Differential effects of \textit{d}-sotalol on normal and infarcted myocardium: an experimental study using epicardial mapping

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Abstract

Objective: The aim of the study was to investigate the differential effects of the class III agent, \textit{d}-sotalol, on conduction and refractoriness on normal and infarcted areas of the canine ventricle. Methods: Epicardial mapping studies were performed in 6 dogs 5–7 days after ligation of the left descending coronary artery using a specially designed patch electrode which contained 192 bipolar electrodes. Normal and infarcted areas were differentiated with respect to their macroscopic appearance and electrophysiological properties. Activation maps and local effective refractory periods (ERP) were determined before and after the administration of \textit{d}-sotalol (1.5 mg/kg) at cycle lengths of 250, 300 and 350 ms. Results: Conduction and refractoriness were relatively homogeneous in the normal zone (NZ), contrasting with inhomogeneity in the infarct zone (IZ). In 2 dogs \textit{d}-sotalol produced regional delay and block of conduction, exclusively in the IZ. The relative increase in refractoriness ($\Delta$ERP) after \textit{d}-sotalol was significantly more pronounced in the IZ than in the NZ. In the NZ, $\Delta$ERP was most prominent at the longest (350 ms) and least prominent at the shortest (250 ms) basic pacing cycle lengths ($11.5 \pm 2.8$ vs. $7.3 \pm 1.4\%$; $P < 0.05$). The effect of \textit{d}-sotalol in the IZ was independent of the basic pacing cycle length. Conclusions: \textit{d}-Sotalol preferentially prolonged refractoriness in the IZ of the canine ventricle. This effect and the lack of rate-dependence in the IZ could provide a possible explanation for both the potent antiarrhythmic and potential proarrhythmic effect of \textit{d}-sotalol.

Keywords: Heart rate; Mapping; Antiarrhythmic drugs; \textit{d}-Sotalol; Myocardial infarct; Conduction; Refractory period; Dog, anesthetized

1. Introduction

The Cardiac Arrhythmia Suppression Trial [1] led to a general appreciation of the proarrhythmic potential of sodium channel blockers. As a consequence the interest in class III antiarrhythmic agents increased, which are believed to exhibit little or no arrhythmogenicity. The prevailing confidence in the beneficial effects of these compounds had a major impact on the design of the SWORD trial, in which patients with previous myocardial infarction and impaired left ventricular function were placed on \textit{d}-sotalol or on placebo, irrespective of the presence or absence of ventricular arrhythmias on 24-hour Holter recordings [2]. Unfortunately, the study had to be prematurely stopped due to an excess mortality in the \textit{d}-sotalol group. Thus, we are left with the notion that even class III antiarrhythmic drugs are potentially harmful and that our current knowledge of their basic mechanisms of action is not sufficient to ensure safe clinical use.

The bulk of data on the pharmacodynamics of antiarrhythmic drugs, including the information on which the Vaughan Williams classification [3] is based, was collected in normal cardiac tissue. There is, however, substantial evidence that antiarrhythmic drugs preferentially affect compromised myocardium [4–8]. Differential effects of antiarrhythmic drugs might predominantly affect pre-existing re-entrant circuits as areas of slow conduction and conduction block, which are prerequisites for re-entry, are typically found in functionally abnormal tissue [9–11]. If, however, the given conditions do not primarily allow re-entry to occur, a preferential drug effect on functionally abnormal myocardium might increase local inhomogeneity of conduction and refractoriness and, thus, facilitate re-entry.
As a class III agent, \( d \)-sotalol is expected to prolong repolarization, and some data suggest that this prolongation of repolarization is more pronounced in ischemic than in normal myocardium [7,12]. Both in vitro [13–15] and in vivo [16–18] studies have shown that action potential or QT interval prolongation with \( d \)-sotalol decreases at rapid pacing or heart rates. This so-called ‘reverse’ rate-dependence has been described for most drugs with predominant class III properties and would cause at least theoretical concern about their efficacy at faster heart rates (i.e., during sustained ventricular or supraventricular tachycardias). Data by Schmitt et al. on \( d,l \)-sotalol suggest, however, that reverse rate-dependence seen in normal myocardium might be abolished in ischemic tissue [17]. If confirmed, this finding would offer an explanation for the preserved clinical efficacy of class III agents at faster heart rates.

In the present study we used epicardial mapping techniques in dogs with subacute anterior wall infarction to address the following questions: What are the electrophysiological differences between normal and infarcted areas of the canine ventricle? Is there a differential effect of \( d \)-sotalol on conduction and refractoriness in respective areas, and are there differences in the rate-dependence of the drug’s effects on normal and ischemic myocardium?

