg-idx was higher in the drug-combination group than in the other two groups. The 4 animals in the drug combination group with early termination of VF also had a higher reg-idx than the other 2 animals. We observed that there was sudden and extreme reg-idx variation per second before each VF termination. The effects of sotalol in decreasing conduction velocity and increasing refractoriness combined with the effects of verapamil in inhibiting Ca^{++} slow inward current which may inhibit the wavefronts propagation and divide the activation in many small area in ventricles with a very slow rate. Some area might be triggered by EAD or DAD with abrupt increased activation rate before VF activation were terminated in the whole heart.

Study Limitations

There are several limitations in this study. We only investigated the effects of sotalol and verapamil at a fixed dosage. Therefore, the dose dependent effect of the combination on VF activation patterns was not detected. The current study was conducted in healthy swine hearts, while VF usually occurs in diseased hearts. Many pathological changes will impact the effect of antiarrhythmic drugs on the heart. Whether the same effects are associated with myocardial infarction or heart failure should be examined in future studies. While the defibrillation threshold (DFT) after monotherapy of these two drugs has been investigated, the effects of combinations of drugs on the DFT should also be investigated in future studies. As with all animal studies, further studies in humans are required before the combinative therapy of intravenous sotalol and verapamil will be applied in the clinical settings.

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Figure 1. Snapshots of activation of VF before and after drugs

Column I represents snapshots of activation of VF in one animal at baseline (panel A), after sotalol (panel B) and with sotalol in combination with verapamil (panel C). The numbers show the time, with time 0 beginning 2 s after VF onset. Each color indicates an individual wavefront, except in panel A where all of the wavefronts that are part of a reentrant pathway are shown in red. The arrows in panel A indicate the direction of wavefront movement in a counterclockwise reentrant circuit. Recordings from the same four electrodes are shown below the activation maps for each panel for 2 s beginning at time 0 ms (arrows). The broad

picture of the dynamics are as follows: Panel A, large spatial and temporal variation; Panel B, more organized activation patterns with less fractionation and collision; Panel C, no further improvement in reduction of the complexity of VF activation patterns compared to sotalol.

Figure 2. Evolution of mean CL, synchronicity index (syn-idx) and regularity index (reg-idx) as VF progressed

Figure 2A shows VF-CL is significantly increased by the combination of verapamil and sotalol during the entire duration of the VF episode. The syn-idx is slightly but significantly lower during the first 300 sec of VF in the drug combination group (Figure 2B) when compared with control and verapamil alone groups. Figure 2C shows that the reg-idx in the drug combination group is significantly higher during the first 300 sec of VF when compared with the control group and verapamil alone group.

Figure 3. Earlier spontaneous termination of LDVF after sotalol and verapamil

In Panel A, each color indicates an individual wavefront and each colored pixel indicates an electrode site. Panel B represents the recordings from four electrodes for 10 s. The red frame in Panel B indicates the activation mapping time in Panel A. In this treated heart, LDVF was suddenly spontaneously terminated after 3'04".

Figure 4. Evolution of mean CL, synchronicity index (syn-idx) and regularity index (reg-idx) as VF progressed in the combination group

Figure 4A shows that 4 out of 6 LDVFs spontaneously terminate before the end of the 10 min recording period. The 2 animals with VF lasting 10 min have the shorter VF cycle length. The syn-idx is also lower in animals in which VF terminates before 10 min (Figure 4B). The 4 animals with early termination of VF have a higher reg-idx than the other 2 animals. Before each early VF terminates, there is a huge variation of the regularity index (Figure 4C).

Table 1

ARI and CV before and after Sotalol at PCL of 300 ms

	ARI (ms)			CV (m/s)		
	Mean	SD	Dispersion SD/Mean	Mean	SD	Dispersion SD/Mean
Baseline	193±13	7.6±1.8	4.0%±0.8%	0.56±0.12	0.19±0.06	34%±9%
Sotalol	216±13 ^{***}	5.5±1.1 ^{***}	2.5%±0.6% ^{***}	0.50±0.08	0.16±0.04	32%±8%
Sotalol+Verapamil	197±10	4.5±0.7 ^{***}	2.3%±0.4 ^{***}	0.54±0.06	0.15±0.03 ^{***}	28%±5% ^{***}

^{***} : P<0.01, compared to baseline.

Table 2

Effects of Verapamil on Activation Patterns During 20 s VF Episodes in Pigs

	Baseline	Sotalol		Sotalol + Verapamil vs Baseline		Sotalol+ Verapamil vs Sotalol	
		% change	P Value	% change	P Value	% change	P Value
Wavefront #	84±15	67±11	20% ↓ <0.01	73±19	13% ↓ <0.01	9% ↑ NS	NS
Fractionation incidence	0.14±0.03	0.12±0.02	14% ↓ <0.01	0.10±0.04	29% ↓ <0.001	17% ↓ <0.01	<0.01
Multiplicity	13.4±3.3	10.2±2.8	24% ↓ <0.001	9.2±1.7	31% ↓ <0.001	10% ↓ <0.01	<0.01
Activation rate	9.4±0.87	7.8±1.0	17% ↓ <0.01	8.5±0.94	10% ↓ <0.01	9% ↑ NS	NS
Conduction velocity	0.45±0.06	0.40±0.07	11% ↓ <0.01	0.43±0.07	4% ↓ NS	8% ↑ NS	NS

Table 3

Duration of VF from initiation to spontaneous termination in swine hearts

	no anti-arrhythmic dugs	verapamil alone	Sotalol+verapamil
VF lasting < 10 min	0 (0%)	1 (17%)	4 (67%)
VF lasting > 10 min	6 (100%)	5 (83%)	2 (33%)