Amiodarone Reduces Transmural Heterogeneity of Repolarization in the Human Heart

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Objectives. The present work was designed to test the effects of amiodarone therapy on action potential characteristics of the three cell types observed in human left ventricular preparations.

Background. The electrophysiologic basis for amiodarone’s exceptional antiarrhythmic efficacy and low proarrhythmic profile remains unclear.

Methods. We used standard microelectrode techniques to investigate the effects of chronic amiodarone therapy on transmembrane activity of the three predominant cellular subtypes (epicardial, midmyocardial [M] and endocardial cells) spanning the human left ventricle in hearts explanted from normal, heart failure and amiodarone-treated heart failure patients.

Results. Tissues isolated from the ventricles of heart failure patients receiving chronic amiodarone therapy displayed M cell action potential duration (404 ± 12 ms) significantly briefer (p < 0.05) than that recorded in tissues isolated from normal hearts (439 ± 22 ms) or from heart failure patients not treated with amiodarone (449 ± 18 ms). Endocardial cells from amiodarone-treated heart failure patients displayed longer (p < 0.05) action potential duration (363 ± 10 ms) than endocardial cells isolated from normal hearts (330 ± 6 ms). As a consequence, the heterogeneity of ventricular repolarization in tissues from patients treated with amiodarone was considerably smaller than in the two other groups, especially at long pacing cycle lengths.

Conclusions. These findings may explain, at least in part, the reduction of ventricular repolarization dispersion and the lower incidence of torsade de pointes observed with chronic amiodarone therapy as compared with other class III agents.

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Antiarrhythmic drugs can induce unexpected and sometimes fatal reactions by either aggravating existing arrhythmias or by producing new symptomatic arrhythmias. Torsade de pointes (TdP) is the most common arrhythmia induced by agents with class III actions (for recent review, see reference 1). In contrast to most class III drugs, amiodarone has been found to be safer in this regard and has even been used successfully in patients who had developed TdP with other agents (2,3). The electrophysiologic mechanism(s) responsible for the salutary effects of amiodarone and its low arrhythmogenicity remain incompletely understood (1).

The mechanisms that underlie sustained episodes of TdP are not well understood. The role of early afterdepolarizations (EADs)-induced triggered activity is far from clear. The development or accentuation of dispersion of repolarization between contiguous myocardial areas leading to circus movement of reentry as a mechanism for TdP has also been suggested (4) and are potentiated by bradycardia (5,6). Some have suggested that TdP may be due to triggered activity originating at two independent foci (7). A spiral wave of reentrant excitation migrating along the epicardial surface is another possible mechanism (8).

Recent studies have shown that EAD activity can be induced in a select population of cells located in the midmyocardial (M) region of the canine ventricle. M cells, but not epicardial and endocardial cells, display a marked action potential prolongation and/or EADs in response to antiarrhythmic drugs such as quinidine or dl-sotalol (9). The preferential prolongation of the M cell action potential results in a marked dispersion of ventricular recovery, creating a zone of functional refractoriness in the midmyocardial layers of the ventricular wall, an ideal substrate for reentry (for review, see reference 10). We have also identified these three cell subtypes in the human heart (11).

It has been suggested that the negligible tendency of amiodarone to induce TdP may derive from the fact that it not only prolongs ventricular repolarization, like other class III agents, but also reduces dispersion of repolarization (12). Another hypothesis could be that, unlike other class III drugs, chronic amiodarone reduces, rather than augments, transmural dispersion of repolarization at slow rates. The present study was designed to test this hypothesis using tissues from hearts explanted from patients with and without chronic amiodarone therapy.
Methods

Patient characteristics are summarized in Table 1. Electrophysiologic studies were performed on tissues isolated from explanted hearts of 1) patients with normal hearts (group NH), 2) patients with heart failure not treated with amiodarone (group HF) and 3) patients with heart failure treated with amiodarone (group AM). Even chronically administered, the duration of the amiodarone therapy was rather short (mean 12 weeks, range 3 to 32 weeks) without loading dose. The mean daily dosage was 182 ± 16 mg/day (range 170 to 200). Cardioplegia was not performed. The normal hearts were obtained from three patients with cystic fibrosis undergoing heart-lung transplantation and from one donor whose heart could not be matched.