2. Methods

All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.1. Model preparation

Nine adult mongrel dogs of either sex weighing between 12 and 20 kg were anesthetized with intravenous pentobarbital (0.5 mg/kg), intubated and ventilated with nitrous oxide and oxygen. To induce anterior wall infarction under aseptic conditions, the heart was exposed by a left thoracotomy and pericardectomy, then the left anterior descending artery (LAD) was ligated proximal to the first diagonal branch. The chest was then closed and the animals were allowed to recover. Routine postoperative care included prophylactic antibiotic therapy and analgetics. Details of the surgical procedure have been previously described [19].

2.2. Recording techniques

To allow simultaneous recording of 192 bipolar epicardial electrograms, a special patch electrode was designed. The patch consisted of a flexible 4 × 3 cm teflon matrix. A total of 384 silver wires were sewn onto the patch to form 192 bipolar electrodes organized in 12 rows and 16 columns. This resulted in an interpolar distance of 1–2 mm and an inter-electrode distance of 2–3 mm. The silver wires were teflon-coated except for their tips and had a total diameter of 0.005 inches.

The patch electrode was connected to a 256-channel computerized multiplexer recording system developed at the University of Limburg, The Netherlands [20]. With this system, local activation times were determined on the basis of the maximal first derivative of the intrinsic deflection of the local electrogram. The maximal first derivative was marked automatically, but each recording was visually controlled to trace possible errors in computer selection. Activation times were calculated relative to a time reference chosen by the investigator. From these activation times, isochronal epicardial activation maps were constructed manually at 10-ms intervals. As detailed elsewhere, conduction block was assumed if the difference in activation time at adjacent electrode sites was 40 ms or more and if local electrograms in these regions demonstrated characteristic double potentials [21–23]. Slow conduction was defined as an area of crowded isochrones spanning at least 3 consecutive electrode sites, with adjacent electrode sites separated by at least one isochrone [21–23].

As in comparable studies [21,22], local refractory periods were determined using the extrastimulus technique, with both the \( S_1 \) and the \( S_2 \) being applied at the site of recovery of the local electrogram. Stimuli at twice diastolic threshold amplitude were provided by a digital stimulator (Biotronic 2000). After 8 basic beats (\( S_1 \)) at a constant cycle length, an extra beat (\( S_2 \)) was introduced, decreasing the \( S_1S_2 \) coupling interval in steps of 5 ms. The maximal \( S_1S_2 \) interval that failed to evoke a propagated ventricular response to \( S_2 \) was taken as the effective refractory period (ERP) of \( S_1 \).

2.3. Mapping studies

Mapping studies were performed 5–7 days after LAD ligation. The dogs were re-anesthetized, and the heart was exposed through an extended midsternal approach. After pericardectomy, the infarcted area could be visually identified. This allowed for proper placement of the patch electrode over both normal and infarcted areas, with the rows and columns of bipolar electrodes running parallel and perpendicular to the LAD, respectively (Fig. 1). The latter arrangement was supposed to reflect fiber orientation, as the epicardial muscle fibers of the anterior wall are known to be oriented with their long axis perpendicular to the LAD [24]. Several sutures along its edges kept the patch in place. The chest cavity was closed, and the body temperature was adjusted to 37°C with a heating lamp.

2.4. Electrophysiological measurements

The epicardial spread of activation under the patch was determined during constant pacing at 3 different cycle
lengths (350, 300 and 250 ms). Longer pacing cycle lengths could not be regularly applied due to the relatively fast intrinsic heart rates. We refrained from inducing total AV block since the effects of d-sotalol were to be tested under ‘physiologic’ conditions. Furthermore, previous studies with d,l-sotalol had been able to demonstrate differential use-dependent effects on normal and infarcted tissue within a comparable range of pacing rates [17].

Stimulation was consecutively applied through two electrode pairs on the patch, located at the center of the top row and of the far left column. Thus, the effect of fiber orientation on the spread of activation could be analyzed.

To determine local refractory periods in normal and infarcted areas, 15 ± 3 electrode sites were randomly selected for stimulation. At each site, stimulation thresholds and local refractory periods were measured during basic stimulation at 3 different cycle lengths (350, 300 and 250 ms). Electrode sites with a stimulation threshold of more than 10 mA were excluded in an effort to avoid far field stimulation.

