Electrophysiological Effects of Dronedarone (SR33589), a Noniodinated Benzofuran Derivative, in the Rabbit Heart
Comparison With Amiodarone

Wei Sun, MD; Jonnalagedda S.M. Sarma, PhD; Bramah N. Singh, MD, DPhil

Background—To overcome the side effects of amiodarone (AM), its noniodinated analogue, dronedarone (SR), was synthesized. In this study, its electrophysiological effects were compared with those of AM in rabbit hearts.

Methods and Results—Five animal groups (n=7 each) for 3 weeks received daily oral treatment of 1 of these regimens: (1) control, vehicle only; (2) AM 50 mg/kg (AM50); (3) AM 100 mg/kg (AM100); (4) SR 50 mg/kg (SR50); and (5) SR 100 mg/kg (SR100). ECGs were recorded before drug and at 3 weeks of drug before euthanasia. Action potentials were recorded from isolated papillary muscle and sinoatrial node by microelectrode techniques. The short-term effects were studied in controls (n=5) at various concentrations of SR (0 to 10 μmol/L) in tissue bath. Action potential duration at 50% (APD50) and 90% (APD90) repolarization and upstroke dV/dt (Vmax) at various cycle lengths were compared by ANOVA with repeated measures. Compared with control, AM and SR increased RR, QT, and QTc intervals (P<0.0001 for all). Ventricular APD50 and APD90 were lengthened by 20% to 49% as a function of dose (P<0.005 to <0.0001) and cycle length (P<0.001). SR100 effects were greater than those of AM100 (P<0.002). Vmax was decreased by both AM100 (P<0.0001) and SR100 (P<0.01). Sinoatrial node automaticity was slowed in treated groups compared with that of the control group (P<0.0001 for all).

Conclusions—The electrophysiological effects of dronedarone are similar to those of AM but more potent, despite deletion of iodine from its molecular structure, a finding of importance for the development of future class III antiarrhythmic compounds. (Circulation. 1999;100:2276-2281.)

Key Words: potentials ■ amiodarone ■ dronedarone ■ electrophysiology ■ drugs

Amiodarone (AM) has now emerged as an unusually effective antiarrhythmic agent for controlling ventricular and supraventricular tachyarrhythmias.1 The fact that it might act by prolonging the myocardial action potential duration (APD) after long-term administration was initially suggested in 1970.2 Subsequently, it was found that beyond this class III action by inhibiting potassium channels,3,4 the drug exhibited all other known classes of antiarrhythmic mechanisms described by Singh et al.2,5,6 These include antiadrenergic activity and inhibition of fast sodium and slow calcium channels.6 Which of these effects might be responsible for the unique clinical antiarrhythmic and low proarrhythmic potentials of the drug remains unclear. Although the role of AM now is well entrenched in clinical practice, its side-effect profile remains of concern.7

AM is an iodinated compound. Its major toxicity profile after drug ingestion as a function of time might be due to iodine.1 The development of ocular and serious pulmonary toxicity7 or thyroid dysfunction8,9 has been attributed to the iodinated nature of the molecule.8 However, iodine as an integral component of the AM molecule might have other consequences.10 Singh and Vaughan Williams2 found that the ventricular APD prolongation in rabbits treated long-term with AM was abolished by administration of thyroxine. There is evidence that the effect of AM might be due in part to cardioselective inhibition of thyroid hormone action in cardiac muscle.11–13 The question arose as to whether the unique long-term electrophysiological effects of AM might stem from its molecular interaction with thyroid hormone receptors independently of iodine in the compound. The development of the noniodinated benzofuran derivative SR33589 (SR), or dronedarone (Sanofi-Recherche), structurally related to AM (Figure 1), provided the opportunity to examine this possibility.

The short-term effects of SR are similar to those of AM. In anesthetized animals,14 SR inhibited ischemia-induced arrhythmias, reduced heart rate, and exerted sympatholytic effects characteristic of AM.15 The present study compares the cellular electrophysiological and ECG actions of SR and AM after 3 weeks of oral administration. The short-term
The maximum slope of action potential upstroke ($V_{\text{max}}$) was obtained by amplified and displayed on an oscilloscope (Tektronics 2201). Signals were amplified and displayed on a computer with pClamp software (Axon Instruments).

