Endothelin receptor—A blockade decreases ventricular arrhythmias after myocardial infarction in rats


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Abstract

Objective: Endothelin-1 (ET-1) production increases during acute myocardial infarction (MI) and may contribute to the genesis of ventricular tachycardia (VT) and ventricular fibrillation (VF). However, the antiarrhythmic effects of ET-1 receptor blockade, examined shortly after MI, have been debated. In the present study, we examined the effects of such treatment on VT/VF during the first 24 h post-MI.

Methods: Thirty-five Wistar rats (223 ± 22 g) were randomly allocated to either the ET-1 receptor-A (ETA) antagonist BQ-123 (0.4 mg/kg, BQ-123 group, n = 17), or normal saline (control group, n = 18) and were subjected to coronary artery ligation. A single-lead electrocardiogram was continuously recorded for 24 h post-MI, using an implanted telemetry system, and episodes of VT/VF were analyzed. Monophasic action potential (MAP) recordings were obtained from the left (LV) and right (RV) ventricular epicardium at baseline, 5 min after treatment and 24 h post-MI.

Results: There were 15.94 ± 19.35 episodes/h/rat of VT/VF in the control group and 1.66 ± 2.22 in the BQ-123 group (p = 0.010), resulting in a lower (p = 0.030) arrhythmic mortality in treated animals. The mean episode duration was 7.40 ± 7.16 s for the control group and 2.30 ± 1.37 s for the BQ-123 group (p = 0.011). The maximum decrease in VT/VF was observed during the 1st, 5th and 6th hours post-MI. In the control group, LV MAP duration increased 24 h post-MI, displaying an increased beat-to-beat variation, but remained unchanged in the BQ-123 group.

Conclusion: Acute ETA blockade reduces the incidence of VT/VF during the first 24-h post-MI in the rat, through a decrease in the dispersion of repolarization.

Keywords: Arrhythmia (mechanisms); Endothelins; Infarction

1. Introduction

Acute myocardial infarction (MI) remains a common cause of morbidity and mortality worldwide [1]. Early reperfusion strategies decrease mortality, but approximately one-third of patients with MI die shortly after acute coronary occlusion, before receiving medical attention [1,2]. The vast majority of these deaths are caused by polymorphic ventricular tachycardia (VT) and ventricular fibrillation (VF) [1,2].

In the constellation of mechanisms leading to ischaemia-induced ventricular arrhythmias, endothelin-1 (ET-1) may play a significant role. Experimental [3] and clinical [4,5] studies have shown that the production of ET-1 increases...
markedly during MI, but the pathophysiological significance of this finding is not well understood. In addition to its vasoconstrictive, pro-fibrotic and pro-inflammatory actions, contributing to myocardial necrosis, ET-1 has been implicated in the pathogenesis of VT and VF during the acute phase of MI [3–5]. Based on these observations, it has been hypothesized that blockade of ET-1 receptors during MI may confer antiarrhythmic actions. Previous studies examining this hypothesis produced conflicting results, but the wide variation in the experimental protocols utilized precludes firm conclusions. Moreover, all previously published studies limited the time window for the observation of ventricular arrhythmias to differ from those responsible for ischemic arrhythmias [16,17]. In the present study, we explored further the pathophysiological role of ET-1 during MI. We aimed to examine the effects of acute ET-1 receptor blockade (a) on the infarct size and (b) on ventricular arrhythmias and to provide further insight into the possible electrophysiologic mechanisms of such intervention. To clarify the differences in the previously reported results, we chose, first, to examine the effects of selective ET-1 receptor-A (ET-A) blockade administered acutely at the maximum dosage known to produce local myocardial pharmacologic action and second, to avoid the confounding effects of reperfusion, since the mechanisms of reperfusion-induced arrhythmias are likely to differ from those responsible for ischemic arrhythmias [15]. The animal model used in the present study was the rat model, which offers clear-cut advantages in the study of ventricular arrhythmias. The rat not only exhibits a high frequency of VT and VF in response to myocardial ischaemia, but also the time course of their occurrence corresponds to the arrhythmia time course seen in humans after acute MI [16,17]. In the present study, we extended the previously studied time window and observed the total arrhythmia burden during the first 24 h post-MI.