After excision, the hearts were immediately immersed in oxygenated cold Tyrode’s solution containing (in mM): NaCl, 131; NaH2PO4, 1.8; MgCl2, 0.5; CaCl2, 2.7; KCl, 4; glucose, 5.5; mannitol, 1.1; HEPES (N-[2-hydroxyethyl] piperazine-N’-[2-ethanesulfonic acid]), 5; pH adjusted to 7.35 with Tris ([hydroxymethyl] amiodarone). The hearts were transported to the laboratory in less than 5 min. The experimental preparations consisted of transmural slices of the left ventricular free wall (for detail, see reference 11). They were placed in a tissue bath and superfused with oxygenated (95% O2/5% CO2) Tyrode’s solution (37 °C, pH 7.35) containing (in mmol/liter): NaCl, 131; KCl, 4; NaH2PO4, 1.8; NaHCO3, 18; CaCl2, 2.7; MgCl2, 0.5; D-glucose, 5.5; mannitol, 1.1. The flow rate in the tissue chamber was 12 ml/min resulting in three changes of chamber volume per minute.

Tissues were impaled with 3 M KCl-filled glass capillary microelectrodes. The electrodes were connected via an Ag/AgCl interface to an amplifier having a high input impedance and input capacity neutralization (Biologic VF-102 frame). Output was displayed on a digital storage oscilloscope (Gould 1604, USA) coupled by a 488 IEEE-interface to a plotter (Gould Colorwriter 6300), a chart recorder (Gould 8188) and a modified digital audioprocessor (Sony PCM-501ES, Japan) coupled to a videotape recorder (JVC HR-D755S). The tissue chamber was connected to ground through a salt bridge (3 M KCl) and an Ag/AgCl junction.

Electrophysiologic studies were performed after 2 h of recovery, during which time tissues were paced at a cycle length of 1 s via Teflon-coated bipolar silver wire electrodes. Stimulus pulse width was 1.5 ms and amplitude was twice diastolic threshold. During the experiments, the action potentials (AP) were recorded at pacing cycle lengths (PCL) of 1, 2, 3, 4, 5, 6 and 10 s (always in this sequence). Action potential characteristics were measured at steady state for each PCL. We measured the maximal diastolic potential, the amplitude of phase 0, the maximal rate of rise of phase 0 (Vmax) and the AP duration at 90% of full repolarization (APD90). Only data from impalements that were maintained throughout the course of the experiment were analyzed.

JT interval instead of QT interval was used to assess ventricular repolarization duration on standard electrocardiogram (ECG). This limits the bias linked to the varying QRS duration. The JT interval duration was determined from the end of the QRS complex to the end of the T wave. The end of the T wave was defined as the intersection of the isoelectric line with the tangent to the maximal down slope of the T wave (13). Considering the large range of RR intervals in our study (Table 1), we decided to correct the JT interval using the Fridericia formula (JTc = JT/(RR)1/3). The latter formula has been shown to limit the deficiencies of the Bazett formula (JTc = JT/(RR)1/2) when correcting QT interval duration for a wide range of heart rate (14,15). We used the mean of five consecutive JT intervals on lead V2 from a standard ECG.

Data were expressed as mean ± SE. Statistical analysis was performed using nonparametric Wilcoxon or U–Mann–Whitney tests, for paired or unpaired data, respectively. The