**Methods**

**Long-Term Studies**

New Zealand White rabbits of either sex weighing 1.9 to 2.2 kg were used. Long-term studies were conducted in 5 separate groups, 7 animals per group. Each group was treated orally daily for 3 weeks with 1 of the following regimens: (1) vehicle only, consisting of 7 mL of 75% polyethylene glycol (MW 400) (control group); (2) SR at 50 mg/kg (SR50 group); (3) SR at 100 mg/kg (SR100 group); (4) AM at 50 mg/kg (AM50 group); and (5) AM at 100 mg/kg (AM100 group). The drugs were administered by gavage in a solution prepared fresh every day. Each dose of SR or AM was dissolved in 5.25 mL of 75% polyethylene glycol (MW 400) diluted to 7.0 mL with distilled water before administration. ECGs recorded from conscious restrained rabbits were stored in digitized form. The QTc was obtained by Bazett’s formula.

After completion of treatment, the rabbits were anesthetized with sodium pentobarbital (30 mg/kg IV), and hearts were rapidly removed and dissected in cold oxygenated Tyrode’s solution. Tissue blocks (2×3 mm) from the middle part of the sinoatrial (SA) node region and the papillary muscles (0.4 to 0.6 mm in diameter and 3 to 4 mm long) from right ventricle were mounted in a tissue bath (10 mL volume) and superfused with Tyrode’s solution (15 mL/min) at 37°C. Whole-cell recordings were made using a patch pipette filled with 3 mol/L KCl, tip resistance 10 to 20 MΩ. The electrode was connected by Ag-AgCl wire to a high-input impedance amplifier (Warner Instruments). Signals were amplified and displayed on an oscilloscope (Tektronics 2201). The maximum slope of action potential amplitude, $V_{\text{max}}$, and APD at 50% and 90% repolarization (APD50 and APD90, respectively) were measured from the papillary muscles. Maximal diastolic potential, spontaneous cycle length, and $V_{\text{max}}$ were measured from the SA node. The frequency-dependent effects of SR and AM in the papillary muscles were evaluated at cycle lengths of 1200, 900, 600, and 300 ms. Action potential recordings were obtained after 5 minutes of steady stimulation at each cycle length. Data were digitized and stored on a computer with pClamp software (Axon Instruments).

**Data Analysis**

The data are presented as mean±SD. The intergroup comparisons of the cycle length–dependent effects on APD50, APD90, and $V_{\text{max}}$ in papillary muscles were made by ANOVA with repeated measures, with cycle length as the within factor and the treatment as the grouping factor. By use of this analysis, the effects of treatment, the effects of cycle length, and the interaction between treatment and cycle length were evaluated simultaneously. All other parameters, including the ECG and the SA nodal parameters, were evaluated by 1-way ANOVA. If ANOVA indicated significant differences among the groups, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons. BMDP biomedical statistical software was used (SPSS Inc).

**Results**

**Whole-Animal Data**

All animals remained active during treatment and gained weight, an average of 0.59±0.18 kg. There were no significant differences in ECG parameters or RR, PQ, QT, or QRS intervals among the groups before treatment. There was a significant prolongation of RR, QT, and QTc intervals in all drug-treated groups compared with control (Table 1). However, the dose-related and drug-specific changes in the RR, QT, or QTc intervals among the treated groups did not attain statistical significance.

**Effects on Ventricular Action Potential Characteristics**

The mean values of the parameters measured from the papillary muscles are summarized in Table 2. Representative traces of action potentials at various cycle lengths for control, SR100, and AM100 groups are presented in Figure 2. The mean data on APD50 and APD90 for all groups are plotted against the cycle length in Figure 3. Both APD50 and APD90 were prolonged significantly, by 31% to 56% and 28% to 47%, respectively, in the drug-treated groups compared with control ($P<0.0001$). The patterns of cycle length versus APD curves shown in Figure 3 were significantly different (ie, significant interaction between treatment and cycle length) between treatment groups and control ($P<0.001$). The effects of drug treatment were significantly cycle-length–dependent in all treated groups. The slopes of the APD50 and APD90 plots against the cycle length of treated groups were not significantly different. The APD50 and APD90 of the SR100 group were significantly more prolonged than those in the AM100...
group (P<0.002). At the lower dose, there was a significantly greater prolongation only in the APD_{50} of the SR50 group compared with that of the AM50 group (P<0.03). The prolongations in APD_{50} and APD_{90} were significantly dose-dependent for both drugs (P<0.005 to <0.0001). The effective refractory period (ERP) measured at 900-ms cycle length was highly correlated with the APD_{90} across the treatment groups (R=0.988; P<0.0001), with ERP at 84% of APD_{90}. Therefore, ERP data were not analyzed separately.