2. Methods

The study was conducted in 35 female Wistar rats, 20±2 weeks old, weighing 200–250 g. The animals received appropriate care and the investigation conforms 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 1996). All rats were housed in individual cages in a climate-controlled environment, with a 12:12-h light–dark cycle and were given water and standard rat chow ad libitum.

2.1. Implantation of telemetry transmitter

A continuous electrocardiogram (ECG) telemetry transmitter (Dataquest, Data Sciences International, Transoma Medical, Arden Hills, MN, USA) was implanted in the abdominal cavity, using a previously described method [16]. Under ether anaesthesia, the animals were intubated and mechanically ventilated using a rodent ventilator (model 7025, Ugo Basile, Comerio, VA, Italy). Anaesthesia was maintained with a mixture of oxygen and 2% isoflurane. The transmitter was secured in the abdominal cavity, and the leads were tunneled under the skin and attached to the underlying tissue. The positive electrode was placed in a V4–V5 position and the negative electrode was placed under the right axilla. All incisions were sutured in two layers and the rat was then housed in an individual cage, placed on a receiver that continuously captured the signal, independently of animal activity. The ECG signal was displayed in real-time with the use of a computer program (A.R.T. 2.2, Dataquest, Data Sciences International, Transoma Medical, Arden Hills, MN, USA). After confirmation of a good quality ECG signal, the data were recorded continuously for the following 48 h and stored for analysis.

2.2. Monophasic action potential recordings

Twenty-four hours after the telemetry transmitter implantation, the rats were re-anesthetized and a left thoracotomy was performed, allowing dissection of the pectoral muscles. The heart was exposed and the pericardium was carefully removed. Monophasic action potential (MAP) epicardial signals were recorded, as described previously [18]. A MAP probe (model 200, EP Technologies, Sunnyvale, CA, USA) was placed on the lateral left (LV) and right (RV) ventricular walls, exerting mild, constant pressure against the epicardium to eliminate electrical artifacts. The signal was amplified with the use of a preamplifier (model 300, EP Technologies, Sunnyvale, CA, USA) and filtered at 50 Hz, using a digital notch filter. A continuous data stream was fed into a personal computer equipped with an analog-to-digital converter (BNC 2110, National Instruments Corporation, Dallas, TX, USA) and 2-min recordings were obtained, as soon as a clear, steady state signal was achieved. The recording software utilized in this study has been developed at our Institution, using Labview 6.1. This software has been validated with the use of a pulse generator, capable of producing a sequence of square waves with 1 mV amplitude, as described previously [19]. Furthermore, this software program facilitates the analysis of MAP recordings and details of these features are presented elsewhere [19]. For purposes of this study, maximal signal amplitude and action potential duration at 90% of repolarization (APD90) were measured. These measurements were performed as follows: at baseline, 5 min after drug administration and 24 h after MI. During analysis of the MAP recordings, non-sinus beats were excluded and 50 consecutive sinus beats per recording were analyzed. The standard deviation of APD90 was calculated for each recording, as a measure of beat-to-beat variation, indicating electrical alternans [18,20,21].
2.3. Drug administration and generation of MI

Following baseline MAP recordings, the rats were randomized in 1:1 fashion to receive either normal saline or BQ-123, the most selective ETA peptide antagonist [22] (Cyclo (D-Asp-Pro-D-Val-Leu-D-Trp) \cdot Na, C_{31}H_{41}N_{6}O_{7} \cdot Na, molecular weight 632.7, EMD Biosciences, Inc, Merck KGaA, Darmstadt, Germany). The dosage used was 0.4 mg/kg, which corresponds to that previously found to produce maximal pharmacological effects locally in the myocardium, but without any significant systemic effects [8,23–25]. In order to achieve a fast onset of a pharmacologic action, a slow (within 3 min) infusion, directly in the LV cavity was performed [26]. Minor bleeding, occasionally seen at the puncture site, stopped after light pressure was applied locally.

