LABORATORY INVESTIGATION
ARRHYTHMIA

The antiarrhythmic efficacy of amiodarone and desethylamiodarone, alone and in combination, in dogs with acute myocardial infarction

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ABSTRACT Antiarrhythmic activity of amiodarone’s desethyl metabolite, which accumulates during oral amiodarone therapy, has been postulated to explain the delayed onset of antiarrhythmic effects during long-term amiodarone therapy. To determine their relative antiarrhythmic efficacy, amiodarone and its desethyl metabolite, desethylamiodarone, were administered to mongrel dogs with ventricular tachycardia 24 hr after ligation of the left anterior descending coronary artery. Cumulative doses of amiodarone, desethylamiodarone, a combination of amiodarone and desethylamiodarone, or the vehicle for drug administration were given at 1 hr intervals. Both amiodarone and desethylamiodarone suppressed ventricular arrhythmias in a dose-dependent fashion. The metabolite, however, was more potent with a 50% effective concentration for suppression of premature ventricular complexes of 1.4 mg/liter compared with 4.6 mg/liter for the parent compound. Plasma and myocardial drug concentrations were similar to those measured during long-term amiodarone therapy in man, with desethylamiodarone producing greater myocardial concentrations than amiodarone for a given plasma concentration. Co-administration of the metabolite along with the parent drug resulted in suppression of arrhythmias at lower doses of amiodarone than when the latter was administered alone, and concentration-response analysis indicated an additive antiarrhythmic effect. These experiments suggest that the accumulation of desethylamiodarone that occurs with long-term oral amiodarone therapy contributes importantly to the antiarrhythmic effects of the drug, and may account for the gradual increase in antiarrhythmic action during the course of amiodarone therapy.


OVER THE PAST few years, amiodarone has emerged as a particularly valuable drug for the treatment of refractory ventricular tachyarrhythmias.1, 2 Maintenance doses of amiodarone are associated with an important delay to the onset of antiarrhythmic action. Intravenous3, 4 and oral5 loading doses can shorten this delay, but differences between early and later antiarrhythmic effects remain, despite the achievement of comparable plasma amiodarone concentrations.3–5 Similar differences between the antiarrhythmic effects of short- and long-term amiodarone therapy have been observed in a canine preparation of sudden coronary death.6 Desethylamiodarone, amiodarone’s N-desethyl metabolite, accumulates in the plasma of patients on sustained amiodarone therapy. Heger et al.7 reported that patients treated with amiodarone for at least 3 months had plasma concentrations of the desethyl metabolite averaging 78% of those of the parent compound. We have shown that desethylamiodarone has more potent effects than amiodarone on fast-channel tissues in both rats8 and dogs.9 The mechanisms of amiodarone’s antiarrhythmic actions are incompletely known, but use-dependent sodium channel-blocking effects10 may play an important role. It is therefore likely that some of the long-term antiarrhythmic effects of amiodarone are due to accumulation of its metabolite.

The experiments described in this article were designed to evaluate the relative antiarrhythmic potency of amiodarone and its desethyl metabolite in a canine preparation showing ventricular tachyarrhythmias. There is evidence that the electrophysiologic effects of combinations of a parent drug and its metabolite may sometimes differ from the effects expected based simply on additive actions.11 We therefore studied the results of administering amiodarone, desethyl-
amiodarone, or combinations of the two to dogs 24 hr after acute anterior myocardial infarction.

Methods

Experimental preparation. Mongrel dogs of both sexes were anesthetized with sodium pentobarbital (30 mg/kg iv, modified as necessary to induce anesthesia). Subsequent doses of pentobarbital were administered as required to suppress the blink reflex. Mechanical ventilation was instituted after endotracheal intubation. A left thoracotomy was performed under aseptic conditions and the left anterior descending coronary artery was isolated distal to the first diagonal branch. Two silk ligatures were placed around the vessel, and the artery was occluded in two stages 30 min apart. The first ligation was performed around a 20-gauge needle blunted at the tip, and the second stage involved a tight and permanent ligature around the artery. One hour was then allowed for resolution of acute ischemic arrhythmias, the chest was closed in layers, and the pneumothorax was evacuated. The dogs were returned to the animal quarters, and treated with levorphanol tartrate (a long-acting opiate narcotic) as needed for pain.