All measurements were repeated after intravenous administration of d-sotalol at a dose of 1.5 mg/kg over 10 min. This dose has been shown to significantly affect electrophysiological parameters in both dogs and human beings and is commonly applied for the treatment of ventricular and supraventricular arrhythmias in patients [12,25,26].

The aim of our study was to determine the differential effect of d-sotalol on normal and infarcted tissue and not its antiarrhythmic efficacy. Thus, the pacing protocol did not include attempts to induce ventricular tachycardia. In addition, previous studies have convincingly demonstrated the difficulties in producing stable VT in both open- and closed-chest dogs [27,28]. In fact, the success rate was as low as 14% in open-chest dogs [28]. Therefore, it would have taken more than 40 experiments to finally gain 6 dogs with inducible sustained tachycardia.

2.5. Statistical analysis

Data are expressed as mean ± standard deviation. Basic comparative statistics were performed using a Student t-test for paired or unpaired data. A confidence level of 95% was considered statistically significant.

3. Results

Of the 9 dogs subjected to LAD ligation, 6 survived the procedure and were subsequently included in the mapping studies. On gross examination, all dogs showed evidence of anterior wall infarction involving the subepicardial layer of muscle.

3.1. Electrophysiological differences between normal and infarcted areas

Normal and infarcted areas were not only different with respect to their macroscopic aspect, but also with respect to their electrophysiological properties. This included the spread of activation, local electrograms, tissue excitability and refractory patterns. In general, conduction was relatively fast and homogenous in normal tissue, contrasting with inhomogeneity of conduction and local conduction delay in the infarct zone. Local electrograms recorded from areas of slow conduction were typically multiphasic, fractionated and of long duration. Due to the inhomogeneity of conduction, it did not seem appropriate to determine average conduction velocities. The site of stimulation did not seem to affect the location of the area of slow conduction, but rather the extent of the conduction delay. Thus, anisotropic tissue properties obviously contributed to slow conduction, but did not account exclusively for it.

A representative example of an epicardial activation map during constant pacing from two different sites is shown in Fig. 2.

Regarding tissue excitability, a considerable percentage (38 ± 8%) of electrode sites primarily selected for refractory measurements in the infarct zone had to be abandoned due to a stimulation threshold above 10 mA. In the normal zone, this was only true for 6 ± 2% of all selected sites (P < 0.05). Despite this obvious bias, the mean threshold for stimulation at so-called ‘excitable’ sites (threshold = 10 mA) was still significantly higher in the infarct zone (3.6 ± 0.8 mA) than in the normal zone (2.3 ± 0.6 mA).

Based on measurements at these excitable sites, mean effective refractory periods did not show significant differences between normal and infarcted areas, irrespective of the basic pacing cycle length (178 ± 17 vs. 179 ± 19 ms, 157 ± 26 vs. 163 ± 26 ms, and 147 ± 27 vs. 155 ± 32 ms at basic pacing cycle lengths of 350, 300 and 250 ms, respectively). However, the refractory pattern in the infarct zone was inhomogeneous, with areas of short and long
re refractoriness found in close proximity, contrasting with a more gradual transition from areas of shorter to areas of longer refractoriness in the normal zone. A representative example is shown in Fig. 3.

3.2. Effects of d-sotalol on the spread of activation

As a class III agent, d-sotalol is not primarily expected to affect conduction velocity. There are, however, at least two possible mechanisms by which prolongation of repolarization might result in reduction of apparent conduction time: induction of local conduction blocks at a microscopic level, forcing activation to proceed in a 'zig-zag' pattern, thereby increasing the actual path length; and conduction of cells at their relative refractory period, with a consequent reduction of upstroke velocity of the action potential. In 4 of the 6 dogs, d-sotalol had apparently no effect on the spread of activation. In fact, the total activation time for the surface area covered by the patch electrode remained virtually unchanged (82 ± 24 vs. 84 ± 25 ms). In 2 dogs, however, administration of d-sotalol produced both regional delay and conduction block, exclusively in the infarct zone. Fig. 4 gives a representative example. During baseline (top panel) and following administration of d-sotalol (bottom panel), stimulation was applied through an electrode site in the upper left corner of the patch. The basic cycle length was 250 ms. As suggested by the moderate slowing of conduction in that area during baseline, the infarct zone was covered by the right half of the patch. d-Sotalol had no apparent effect on the normal zone, but led to rather inhomogeneous conduction in the infarct zone. Areas of faster and slower conduction were now found next to each other, and an arc of functional conduction block (indicated by the heavy solid line) developed in an area where some regional conduction delay had been evident at baseline (the 50 and 60 ms isochrone in the
top panel). The arc of functional conduction block forced the activation wavefront to split into two components which initially spread in opposite directions, then turned around both ends of the arc of block and converged on its distal side. As a result, the central portion on the distal side of the arc of block was only activated after 110 ms, 60 ms after respective areas on the proximal side (the 50 ms isochrone) had been depolarized. This difference in activation time was obviously not sufficient for the tissue on the proximal side of the arc to regain excitability. Therefore, the wavefront was blocked instead of breaking through the arc of block to re-excite previously depolarized tissue. However, it is well conceivable that with a longer arc of block and/or slower conduction around it, re-entry could have occurred.