Five minutes after drug administration, coronary artery ligation was performed, as described previously [27]. In brief, the heart was exteriorized from the thorax and the left coronary artery was encircled and ligated using a 6-0 suture, placed between the pulmonary artery cone and the left atrial appendage. The heart was returned to its normal position and the thorax was closed in two layers. The remaining air was aspirated from the thorax, allowing the rats to resume spontaneous respiration. A six-lead ECG was obtained and ST elevation in 2 or more leads was considered a proof of induced MI.

The animals were returned to their cage and ECG recording was continued for another 24 h, or until spontaneous death. Bradyparrhythmic death occurring during the first 5 min after induction of MI was attributed to the surgical procedure or to cardiogenic shock [16] and these animals were excluded from the study. No resuscitation attempts were allowed at any time during the study.

Twenty-four hours after the generation of MI, the survivors were re-anaesthetized and the site of previous left thoracotomy was reopened. The heart was exposed and MAP recordings were repeated at the same sites. The rats were then sacrificed using a lethal dose of potassium chloride and the heart was harvested for measurement of infarct size. The study protocol is depicted in Fig. 1.

2.4. Infarct size

The method used for measurement of infarct size has been described previously [28]. In brief, the heart was excised, frozen (in −20 °C for 1 h) and hand-cut simultaneously in five 2 mm slices. They were incubated in triphenyltetrazolium chloride for 15 min at 37 °C and fixed (in 10% formalin for 20 min). The slices were scanned with the use of a high resolution scanner (Scanjet 4570c/5500c, Hewlett-Packard, Palo Alto, CA, USA). The areas of the infarcted and non-infarcted myocardium were determined by planimetry, using a previously validated software program (Image Tool, University of Texas, USA). Infarct size, expressed as a percentage of the LV cross sectional area, was defined as the ratio between the infarcted area, divided by the total LV area.

2.5. Heart rate

Because heart rate (HR) in the conscious rat can be variable, we analyzed continuous 5-min ECG recordings, from which non-sinus beats were excluded. The mean value of these RR intervals was used to determine HR. HR was calculated at baseline, at the 5th, 30th and 60th minute post-MI and hourly thereafter.

2.6. Arrhythmia analysis

The acquired single-lead ECG tracings were displayed and analyzed off-line independently by two of the authors (G.G.B., D.E.). The stored tracings were manually scrolled on a computer screen and the observers recorded all arrhythmic events. Ventricular arrhythmias were recorded as single ventricular ectopic beats (VEB’s), couplets, triplets, VT and VF, according to the guidelines provided by the Lambeth Conventions for determination of experimental arrhythmias [29]. Even with these guidelines, separating VF from VT was often difficult, and this has been the experience of others [16]. Therefore, in this study, we report the sum of VT and VF episodes. The duration of each VT and VF episode was measured using the time-scale provided by the recording software. To account for the censoring effect associated with differential survival, the arrhythmia frequencies are reported as mean values per hour per rat.

2.7. Statistical analysis

All values are given as mean ± one standard deviation, unless otherwise specified. Mortality rates were compared using Yates’ corrected chi square. Differences in continuous
variables were compared using Student’s $t$-test, or the analysis of variance for repeated measures, followed by Tukey’s multiple comparisons test, as appropriate. The continuous variables describing the arrhythmia frequencies were not normally distributed and were compared using the Mann–Whitney $U$-test. Statistical significance was defined at an alpha level of 0.05.

3. Results

We studied female 40 Wistar rats, weighing 224±21 g. Two rats (one had received BQ-123 and one normal saline) died during the surgical procedure and were excluded from the study. Three further rats (two had received BQ-123 and one normal saline) died within the first 5 min of complete atrioventricular block (probably attributable to cardiogenic shock) and were also excluded. Thirty-five animals were included in the study, of which 17 rats (223±21 g) were randomized to receive BQ-123 (BQ-123 group) and 18 rats (224±22 g) to receive normal saline (control group).

3.1. Mortality

During the 24-h period following MI, none of the animals in the BQ-123 group (0/17, 0%) died due to tachyarrhythmias, whereas 6 animals (6/18, 33%) in the control group had a fatal episode of VF ($p=0.030$). Two rats in the BQ-123 group (2/17, 11%) and one rat in the control group (1/18, 5%) died due to bradycardia associated with complete atrioventricular block. The overall mortality did not differ significantly between the two groups ($p=0.14$).