The next day, dogs were anesthetized with morphine (2 mg/kg im) and a-chloralose (100 mg/kg iv, modified as necessary) and returned to the electrophysiology laboratory. Dogs were ventilated mechanistically with a cuffed endotracheal tube. A ventilatory rate of 10/min was used, along with a tidal volume obtained from a nomogram relating minute ventilation to body weight. Arterial blood gases were measured to ensure adequate oxygenation (arterial O2 saturation >90%) and physiologic pH (7.38 to 7.45). Two femoral intravenous catheters and a femoral arterial catheter were inserted. Arterial pressure was monitored with a Statham P23 1D transducer, and at least two electrocardiographic leads were recorded with either a pen recorder (Grass Instruments, Quincy, MA) or a Mingograf 80 inkjet recorder (Siemens Inc., Toronto, Canada).

Experimental protocol. Three 1 min electrocardiographic (ECG) recordings were obtained at 5 min intervals before drug administration. Amiodarone, desethylamiodarone, a combination of the two, or the vehicle for drug administration was then given. All doses of amiodarone and desethylamiodarone were dissolved in 2 ml of 50% ethanol, as previously described. Animals receiving amiodarone or desethylamiodarone were given doses of 5, 10, and 20 mg/kg, with each dose being given intravenously over 20 min and with a 60 min observation period after the end of each dose. Dogs given a combination of the drugs received 2.5, 5, and 10 mg/kg of each agent simultaneously. The number of dogs given amiodarone, desethylamiodarone, the combination of the two, and the vehicle for drug administration alone was 6, 6, 5, and 6, respectively. Each animal was allocated to only one treatment group.

One minute ECG strips were recorded immediately after the end of drug administration and at 5, 10, 15, 30, 45, and 60 min after each dose. Blood for determination of plasma drug concentrations was drawn before drug administration (blank) and at 30 and 60 min after each dose. At the end of each experiment, the dog was killed with an overdose of pentobarbital. Atrial and ventricular samples were obtained for measurement of tissue drug concentrations, and myocardial infarct size was measured to ensure comparability of infarct size between groups.

Data analysis. Analysis of antiarrhythmic effects was based on the ECG recordings obtained before and after drug administration. Ventricular ectopic complexes were defined as broad QRS complexes with a morphology different from sinus beats and either no preceding P waves or an inconsistent PR interval for a given morphology. The number of ventricular ectopic complexes in each 1 min strip was counted. Ventricular tachycardia was invariably present before drug administration. The rate of ventricular tachycardia was determined by calculating the mean RR interval for a sequence of 11 tachycardia beats before drug administration, and for the longest sequence of beats (up to 10 cycles) at each observation point after drug administration. Ventricular tachycardia was defined as a sequence of three successive ventricular ectopic complexes at a rate faster than that of the underlying sinus rhythm. The mean of the three values obtained during each 1 min control recording was taken to represent control values for each dog.

Plasma and tissue concentrations of amiodarone and desethylamiodarone were measured by high-pressure liquid chromatography (HPLC), as previously described. Samples of atrial tissue, right ventricular myocardium, and normal and infarcted left ventricular myocardium were obtained at the end of each experiment and frozen for subsequent drug assay. Initially, the left ventricular samples were obtained after infarct delineation by vital staining (see below). Samples obtained in this fashion were found to be unsuitable for assay by HPLC because of interference between the vital dye and the assay procedure. Subsequently, therefore, normal and infarct zone samples from the left ventricle were selected for assay based on differences in gross appearance, with later confirmation of correct identification by the vital staining procedure.

Equilibrium is established slowly between plasma and myocardial amiodarone concentrations, with 90% of equilibrium occurring 80 min after bolus intravenous administration. We analyzed the antiarrhythmic effects of amiodarone, desethylamiodarone, and the combination in relationship to plasma concentrations measured 80 min after the onset of drug administration (60 min after the end of drug infusion). The percent reduction in ventricular ectopic complexes at the end of each observation period compared with control values was transformed into probit units and related to plasma drug concentrations by linear regression analysis for experiments evaluating the effects of individual agents. The resulting concentration-response equations were used to estimate the effects expected from the amiodarone and desethylamiodarone concentrations achieved at the end of each observation period in each experiment studying combination administration. The results predicted by a simple additive effect model for the combination were then compared with the results observed after each dose of the combination.