When APD data were compared at the shortest cycle length (300 ms), the APD_{50} and APD_{90} of the AM50 group were not significantly prolonged compared with control, whereas the AP of SR50, SR100, and AM100 were significantly prolonged over control (Figure 3). The relative prolongation of APD over mean control values in the treated groups are shown in Table 2. The V_{max} values of the papillary muscle preparations were significantly lower with the shortening of the cycle length in all groups (P<0.0001). However, the relative differences among all groups, including the control group, were not significantly cycle-length–dependent (Figure 5). Significant reduction of V_{max} compared with that in the control was observed in SR100 (P<0.0001) as well as AM100 groups (P<0.01). The dose-dependent reduction of V_{max} was significant in the case of SR (P<0.01), but not AM (P=NS).

### Effects of SR and AM Treatments on the SA Nodal Preparations

The mean data are summarized in Table 3. Representative traces of relevant action potential recordings are presented in Figure 6. There were no differences among the groups with respect to maximum diastolic potential, action potential amplitude, or V_{max} of the SA nodal preparations. However, the spontaneous cycle length was significantly prolonged in the treated groups compared with those in the control group (P<0.0001 for all). Spontaneous cycle length was significantly more prolonged with SR than with the corresponding dose of AM (P<0.0005) at both the lower (50 mg·kg^{-1}·d^{-1}) and higher (100 mg·kg^{-1}·d^{-1}) doses.

### Short-Term Studies

The results are summarized in Figure 7. In contrast to long-term studies, both APD_{50} (Figure 7A) and APD_{90} (Figure 7B) were shortened in a dose-dependent manner over the range of 1 to 10 μmol/L SR concentration and 300- to 1200-ms stimulation cycle lengths. However, consistent with the long-term study, V_{max} measured at a stimulation cycle length of 900 ms decreased in a dose-dependent manner over the entire range of concentrations (Figure 7C).

### Table 1: Effect of Long-Term Oral Administration of AM or SR on Rabbit Surface ECG Parameters in the Conscious State

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RR, ms</th>
<th>PQ, ms</th>
<th>QRS, ms</th>
<th>QT, ms</th>
<th>QTc, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>235±20</td>
<td>57±7</td>
<td>43±4</td>
<td>140±9</td>
<td>289±15</td>
</tr>
<tr>
<td>AM, mg · kg^{-1} · d^{-1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>283±18*</td>
<td>59±5</td>
<td>46±4</td>
<td>176±9*</td>
<td>330±17*</td>
</tr>
<tr>
<td>100</td>
<td>285±18*</td>
<td>58±7</td>
<td>47±4</td>
<td>181±12*</td>
<td>338±11*</td>
</tr>
<tr>
<td>SR, mg · kg^{-1} · d^{-1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>286±17*</td>
<td>60±6</td>
<td>47±4</td>
<td>177±10*</td>
<td>331±14*</td>
</tr>
<tr>
<td>100</td>
<td>288±20*</td>
<td>59±5</td>
<td>47±4</td>
<td>183±9*</td>
<td>340±11*</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=7 for each group. Drugs were administered for 3 weeks.

### Table 2: Effects of Long-Term Oral AM or SR on Transmembrane Action Potentials of Rabbit Papillary Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RP, mV</th>
<th>APA, mV</th>
<th>V_{max}, V/s</th>
<th>APD_{50}, mV</th>
<th>APD_{90}, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>−85±5</td>
<td>101±5</td>
<td>210±20</td>
<td>103±13</td>
<td>130±15</td>
</tr>
<tr>
<td>AM, mg · kg^{-1} · d^{-1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>−85±4</td>
<td>101±4</td>
<td>200±21</td>
<td>124±24†</td>
<td>155±27†</td>
</tr>
<tr>
<td>100</td>
<td>−85±6</td>
<td>101±5</td>
<td>191±21†</td>
<td>138±27†</td>
<td>172±29†</td>
</tr>
<tr>
<td>SR, mg · kg^{-1} · d^{-1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>−85±5</td>
<td>101±6</td>
<td>199±22*</td>
<td>133±23†</td>
<td>160±24†</td>
</tr>
<tr>
<td>100</td>
<td>−85±5</td>
<td>101±5</td>
<td>183±20†</td>
<td>154±25†</td>
<td>183±28†</td>
</tr>
</tbody>
</table>

RP indicates resting transmembrane potential; APA, action potential amplitude. The V_{max}, APD_{50}, and APD_{90} values were averaged over 4 cycle lengths (1200, 900, 600, and 300 ms). Values are mean±SD. n=7 for each group. Drugs were administered for 3 weeks.