3.2. Heart rate

HR was not significantly different between the two groups during the entire 24-h observational period ($F=1.34$, $p=0.22$, Fig. 2).

3.3. Infarct size

Infarct size was calculated for the 26 survivors, 15 in the BQ-123 group and 11 in the control group. Mean infarct size was 39.7±4.2% for the BQ-123 group and 38.6±5.0% for the control group ($p=0.56$).

3.4. Ventricular arrhythmias

No statistically significant differences were found between the two groups in the number of VEBs, couplets or triplets. There was a significant reduction in the incidence of VT+VF episodes, from 15.94±19.35 episodes/h/rat in the control group to 1.66±2.22 episodes/h/rat in the BQ-123 group ($p=0.010$). Furthermore, there was a significant decrease in the mean duration of each episode, from 7.40±7.16 s in the control group to 2.30±1.37 s in the BQ-123 group ($p=0.011$). The hourly distribution of total VT+VF duration (number of episodes times duration of each episode) is shown in Fig. 3. Significant differences between the two groups were observed during the first ($p=0.0033$), fifth ($p=0.0085$) and sixth ($p=0.017$) hours post MI. This timing reflects the time period of maximum treatment action in conjunction with the highest incidence of ventricular arrhythmias.

3.5. Monophasic action potential duration and amplitude

No changes in RV APD90 were found in either group ($F=0.26$, $p=0.77$). There was a significant variance in LV APD90 ($F=71.7$, $p<0.0001$), that was due to a significant increase in LV APD90 24 h post-MI in the control group, compared to baseline (Table 1). In contrast, no significant changes in LV APD90 were observed over time in the BQ-123 group (Table 1, Fig. 4A and B). Furthermore, a significant variance was found in beat-to-beat variability of LV APD90 ($F=34.0$, $p<0.001$). This variance was due to a significant increase in beat-to-beat variability of LV APD90 24-h post-MI in the control group, compared to
These findings were associated with the occurrence of early afterdepolarizations (Fig. 4C). In contrast, no significant changes in beat-to-beat variability were observed over time in the BQ-123 group. LV MAP amplitude decreased significantly 24 h post-MI in both groups, with no significant differences between them. All values are shown in Table 1.

### 4. Discussion

Sudden cardiac death accounts for approximately 50% of the estimated 500,000 cardiovascular deaths that occur annually in the United States, and similar figures apply in Europe [2]. Acute MI is often responsible for sudden death in patients without a prior history of heart disease, in whom a fatal ventricular arrhythmia may be the first manifestation of coronary atherosclerosis.

The pathophysiology of ischaemia-induced ventricular arrhythmias is complex, and several aspects remain obscure. Within seconds of coronary occlusion, metabolic and ionic changes occur locally in the ischaemic ventricular myocardium, resulting in dispersion of conduction and refractoriness, which, in turn, favour the onset of reentrant ventricular arrhythmias [17].

ET-1 is a 21-amino acid peptide, acting on two specific receptors, namely ET\textsubscript{A} and ET\textsubscript{B}, located in the vasculature and cardiac muscle. Among other disease states, ET-1 levels are elevated in acute coronary syndromes [3–5]. Experimental studies have shown increased ET-1 expression in the infarcted area from 1 h up to 7 days after coronary occlusion [3]. Similarly, elevated plasma concentrations of ET-1 have been reported in patients with acute MI and have been shown to predict 1-year mortality [4,5]. This rise peaks 6 h after coronary artery occlusion and returns to normal values 24 h later, but it can be prolonged in infarcts complicated with heart failure [4]. Animal studies suggest that ET-1 may contribute to the induction of VT and VF independently of ischaemia, [8–14,30,31] but these direct electrophysiologic actions of ET-1 are still debated [8,13]. This issue is further complicated by the fact that conclusions have been drawn from exogenously administered ET-1 in some studies [31] and from the actions of endogenous ET-1 in others [9,10].