Myocardial infarct size was measured by use of triphenyltetrazolium chloride and previously described methods. Analysis of variance with Duncan’s range test was used to test the significance of differences between multiple repeated observations on a single group. Paired t tests were used for comparisons between groups when only one pair of values was being compared. Group averages are expressed in this manuscript as the mean ± SE, and a probability of less than 5% was taken to indicate statistical significance.

Results

Comparative antiarrhythmic activity of amiodarone and desethylamiodarone. Amiodarone and desethylamiodarone both produced dose-dependent reductions in the prevalence of ventricular ectopy (figure 1). These effects were statistically significant only after the last dose of each agent. The frequency of ventricular ectopy was constant over time among control dogs given the vehicle (figure 1). Both amiodarone and desethylamiodarone substantially reduced the intrinsic fre-
frequency of ventricular tachycardia, as shown in figure 2. Statistical analysis of changes in rate of ventricular tachycardia after the largest dose of each agent was not possible because of extensive data dropout, with continuous sinus rhythm observed (and therefore no ventricular tachycardia manifest) for at least one 1 min observation period after the last dose in three of six dogs receiving amiodarone and five of six dogs receiving desethylamiodarone. Desethylamiodarone reduced the prevalence of ventricular ectopy to a greater extent than did amiodarone (figure 1), but differences in percent reduction in premature ventricular complexes (PVCs) between the agents were not statistically significant. Neither amiodarone nor desethylamiodarone significantly altered blood pressure, although there was a tendency for blood pressure to increase when sinus rhythm was restored.

**Antiarrhythmic activity of a combination of amiodarone and desethylamiodarone.** With long-term oral amiodarone therapy in man, the effects observed result from the accumulation of both the amiodarone administered and the desethylamiodarone produced by hepatic bio-transformation. We attempted to simulate this situation by administering equal doses of amiodarone and desethylamiodarone in total quantities equal to those studied for single agents. The change in PVC frequency produced by the combination is compared with the effects of amiodarone and desethylamiodarone alone in figure 3. The effects of the combination are of the same order as those produced by single agents. In fact, the mean number of PVCs per minute for dogs given each of the single agents falls within the 95% confidence limits for the effect of the combination at all but one observation point. Changes in rate of ventricular tachycardia produced by the combination were also of the same order as changes produced by single agents (figure 2).

**Quantitative analysis of antiarrhythmic potencies from the plasma concentration-response curve.** In order to further analyze the relative antiarrhythmic potency of amiodarone and desethylamiodarone, and to better understand the effects of the combination, plasma concentration-effect curves for single agents were constructed (figure 4). For each experiment, the plasma concentration 60 min after the end of each infusion was related to the resulting percent reduction from control in PVC frequency. Antiarrhythmic effects were consistently seen at lower concentrations...
of desethylamiodarone than of amiodarone. Probit transformation and regression analysis indicated a 50% effective concentration (EC\textsubscript{50}) of 4.58 mg/liter for amiodarone and 1.44 mg/liter for desethylamiodarone. The concentration-response equations for single agents were then used to estimate the expected effects of the amiodarone and desethylamiodarone concentrations achieved by combination administration, and the change in PVC frequency predicted to result from the combination assuming a simple additive effect model. The percent reduction in PVCs predicted by an additive effect model was in close agreement (table 1) with the changes observed after administration of the combination of amiodarone and desethylamiodarone.

**Myocardial drug concentrations and infarct sizes.** Myocardial concentrations of desethylamiodarone were substantially greater than those of amiodarone in relationship to plasma concentrations (table 2). This observation applied both to dogs given single agents and to dogs receiving a combination of the two drugs. Infarct zone drug concentrations were lower than normal left ventricular concentrations for both agents. There were no significant differences in infarct size between groups of dogs, with the percentage of left ventricular mass infarcted averaging 21.0 ± 4.4% for control dogs compared with 25.7 ± 1.2% for amiodarone-treated dogs, 17.8 ± 3.5% for desethylamiodarone-treated dogs, and 17.5 ± 2.6% for dogs receiving a combination of the two agents.