*P<0.05, †P<0.001 vs vehicle group.
Discussion

Major Findings
The main findings of the study indicate that despite the deletion of iodine from the molecule compared with that in AM, the major electrophysiological properties of SR are very similar to those of AM. During short-term superfusion, SR shortened the APD, as reported for AM, but reduced the ventricular V_max. In contrast, after 3 weeks of oral administration of both drugs, there was significant slowing of the sinus frequency in vivo and in vitro associated with a significant prolongation of ventricular APD. In the sinus node, the rate slowing after long-term treatment was due to the depression of phase 4 depolarization and lengthening of the APD. Both SR and AM produced comparable degrees of depression of V_max as an index of inhibition of the ventricular myocardial sodium channel activity. Thus, the overall data show that SR is at least as potent as AM in its ability to alter the electrophysiological properties of ventricular muscle and those of the sinus node.

Frequency-Dependent Electrophysiological Effects
It is well known that the APD-lengthening effect of most class III antiarrhythmic drugs is reduced by increases in rate and duration of stimulation of cardiac muscle. Such an effect has been described as reverse rate- and use-dependency, in contrast to the increases in the effects of class I agents on blocking sodium channel function. Hondeghem and Snyder suggested that reverse use-dependency may be responsible for a high incidence of torsade de pointes associated with most class III antiarrhythmic agents. This is especially so in the case of those agents that exert their predominant repolarization-blocking effects by inhibiting the rapid component of the delayed rectifier K current, $I_{Kr}$. In this regard, the long-term effects of AM differ from those of most other class III agents in inducing a negligible incidence of torsade de pointes, an effect that has been attributed to marked inhibition of the slow component of the delayed rectifier K current, $I_{Ks}$. Whether SR might also act by a similar or identical action on the $I_{Ks}$ is currently under study. However, our present study showed that SR and AM both prolonged APD_50 and APD_90 in a cycle length-dependent manner while exhibiting a minimal degree of reverse use-dependency. An unusual observation was that the percent prolongation at the shortest cycle length (300 ms) studied in our experiments was significantly greater with SR than that with AM at the higher drug dose of 100 mg·kg^{-1}·d^{-1} tested. Thus, under the conditions of our study, SR exhibited even less reverse use-dependency of repolarization than that found with AM, which has been shown to display minimal reverse use-dependency under in vivo conditions.
TABLE 3. Effects of Long-Term Oral Administration of AM or SR on the Transmembrane Potential of Rabbit SA Nodal Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDP, mV</th>
<th>APA, mV</th>
<th>Vmax, V/s</th>
<th>SCL, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>−63±6</td>
<td>73±7</td>
<td>10±3</td>
<td>425±9</td>
</tr>
<tr>
<td>AM, mg·kg⁻¹·d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>−62±7</td>
<td>74±7</td>
<td>10±3</td>
<td>461±11†</td>
</tr>
<tr>
<td>100</td>
<td>−62±6</td>
<td>73±7</td>
<td>10±3</td>
<td>496±13‡</td>
</tr>
<tr>
<td>SR, mg·kg⁻¹·d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>−62±7</td>
<td>73±7</td>
<td>10±3</td>
<td>487±16‡</td>
</tr>
<tr>
<td>100</td>
<td>−62±9</td>
<td>74±6</td>
<td>10±3</td>
<td>548±11†‡</td>
</tr>
</tbody>
</table>

MDP indicates maximum diastolic potential of SA nodal cells; APA, action potential amplitude; and SCL, spontaneous cycle length of the SA nodal preparation. Values are mean±SD, n=7 for each group. Drugs were administered for 3 weeks.

*Significantly prolonged vs control group (P<0.0001).
†Significantly prolonged vs the lower-dose group (P<0.001).
‡Significantly prolonged vs the corresponding dose of AM (P<0.005).