In the present study, we hypothesized that acute administration of BQ-123, an ET\textsubscript{A} antagonist, may exert antiarrhythmic effects, by inhibiting the arrhythmogenic potential of endogenous ET-1. We tested this hypothesis in a rat model of MI; the rat exhibits a high frequency of ischaemic ventricular arrhythmias in a repetitive, self-

![Fig. 4. Representative examples of monophasic action potential recordings from the left ventricular epicardium in a rat at baseline (A), 24 h post-myocardial infarction in a rat in the BQ-123 group (B) and in the control group (C). Note the increase in duration and the beat-to-beat variability in the duration of the signal in C but not in B. Arrows indicate early after depolarizations.](image)

### Table 1

<table>
<thead>
<tr>
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<th>BQ-123</th>
<th>Control</th>
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<tr>
<td><strong>APD90</strong> baseline (ms)</td>
<td>107±7</td>
<td>106±7</td>
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<tr>
<td><strong>APD90</strong> after injection (ms)</td>
<td>106±5</td>
<td>106±6</td>
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<tr>
<td><strong>APD90</strong> 24-h post-MI (ms)</td>
<td>109±5</td>
<td>131±10*</td>
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<tr>
<td>Beat-to-beat variability&lt;br&gt; baseline (ms)</td>
<td>2.8±0.6</td>
<td>3.7±0.6</td>
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<tr>
<td>Beat-to-beat variability&lt;br&gt; after injection (ms)</td>
<td>3.1±0.9</td>
<td>3.8±1.5</td>
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<tr>
<td>Beat-to-beat variability&lt;br&gt; 24-h post-MI (ms)</td>
<td>4.7±1.9</td>
<td>14.9±5.6*</td>
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<tr>
<td>Amplitude baseline (mV)</td>
<td>7.7±0.4</td>
<td>7.8±0.4</td>
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<tr>
<td>Amplitude after injection (mV)</td>
<td>7.7±0.4</td>
<td>7.8±0.4</td>
</tr>
<tr>
<td>Amplitude 24-h post-MI (mV)</td>
<td>3.8±0.7*</td>
<td>3.5±0.4*</td>
</tr>
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Data are given as mean±standard deviation.

* $p<0.05$ compared to baseline.

† APD90: action potential duration at 90% of repolarization.

‡ Expressed as the standard deviation of APD90 in 50 consecutive sinus beats.
terminating manner, rendering this model ideal in the assessment of various therapeutic interventions [16]. Furthermore, the miniature telemetry recording system used in our study facilitates the study of arrhythmias for extended periods of time in the conscious rat, without the confounding effects of anaesthesia [16].

We report a significant reduction in the incidence of summed VT and VF episodes after acute ETₐ blockade and a significantly shorter duration of each of these episodes. This reduction in the total arrhythmic burden decreased arrhythmic mortality. As expected from the comparable infarct size, mortality due to cardiogenic shock was similar in the two groups, resulting in a modest, non-significant decrease in total mortality.

These findings cannot be explained by a decrease in the necrotic myocardial mass, as no significant difference in the infarct size was found in our study, and this is in accordance with previously reported results [13,32]. Similarly, the antiarrhythmic effect observed in the present report cannot be attributed to a lesser sympathetic stimulation in treated rats, as suggested by studies evaluating the effects of chronic ET-1 receptor blockade post-MI [33]. This view is based on the absence of significant differences in heart rate between the control group and the BQ-123 treated group found in our study, as well as in previous studies of acute ET-1 receptor blockade post-MI [15,32].

The reduction in the incidence of VT and VF episodes could be attributed to the haemodynamic effects of ETₐ blockade. Indeed, a significant reduction in LV end-diastolic pressure was reported after administration of a dual ET-1 receptor antagonist early post-MI [32]. In the present study, we did not perform haemodynamic measurements; hence, such a mechanism cannot be excluded. However, we feel that this is unlikely, because the dose of BQ-123 used in this study was carefully selected to produce effective ETₐ blockade locally in the myocardium, without any systemic effects [23–25]. Furthermore, Sharif et al. [8] using a total dose of BQ-123 identical to ours, reported no effects on blood pressure in the rat model of MI.