3.3. Effects of d-sotalol on local refractoriness

As mentioned earlier, there was a significant difference in baseline pacing thresholds of normal and infarcted areas, whereas respective values for the mean effective refractory period were similar at all 3 basic pacing rates. Pacing thresholds were not affected by d-sotalol (2.3 ± 0.6 vs. 2.6 ± 0.8 mA for the normal zone and 3.6 ± 0.8 vs. 3.5 ± 0.8 mA for the infarct zone, respectively. However, at any given pacing rate, d-sotalol significantly prolonged local refractory periods in both the normal and the infarct zone. Data are summarized in Fig. 5.

Regarding the relative increase in local refractoriness, the effect of d-sotalol was significantly more pronounced in the infarct zone than in the normal zone. Again, this was true for any given pacing rate (Fig. 6).

3.4. Rate-dependence of the effects of d-sotalol

Fig. 6 also illustrates that the relative increase in local refractoriness in the normal zone was most prominent at the longest (350 ms) and least prominent at the shortest (250 ms) basic pacing cycle length (11.5 ± 2.8 vs. 7.3 ± 1.4%, P < 0.05). In contrast, the effect of d-sotalol was independent of the basic pacing cycle length in the infarct zone. Thus, the reverse use-dependence of the effects of d-sotalol on normal tissue seems to be abolished in ischemic myocardium.

4. Discussion

The present study demonstrates that normal and ischemic areas in the canine subacute myocardial infarction model exhibit functional differences regarding epicardial spread of activation, electrical excitability, and susceptibility to the effects of the class III antiarrhythmic agent, d-sotalol. Due to a more pronounced effect on local refractoriness in the infarct zone, the drug may produce new functional conduction blocks, preferentially in pre-existing areas of slow conduction. The effects on local refractoriness in the normal zone are inversely related to the pacing rate. This is, however, not the case in the infarct zone. Thus, d-sotalol differentially affects normal and infarcted tissue, not only with respect to the extent, but also with respect to the rate-dependence of the effect on local refractoriness.

Our findings regarding the conduction characteristics in the infarct zone are in accordance with previous studies [7,27–29]. It was unexpected to find comparable values for the mean effective refractory period in normal and infarcted areas. As mentioned earlier, electrode sites with a stimulation threshold of more than 10 mA were disregarded in an effort to avoid far field stimulation. Respective electrode sites were more common in the infarct zone. Since it is conceivable that areas with high stimulation
thresholds are likely to enclose severely compromised tissue with long refractory periods, this might have introduced a significant bias. To allow refractory measurements before and after application of d-sotalol within a reasonable time frame, the number of electrode sites had to be limited. Thus, refractory maps at a high spatial resolution could not be provided. Still, our limited data confirm the findings of Gough et al. [6] in suggesting a greater dispersion of refractoriness in infarcted than in normal areas of the canine ventricle.

The observation that d-sotalol did not affect the spread of activation in 4 out of 6 experiments agrees with the known cellular electrophysiologic effects of the drug [26,30–33]. New conduction blocks in response to d-sotalol probably resulted from prolongation of local refractoriness beyond the basic pacing cycle length, potentially at sites which had previously conducted close to their relative refractory periods.

The demonstrated effects of d-sotalol on local refractoriness in normal and infarcted areas were in line with previous studies, which, in part, had already suggested a preferential effect of the drug on infarcted tissue [7,12,32,33]. However, different from our in vivo mapping study, respective data were either obtained in isolated tissue preparations, or had to be based on indirect means to delineate normal and infarcted areas in the intact heart.

Whereas the reverse rate-dependence of the effects of d-sotalol on normal tissue is well established [26,34–38], the lack of rate dependence in infarcted tissue has not been appreciated so far. Data by Schmitt et al. [17] suggest similar behavior for d,l-sotalol, although their study had to rely only on comparison of monophasic action potential measurements in the right and the left ventricle of dogs with anterior wall infarction.