Significance of Blocking Myocardial Sodium Channels

In the present studies, the Vmax values of papillary muscle transmembrane action potentials were significantly reduced by both AM and SR, indicating inhibition of the fast Na channel. Whether such an additional property might contribute to the overall antiarrhythmic actions of these drugs remains uncertain. In AM, the associated class I antiarrhythmic effect is of moderate potency, 21,23,24 but its rate-dependency has not been as compellingly uniform. 21 Our data indicating that SR, a noniodinated benzofuran derivative, might have a similar potency for blocking the fast channel in ventricular myocardium are of particular interest relative to its similarity to the overall properties of AM.

Potential Mechanisms of Heart Rate Slowing

Although the long-term in vivo effects of AM and SR in terms of increases in RR, QT, and QTc intervals showed trends similar to those of the in vitro data, the differences between the drugs did not attain statistical significance. Also, there were no significant differences between the 2 doses (50 and 100 mg·kg⁻¹·d⁻¹) tested, suggesting a saturation effect. However, our data did not address the issue of whether a more prolonged drug exposure might lead to further increases in the RR intervals. In the case of AM and SR, the slowing of the sinus rate might be attributable to the lengthening of APD with a delayed attainment of the maximal diastolic potential in the sinus pacemaker, accompanied by drug-induced depression of phase 4 depolarization by antiadrenergic actions, as shown for AM in vivo. 25 The present results on the effect of AM on spontaneous cycle length of the SA node are consistent with our previous results. 26 There is evidence that SR also interacts with β-adrenergic receptors of the rat heart at intracellular sites. 27

Benzofuran Derivatives and Thyroid Hormone Interactions

The overall similarities in the electropharmacological effects of AM and its noniodinated derivative SR demonstrated here have potentially important implications for new drug development. There is a structural similarity between AM and thyroid hormones, including the iodine in its aromatic ring (Figure 1). Iodine release in the body after drug ingestion may cause an altered thyroid state, an effect that is clearly related to iodine rather than to the molecular structure of AM. 28 It also has been suggested that several of the most significant side effects of the drug—pulmonary fibrosis, ocular deposits, and skin pigmentation—are related to iodine contained in the AM molecule. Conversely, it is known that the cardiac electrophysiological effects of long-term AM closely resemble those of hypothyroidism. 10 In this regard, the effect of AM on cardiac repolarization appears to have a measure of specificity. 11–13 A direct inhibition of the T3 nuclear receptor binding by AM or its metabolite desethylamiodarone has been postulated to result in a hypothyroid state at a cellular level. 11 AM and its active metabolite have been shown to bind to different isofoms of nuclear T3 receptors with variable affinity. 30 It is noteworthy that the brominated analogue (without the iodine) of AM has been shown to have identical class III antiarrhythmic actions comparable to those of AM. 31,32 Thus, the data raise the possibility that neither the presence of a halogen nor its type might be the basis for the unique electrophysiological properties of benzofuran derivatives as a class for the propensity to homogeneously increase the duration of the cardiac action potential. Our data in the present study demonstrating that the long-term effects of SR, a nonhalogenated benzofuran derivative, closely resemble not only those of long-term AM but also those reported for hypothyroidism. 10 Thus, in the clinical setting, SR therapy...
may not have the same proclivity to induce altered thyroid state or the iodine-related complications seen with AM. Conversely, the similarity between the molecular structure of SR and thyroid hormone, as in the case of AM, does not exclude the possibility that the compound might exert its potentially beneficial electropharmacological effect on cardiac muscle by cardioselective blockade of T3 receptors in cardiac muscle.

Conclusions
The results of this study demonstrate that the major short-term and long-term electrophysiological properties of the noniodinated derivative (SR) of AM on cardiac muscle are very similar to those of the parent compound. After 3 weeks of oral administration, AM and SR reduced sinus frequency in vivo and in vitro with a significant prolongation in the APD in the rabbit ventricular myocardium. This was accompanied by a corresponding increase in the ERP. Both SR and AM produced comparable degrees of depression of the Vmax as an index of inhibition of the myocardial sodium channels. Thus, the overall data show that SR is at least as effective as AM in its ability to alter the electrophysiological properties of ventricular muscle and those of the sinus node. Its actions are not mediated by the presence of iodine, but the similarity between the molecular structure of SR and thyroid hormone, as in the case of AM, suggests the possibility that its beneficial effect may stem from other mechanisms, which may include cardioselective blockade of T3 receptors in cardiac muscle.

Acknowledgments
This study was supported by a grant-in-aid from Sanofi-Winthrop Recherche (Montpellier, France), which also provided amiodarone.

References
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