Two possible mechanisms may explain our findings. First, ETₐ blockade may exert direct antiarrhythmic effects, independently of ischaemia [30,31]. ET-1 activates potassium currents and inhibits L-type calcium currents, an effect mediated by ETₐ stimulation [34,35]. Furthermore, infusion of ET-1 in dogs has been shown to induce sustained polymorphic VT and VF [31]. Thus, acute blockade of ETₐ prior to MI induction, may ameliorate the detrimental effects of the early surge in ET-1 that occurs in the rat model [3] and in humans after MI [4,5]. Our results, in accordance with previous observations, [8–10] point towards a direct antiarrhythmic effect of ETₐ blockade post-MI. This antiarrhythmic effect of BQ-123 occurs within a very narrow dose range, [14] which may, in part, explain the negative results reported previously [11–13]. Other potential explanations include species differences, variations in the anaesthesia and ischaemia–reperfusion protocols, as well as in the route of administration and nature of ET-1 receptor antagonists utilized [8–14]. Of note, BQ-123 may exert a direct electrophysiologic effect unrelated to its action at the ETₐ receptor. This agent has unique antifibrillatory properties among ETₐ receptor antagonists, as previously indicated from findings in isolated rabbit ventricular cardiomyocytes, [36] and in Langendorff-perfused rat hearts [37].

Second, the decreased arrhythmogenicity in the BQ-123 group may be due to a reduction of ischaemia within or around the infarct zone. Previous work has indicated a cardioprotective effect of ETₐ blockade, an effect mediated by increased local release of nitric oxide [38]. In accordance with these observations, we report a significant prolongation of LV APD₉₀ in the control group, indicative of myocardial ischaemia, [17,18] which was eliminated in BQ-123-treated rats. This prolongation may generate early afterdepolarizations that are thought to be involved in the genesis of ET-1-induced arrhythmias [30]. Furthermore, MAP duration in the control group displayed a significant beat-to-beat variation 24 h post-MI, an effect abolished by BQ-123. This alternans in MAP duration is typical of ischaemia and has been linked to the development of ischemic tachyarrhythmias [18,20,21]. Thus, a decreased dispersion of ventricular repolarization, either via a direct antiarrhythmic action of BQ-123, or secondary to a reduction in ischaemia, may explain the antiarrhythmic effect of ETₐ blockade seen in our study.

4.1. Limitations of the study

We feel that our study adds significantly to the current understanding of the pathophysiological role of ET-1 during acute MI. However, apart from the absence of haemodynamic data, discussed earlier, a few more limitations may be apparent. First, our sample size was relatively small, thus our study may be underpowered to detect significant differences in total mortality. Second, we did not assess the effects of acute ETₐ blockade on indices of LV remodelling, which may correlate with the occurrence of ventricular arrhythmias. However, we believe that measurement of such indices as early as 24 h post-MI would be of lesser physiological significance. Third, measurements of MAP recordings at some point during the course of MI, e.g. 1 h post-MI induction, would have permitted better assessment of the underlying pathophysiological mechanisms of ET-1 receptor blockade. However, these measurements were omitted, because they would have interfered with the arrhythmia recording process. Similarly, measurements of the ventricular effective refractory period would have added to the strengths of our study, although no significant differences were found from our group in a previous study in humans with coronary artery disease [39]. Lastly, we did not address a long-standing controversial issue, namely whether selective receptor-A,
or mixed \( \text{ET}_A \) and \( \text{ET}_B \) blockade would result in better effects post-MI.

In conclusion, acute administration of the selective \( \text{ET}_A \) antagonist BQ-123, in the rat model of acute MI, did not affect infarct size, but decreased the incidence of malignant ventricular arrhythmias during the first 24 h post-MI. In keeping with previous suggestions, this effect appears to be the result of a reduction in dispersion of repolarization across the ventricular myocardium. Whether this effect is secondary to a decrease in ischaemia or whether it represents a direct antiarrhythmic effect remains to be seen. The clinical implications of these findings may be significant, as they provide further insight into the pathophysiological mechanisms of sudden cardiac death. Additional studies are needed to determine whether these favourable results are sustained in the presence of reperfusion and to assess the effects of chronic ET-1 receptor blockade.

References