**Discussion**

We have demonstrated antiarrhythmic efficacy for desethylamiodarone in a canine preparation of ventricular tachyarrhythmia. Furthermore, the antiarrhythmic effects of desethylamiodarone have been compared with those of amiodarone in a quantitative fashion and the effects of administration of a combination of amiodarone and desethylamiodarone have been studied.

**The relative activity of amiodarone and desethylamiodarone.** We found that, on the basis of dosage, the antiarrhythmic efficacy of desethylamiodarone was of the same order as that of amiodarone. When analyzed in...
FIGURE 3. Changes in PVC frequency after administration of amiodarone (AMIO), desethylamiodarone (DESETHYL), or the combination of equal doses of both agents at total intravenous doses of 5, 10, and 20 mg/kg for each group at 60 min intervals. The 95% confidence interval for the mean effect of the combination is indicated by solid lines. All but one mean value for PVC frequency after either of the two single agents falls within the confidence interval for the combination, indicating additivity of effects. (Error bars were omitted from the figure to avoid excessive clutter. Error bars for single agent data are shown in figure 1, and the confidence intervals provide information about the variability of combination data.)

terms of plasma concentration (figure 4), the antiarrhythmic potency of desethylamiodarone was clearly greater. The EC$_{50}$ for amiodarone in these experiments was 3.2 times as large as the EC$_{50}$ for desethylamiodarone, suggesting that the parent compound has approximately one-third of the ventricular antiarrhythmic activity (for a given plasma concentration) of the metabolite. Previous experiments have shown that after a single intravenous dose desethylamiodarone is 2.6 times as potent as amiodarone in increasing the QRS duration of rats, and is also more potent than the parent compound in producing rate-dependent QRS prolongation in dogs. This contrasts with the relative actions of these agents on indexes of slow-channel function, which indicate that amiodarone is a more potent calcium-channel blocker.

The mechanism for desethylamiodarone’s greater action on sodium-channel function compared with amiodarone is unknown. Both agents depress maximum upstroke velocity to a comparable extent in vitro, so that a greater affinity of desethylamiodarone for the sodium channel seems unlikely. In the current experiments, as in our previous work, desethylamiodarone accumulated to a greater extent than amiodarone in the myocardium. The right ventricular/plasma concentration ratio for desethylamiodarone in this study was 3.68 times that of amiodarone. This value is similar to the relative antiarrhythmic potency that we observed, and is consistent with previous observations of myocardial drug distribution in rats, dogs, and man. The greater tendency for desethylamiodarone to accumulate in the heart may therefore be responsible for its greater sodium-channel-blocking action at a given plasma concentration compared with amiodarone.

Consideration of the preparation. The preparation used to study ventricular arrhythmia in these experiments, first described by Harris in 1950, has been used extensively to screen and study antiarrhythmic drug action. The spontaneous ventricular arrhythmias 1 day after infarction in this preparation originate in the subendocardial Purkinje fiber network, and are most likely caused by enhanced automaticity. While substrates for both triggered automaticity and reentry exist in these dogs, they require experimental manipulation for demonstration. Furthermore, the
pharmacologic response of these arrhythmias both in vitro and in vivo indicate that automaticity at reduced levels of membrane potential is involved. Part of the evidence for an automatic mechanism of the arrhythmias in this preparation is provided by their susceptibility to overdrive suppression. A similar response to overdrive pacing has been observed for ventricular tachycardias occurring 24 hr after acute myocardial infarction in man, suggesting that our observations may be directly relevant to at least some clinical ventricular tachyarrhythmias.