Survival of myocardial fibers in the epicardial and endocardial regions of healing and healed infarcts is believed to be a major cause of subacute and chronic ventricular arrhythmias [23,24]. These regions exhibit abnormalities in electrophysiological properties that may cause re-entry. Once re-entry is established, the crucial components of re-entrant circuits (that is, areas of slow conduction and arcs of functional conduction block) are typically found in the infarct zone [21,29,39,40]. Areas of slow conduction, on the other hand, seem to be primary targets for antiarrhythmic drugs [40–44]. Thus, it might be more important to assess the effects of antiarrhythmic drugs on structurally damaged myocardium, preferably specified according to the underlying pathologic condition rather than focusing on normal myocardium.

As a class III agent, d-sotalol primarily acts by blocking the delayed rectifier potassium current, thereby prolonging repolarization without affecting upstroke velocity [30–32,45]. The reverse rate-dependence of this effect in normal tissue has already been shown by several in vitro and in vivo studies [26,34–36]. The preferential prolongation of local refractoriness and the lack of rate-dependence in the infarct zone found in our study could provide a possible explanation for both potent antiarrhythmic and potential proarrhythmic effects of d-sotalol.

Whenever the electrophysiological properties at baseline allow for re-entry to occur, preferential effects of the drug on the crucial components of these re-entrant circuits are expected to be beneficial, and these effects will persist even at faster heart rates. This is in accordance with the clinical observation that d-sotalol quite effectively suppresses the inducibility of ventricular tachycardias during electrophysiological testing [25,46–50]. If, on the other hand, conditions at baseline are not suitable for re-entry, drug-induced transformation of an area of slow conduction into an arc of functional conduction block might provide one missing prerequisite for circus movement. This is even more likely at faster heart rates, as the differential rate-dependent effects of d-sotalol are expected to further increase the dispersion of refractoriness between areas of normal and infarcted tissue. Thus, it would seem understandable why treatment with d-sotalol in the SWORD trial was associated with an excess mortality, given the fact that patients randomized to treatment with d-sotalol or placebo were supposed to have had previous myocardial infarction, but not to have malignant ventricular tachyarrhythmias at baseline.

There is ample evidence that antiarrhythmic drugs exhibit differential effects on normal and infarcted tissue [4–6,8,17]. For in vivo preparations, one might argue that this is due to a reduced blood flow to the infarcted region, resulting in a lower tissue concentration of the administered agent. However, in this and in other studies, the drug effect was more pronounced in the infarct zone than in the normal zone. This would be contrary to the assumption of differences in tissue concentrations. Thus, it can be suggested that altered tissue properties are responsible for the increased susceptibility of infarcted areas to the action of antiarrhythmic drugs. In this respect, ischemia-induced changes in the resting membrane potential might be of relevance. Furthermore, alterations of the intra- and extracellular milieu affecting the function of membrane and/or regulatory proteins have to be considered. Structurally different proteins resulting from ischemia-induced changes in their genetic expression are another possibility. Modifications of passive conduction properties might also play a role, due to a loss or malfunction of cell–cell connections, or an accumulation of interstitial fluid or fibrous tissue. Finally, there is increasing evidence of functionally different tissue properties throughout the ventricular wall [51]. In particular, the suspected presence of subendocardial M-cells introduces a marked transmural dispersion of refractoriness. Any alteration which inhomogeneously affects the different muscle layers of the ventricular wall might alter the existing balance between the different cell populations, thereby unmasking the prevailing functional properties of the surviving muscle layer(s).

Several limitations apply to the present study. The
ventricular wall is essentially a three-dimensional structure. Therefore, epicardial mapping is not sufficient to depict the actual spread of activation. However, the rim of epicardial tissue surviving transmural infarction in the canine ventricle is relatively thin [24,37,39], so that at least in the infarct zone the epicardial activation map should reflect the major activation wavefront. Furthermore, areas of slow conduction or conduction block on the epicardium are only detectable if they are not short-circuited by faster intramural conduction. Thus, faster intramural conduction might suspend actual slow conduction or conduction block on the epicardium, but it could certainly not mimic these conduction disturbances.

For refractory measurements, a train of 8 basic beats preceded application of an extra beat. With that, steady-state conditions were probably not reached. However, the emphasis of our study was on the comparison of normal state conditions were probably not reached. However, the seemingly favorable properties of the drug might, in turn, prove detrimental in a situation where conditions at baseline are not (yet) suitable for re-entry, which might then be induced by the drug.

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References