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>N (sex)</th>
<th>Age (yr)</th>
<th>Disease</th>
<th>RR (ms)</th>
<th>JT (ms)</th>
<th>JTc (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>5 (1 F, 4 M)</td>
<td>34–60</td>
<td>1 DC</td>
<td>554 ± 63</td>
<td>228 ± 24</td>
<td>278 ± 22</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group HF</td>
<td>2 (2 M)</td>
<td>55–58</td>
<td>2 DC</td>
<td>726 ± 50</td>
<td>249 ± 2</td>
<td>277 ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(n = 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group NH</td>
<td>4</td>
<td>14–46</td>
<td>3 CF</td>
<td>809 ± 277</td>
<td>295 ± 25</td>
<td>321 ± 19</td>
</tr>
<tr>
<td></td>
<td>(2 F, 2 M)</td>
<td></td>
<td>1 normal donor</td>
<td>(n = 3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CF = cystic fibrosis; CI = cardiac infarct; DC = dilated cardiomyopathy; F = female; group AM = patients with heart failure treated with amiodarone; group HF = patients with heart failure not treated with amiodarone; group NH = patients with normal heart; IC = ischemic cardiomyopathy; M = male; N = number of patients for the study; n = number of ECG available.

Abbreviations and Acronyms

AM = group of patients treated with amiodarone
APD = action potential duration
EAD = early afterdepolarization
ECG = electrocardiogram
HF = group of patients with heart failure
NH = group of patients with normal heart
PCL = pacing cycle length
TdP = torsade de pointes
Vmax = maximal rate of rise of phase 0 of the action potential
effects of PCL on APD$_{90}$ were determined by two-way analysis of variance. A value of $p < 0.05$ was considered significant.

**Results**

**Action potential configuration.** Table 2 summarizes the AP characteristics of the three cell types observed in human left ventricular preparations paced at a cycle length of 1 s. These data are illustrated in Figure 1. There was no significant difference in resting potential and amplitude of phase 0 between the cellular subtypes and from one group to another. $V_{\text{max}}$ was significantly higher in M cells than in epicardial and endocardial cells for all groups. However, $V_{\text{max}}$ was decreased in all cellular subtypes of ventricular preparations from the AM group relative to the NH and HF groups. In all cases, APD$_{90}$ was significantly higher in M cells than in other cell types. However, APD$_{90}$ of M cells was significantly reduced in group AM compared to groups HF ($p < 0.05$) and NH ($p < 0.05$), whereas APD$_{90}$ of epicardial cells was unchanged and APD$_{90}$ of endocardial cells was greater in the HF ($p < 0.05$) and AM ($p < 0.05$) groups than in the NH group.

**Rate-dependence of action potential duration.** Figure 2 shows APD$_{90}$-rate relations of the three cellular subtypes in the three groups studied. Treatment with amiodarone had little effect on epicardial and endocardial cells. However, after treatment with amiodarone, APD$_{90}$ of M cells was significantly reduced ($p < 0.05$), especially at long PCL, and the slope of their APD$_{90}$-rate relation was no longer steeper than that of other cellular subtypes: the typical response of M cells to bradycardia, that is, the large increase of their APD, was lost (see also Fig. 1).

**Discussion**

Amiodarone is a unique antiarrhythmic compound with poorly understood electrophysiologic effects (16). It acts primarily by prolonging ventricular repolarization (16,17), and for this reason has been defined as a class III antiarrhythmic agent, but its electrophysiologic effects fit all four classes (16). Amiodarone differs from other class III agents in reducing QT dispersion (12). Besides its remarkable antiarrhythmic and antifibrillatory efficacy, this compound has a low proclivity to induce proarrhythmia despite its significant class I properties. The precise mechanism for this low incidence of amiodarone-associated proarrhythmia remains speculative, but differential effects on APD of varying lengths in ventricular myocardium could be involved in this unexplained paradox.

**Amiodarone and transmural heterogeneity.** Up to now the effects of amiodarone treatment on M cells in the human heart were not known. This study suggests that chronic treatment with amiodarone reduces the transmural dispersion of ventricular repolarization in human heart. This comes from a differ-
ential effect of amiodarone on repolarization of the different cellular subtypes. In the AM group, the heterogeneity of ventricular repolarization was considerably smaller than in the two other groups, especially at long PCLs. Comparable results were found by Sicouri et al. (18) in dogs chronically treated with amiodarone. These authors showed that APD in M cells was reduced by amiodarone, especially at long PCLs, while APD in endocardium was prolonged. These results, that is, an important decrease in transmural dispersion of repolarization, are typically those obtained in our study. They also showed that the epicardial AP was the least modified by amiodarone, which is what we also observed.