The surviving subendocardial Purkinje fibers in the infarct zone responsible for arrhythmia have increased automaticity that is susceptible to depression by fast-
channel blockers.\(^{26}\) The ability of fast-channel blockers to suppress arrhythmias in vivo 24 hr after infarction in dogs\(^{27}\) is presumably related to their capacity to suppress automaticity occurring at reduced levels of transmembrane potential.\(^{22}\) The relatively greater antiarrhythmic potency of desethylamiodarone compared with amiodarone in the current experiments is therefore consistent with our previous observations suggesting that the metabolite is a more potent sodium-channel blocker in vivo than the parent compound.\(^{8,9}\) The slowing of the rate of ventricular tachycardia observed along with arrhythmia suppression in the current experiments is compatible with an automatic mechanism of arrhythmia. The extent to which the present observations can be extrapolated to the antiarrhythmic effects of amiodarone and its metabolite on arrhythmias due to other mechanisms, such as reentry, remains to be determined. Preliminary data suggest that desethylamiodarone may be effective in a canine preparation exhibiting reentrant ventricular tachycardia.\(^{28}\)

Amiodarone has previously been shown to have antiarrhythmic effects in the dog 24 hr after myocardial infarction.\(^{29}\) Six hours after amiodarone the desethyl metabolite had accumulated to a plasma concentration 8.6% of that of the parent compound.\(^{29}\) As in our study, myocardial/plasma concentration ratios were higher for the metabolite desethylamiodarone than for the parent compound, and the concentration of both agents was less in infarcted than in normal tissue. Other antiarrhythmic agents also produce lower concentrations in infarcted than normal tissue 24 hr after coronary artery ligation in the dog.\(^{30}\) This does not seem to preclude antiarrhythmic efficacy,\(^{30}\) perhaps because the cells from which the arrhythmia arises are in close contact with intracavitary blood.

Amiodarone and its metabolite have other pharmacologic actions that could, in part, have contributed to the arrhythmia reductions observed. Both agents have calcium channel-blocking properties,\(^{16}\) but their relative antiarrhythmic potency is opposite to what would have been expected based on their respective slow-channel blocking ability.\(^{9}\) Furthermore, the slow-channel blocker verapamil is relatively ineffective in this preparation of arrhythmia.\(^{27}\) Recent work has demonstrated that myocardial \(\beta\)-receptor number is reduced 23% and 32% by intravenous amiodarone and desethylamiodarone, respectively.\(^{31}\) Complete \(\beta\)-adrenergic blockade with metoprolol slows the rate of ventricular tachycardia by only 20% in dogs 24 hr after acute myocardial infarction,\(^{32}\) suggesting at most a secondary role for antiadrenergic actions in mediating the 60% to 80% reduction in ventricular arrhythmias that we saw after intravenous amiodarone and desethylamiodarone.

Analysis of the effects of a combination of amiodarone and desethylamiodarone. Since desethylamiodarone appears clinically only in the presence of amiodarone in patients on sustained amiodarone therapy, the contribution of desethylamiodarone to the effects of a combination of

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**TABLE 2**

Myocardial and plasma drug concentrations among dogs given amiodarone, desethylamiodarone, or the combination\(^{A}\)

<table>
<thead>
<tr>
<th></th>
<th>Dogs receiving single agents</th>
<th>Dogs receiving the combination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amiodarone</td>
<td>Desethylamiodarone</td>
</tr>
<tr>
<td>Total dose</td>
<td>35 mg/kg</td>
<td>35 mg/kg</td>
</tr>
<tr>
<td>Plasma concentration</td>
<td>6.0 ± 0.9 mg/l</td>
<td>2.3 ± 0.3 mg/l</td>
</tr>
<tr>
<td>RV concentration</td>
<td>70.9 ± 7.5 mg/l</td>
<td>99.0 ± 9.0 mg/l</td>
</tr>
<tr>
<td>LV/plasma</td>
<td>12.2 ± 2.1 mg/l</td>
<td>44.9 ± 3.1 mg/l</td>
</tr>
<tr>
<td>LV/plasma</td>
<td>64.4 ± 8.8 mg/l</td>
<td>100.3 ± 45.4 mg/l</td>
</tr>
<tr>
<td>LV/plasma</td>
<td>43.3 ± 12.8 mg/l</td>
<td>21.6 ± 4.7 mg/l</td>
</tr>
<tr>
<td>Atrial concentration</td>
<td>51.5 ± 10.5 mg/l</td>
<td>64.7 ± 8.1 mg/l</td>
</tr>
<tr>
<td>LV/plasma</td>
<td>9.9 ± 2.5 mg/l</td>
<td>28.0 ± 2.9 mg/l</td>
</tr>
<tr>
<td>IZ/plasma</td>
<td>3.9 ± 1.6 mg/l</td>
<td>18.3 ± 3.9 mg/kg</td>
</tr>
</tbody>
</table>

\(^A\) The results are for six dogs each given amiodarone and desethylamiodarone as single agents, and five dogs given the combination of amiodarone and desethylamiodarone. All concentrations were measured in samples obtained 60 min after the completion of infusion 3.