It is of interest that previous studies showing that chronic amiodarone prolongs the AP of ventricular myocardium were performed on endocardium isolated from normal animals. A differential effect of amiodarone has also been observed by Yabek et al. (19), who showed that superfusion with amiodarone reduced APD of Purkinje fibers from nontreated dogs, but prolonged APD of the surrounding myocardium.

M cells have electrophysiologic properties similar to those of Purkinje cells: their APs are longer than that of other cellular subtypes and they are markedly prolonged by bradycardia. After treatment with amiodarone, the APD of M cells no longer increases dramatically with PCL. It was shown that M cells, because of their typical response to bradycardia, are more sensitive than other myocardial cells to drugs that prolong repolarization and induce EADs and triggered activity (9). This double effect of amiodarone, that is, to decrease transmural heterogeneity of ventricular repolarization and to suppress M cells sensitivity to bradycardia, may explain its clinical properties. Indeed, the homogenization of ventricular repolarization (Fig. 2) could explain, at least in part, why amiodarone differs from other class III agents such as sotalol or sematilide.

The absence of steeper APD-rate relationship of M cells could lead to a corresponding reduction in the possibility of pharmacologically induced EADs and reentrant mechanisms and may account for the lower incidence of TdP, compared to that after dl-sotalol or other class III which tend to increase APD in M cells to a greater extent than in epicardial and endocardial cells (10). As already noted, it is well known that EADs that give rise to TdP appear to arise in Purkinje network and M cells with transmission to ventricular muscle. Furthermore, it was reported that amiodarone may prove to be a suitable therapeutic option for TdP in patients with drug-induced TdP (2,3).

Another finding of this study is that heart failure does not seem to prolong repolarization of epicardial and M cells. Only endocardial APs were significantly prolonged compared to normal hearts. This prolongation of endocardial APs has already been observed in other studies (20).

**Amiodarone and JTc interval.** Amiodarone is well known to prolong QT interval (21). However, various responses in QTc prolongation depend on both the duration of the treatment and the dose. Pollack et al. (22) found that 70% of the QTc interval lengthening occurred between 9 and 12 months after treatment initiation. Besides, previous reports of patients receiving a mean dose of 380 mg/day showed only an 8% increase in QTc interval (23,24), whereas patients receiving 600 to 1,200 mg/day have shown a 23% increase (25). In our study, amiodarone did not induce a prolongation of the JTc interval, in agreement with our in vitro results. We believe that this effect was due to both a rather short duration of treatment and a small dose of amiodarone. These results are comparable.

![Figure 2](image_url)
to those obtained by Sicouri et al. (18) in dogs. Thus, we can speculate that amiodarone treatment induces in a first time a decrease of the transmural heterogeneity and in a second time a prolongation of ventricular repolarization.

**Limitations of the study.** Aside from the obvious limitations inherent in studying tissues isolated from their native milieu, this study has the additional limitation of not being able to compare the effects of chronic amiodarone in normal hearts. The data, from which the actions of amiodarone are deduced, derive from a heterogeneous mixture of diseased hearts. Another limitation comes from the small number of patients with heart failure. This comes from the fact that amiodarone, or other drugs having major effects on repolarization, is frequently prescribed to patients with severe heart failure. These limitations notwithstanding, the results are sufficiently compelling to warrant further investigation into the problem.

**Conclusion.** In summary, these data indicate for the first time that an important aspect of the antiarrhythmic action of amiodarone in humans may be its differential electrophysiologic effects on the three cellular subtypes, leading to more homogeneous repolarization across the ventricular wall. This may explain at least in part the reduced occurrence of TdP and ventricular fibrillation with amiodarone compared to other class III antiarrhythmic drugs. Finally, we should keep in mind that the electrophysiologic properties of amiodarone are probably more complex and multifactorial.

The authors contributed to this study the following way: Emmanuel Drouin, the in vitro experiments on the human hearts; Gilles Lande, the analysis of the ECGs and discussion; and Flavien Charpentier, the supervision of the work and preparation of the manuscript.

**References**