Left ventricular assays for dogs given single agents were limited by interference from the vital dye (triphenyltetrazolium chloride) used for infarct size measurement. In dogs receiving the combination of agents, samples for drug assay were obtained before vital staining.\(^{A}\) Results were available for two dogs only. \(^{C}\) No results available.

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the compounds is of importance. A cumulative amiodarone dose of 15 mg/kg as a single agent had a minor, statistically nonsignificant antiarrhythmic effect. In contrast, a cumulative amiodarone dose of 12.5 mg/kg along with an equal quantity of desethylamiodarone produced important antiarrhythmic actions. Dose-response data (figure 3) and concentration-response analysis (table 1) suggest that the antiarrhythmic effects of amiodarone and desethylamiodarone are additive.

**Clinical relevance.** The delayed onset of the ventricular antiarrhythmic actions of long-term amiodarone therapy is widely recognized. Intravenous loading produces more rapid suppression of ventricular arrhythmias, but antiarrhythmic effects nonetheless continue to increase with maintained therapy, despite a lack of increase in plasma amiodarone concentrations. Similar observations have been made in a canine preparation of sudden cardiac death. In our experiments, the antiarrhythmic potency of desethylamiodarone was about three times that of amiodarone based on plasma concentration. In light of available information about the plasma concentrations of the two compounds in patients on long-term amiodarone therapy, and assuming relative antiarrhythmic activity in man similar to what we observed in dogs, accumulation of the desethyl metabolite would be responsible for 71% of the ventricular antiarrhythmic action of long-term oral amiodarone therapy. Given the gradual accumulation of the metabolite with long-term amiodarone therapy, our results suggest that the delayed onset of amiodarone’s antiarrhythmic action is largely due to the production of its active metabolite. The potential role of other slowly developing actions, such as antagonism of thyroid hormone and sympathetic effects, in explaining the delayed effects of long-term amiodarone therapy remains to be determined.

Extrapolation of the results of intravenous drug administration in dogs to the effects of long-term oral drug therapy in man requires great caution. The plasma and myocardial drug concentrations achieved in our dogs were in the same range as concentrations occurring with long-term oral amiodarone treatment. Single-dose intravenous amiodarone is known to be much more effective against supraventricular than ventricular arrhythmias. This may be due to the greater effect of amiodarone compared with its metabolite on slow-channel function. Further evidence in support of a role for desethylamiodarone in the ventricular antiarrhythmic actions of sustained amiodarone therapy is provided by the observation that changes in ventricular electrophysiologic properties during amiodarone therapy correlate with plasma desethylamiodarone concentrations, in contrast to changes in atrioventricular nodal function, which correlate with amiodarone concentrations.

We have demonstrated that desethylamiodarone has substantial antiarrhythmic action in a canine preparation of ventricular tachyarrhythmias after acute myocardial infarction. Furthermore, the antiarrhythmic effect of desethylamiodarone occurs at lower plasma concentrations than the antiarrhythmic effect of amiodarone, and the antiarrhythmic effects of the metabolite and the parent compound are additive. These observations suggest a potentially important role for the metabolite in mediating the antiarrhythmic actions of amiodarone therapy in man.

We thank Kathleen Kay and Carol Matthews for excellent technical assistance, Ginette Bleau and Suzanne Morin for secretarial assistance, and Ayerst Pharmaceuticals (Montreal) and Sanofi Corp. (Paris) for providing the purified amiodarone and desethylamiodarone used in these studies.

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The antiarrhythmic efficacy of amiodarone and desethylamiodarone, alone and in combination, in dogs with acute myocardial infarction.

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Circulation. 1988;77:200-208
doi: 10.1161/01.CIR.77.1.200

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