A method for quantitating antifibrillary effects of drugs after coronary reperfusion in dogs: improved outcome with bretylium

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ABSTRACT We developed a quantitative approach to assess the antifibrillary effects of short-term interventions in a canine preparation of ventricular fibrillation caused by coronary reperfusion, and applied it to evaluate the antifibrillary effects of bretylium tosylate. Twenty-five dogs were given 10 mg/kg infusions of bretylium over 10 min, subjected to a 20 min proximal left anterior descending coronary artery ligation followed by sudden release, and compared with 25 animals given saline placebo. Drug infusion was begun 90 min before reperfusion to avoid evaluation of outcome during the phase of drug-induced catecholamine release and to allow adequate time for bretylium uptake in the myocardium. The relationship between the likelihood of ventricular fibrillation during reperfusion and the amount of myocardium perfused by the occluded vessel (myocardium at risk) was analyzed with the logistic risk-regression model. This model was developed to control for the effects of amount of myocardium at risk on outcome. For both the bretylium and the placebo groups the incidence of ventricular fibrillation correlated significantly with amount of myocardium at risk. However, animals treated with bretylium had an improved outcome for a given amount of myocardium at risk. In other words, the curve relating outcome to myocardium at risk was shifted significantly to the right. The amount of myocardium at risk required for half the placebo-treated animals to fibrillate was 20.3 g and that required for half the bretylium-treated animals to fibrillate was 27.9 g, or 37% more than that for the placebo group. This logistic risk-regression analysis format permits quantification of treatment effects while accounting for variability in amount of myocardium at risk.


SUDDEN RELEASE of an acute proximal canine coronary artery occlusion often leads precipitously to ventricular fibrillation. The clinical significance of this laboratory observation is uncertain, but its sudden nature suggests a parallel to the clinical syndrome of sudden cardiac death in man. In this regard, it may represent a useful model for testing interventions specifically designed to be antifibrillary, at least insofar as reperfusion phenomena such as dissolution of platelet plugs or sudden resolution of coronary artery spasm serve as clinical events underlying sudden death in man.2-4

It is possible that antiarrhythmic compounds may vary in their relative efficacy against ventricular extrasystoles and ventricular fibrillation. One compound in clinical use that appears to have little effect on ventricular extrasystoles but may be predominately antifibrillary is bretylium tosylate.5,6 The antifibrillary effect of bretylium has been difficult to prove definitively in man, but support for such an action comes from a variety of studies of animal preparations.7-11 In this regard, bretylium is one of few drugs reported to reduce the incidence of ventricular fibrillation in dogs after release of an acute proximal left anterior descending coronary artery ligation.11 However, the above-mentioned study by Kniffen et al. was limited to small numbers of animals in the control and treatment groups. In light of the substantial variability in outcome with the model of reperfusion-induced ventricular fibrillation,12,13 the reported beneficial effect of bretylium in this preparation warrants substantiation. This variability in outcome after reperfusion has been shown by others to be in part related to different

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duration and location of the ligation, suggesting that the incidence of ventricular fibrillation after reperfusion depends on the amount of potentially ischemic myocardium that results from the ligation. We demonstrated in a previous study that the likelihood of developing ventricular fibrillation after release of a 20 min occlusion of the proximal left anterior descending coronary artery was related to the mass of myocardium perfused by the ligated vessel (myocardium at risk). This relationship was well described by the logistic risk-regression model, which predicts uniformly low and high probabilities of ventricular fibrillation with small and large amounts of myocardium at risk, respectively, and by a direct correlation of outcome with midrange values for myocardium at risk. These findings implied that small differences in amounts of myocardium at risk between groups of animals undergoing different interventions could result in substantial differences in outcome. We also showed that differences in amounts of myocardium at risk could probably not be easily controlled by restricting the size of dogs studied, since there is substantial variability in the coronary artery distribution among dogs of similar weight. Based on this previous experience, we have developed an alternative approach to the assessment of treatment effects on outcome after reperfusion.

In our study we assess the relationship observed between reperfusion-induced fibrillation and myocardium at risk in animals treated with bretylium, and compare it with the relationship observed in control animals. We apply a method of analysis, based on the logistic risk-regression format, that permits quantification of the efficacy of bretylium while accounting for variability in amount of myocardium at risk and also for differences in hemodynamic variables.

Methods

Mongrel dogs weighing between 10 and 20 kg were anesthetized with sodium pentobarbital (30 mg/kg) administered intravenously. The dogs were then intubated and ventilated with room air with the use of a Harvard Apparatus Model 607 respirator set at 20 cycles/sec. The respirator was adjusted to deliver a tidal volume calculated from the formula: $13 \times \text{weight of dog (in kg)} + \text{dead space}$. The right femoral artery was cannulated to monitor the arterial pressure. Lead II of the electrocardiogram was recorded. The right femoral vein was cannulated and used as a route for intravenous administration of drug or saline placebo. Twenty-five animals received bretylium tosylate (10 mg/kg in 20 ml saline) infused over a period of 10 min while 26 others were given saline alone. The chest of each dog was opened in the fourth left intercostal space. A small incision was made into the pericardium and the left atrial appendage was reflected from the operating field. The left anterior descending coronary artery (LAD) was carefully dissected free at its origin, and a silk suture was passed underneath the vessel just distal to the septal artery. The two ends of the suture were then threaded through a small polyethylene tube so that the coronary artery could be occluded by pulling the two ends of the suture and securing the ligation with a bulldog clamp. Drug was administered 90 min before release of the occlusion to allow heart rate and blood pressure to return to baseline and to permit accumulation of drug in the myocardium. The LAD was occluded in a single stage 70 min after the drug infusion was started and the occlusion was maintained for 20 min. After abrupt reperfusion, the dogs were observed for 5 more min. If they reached the end of this period without ventricular fibrillation, they were considered reperfusion survivors. Survivors were given 5000 units of heparin (1:1000 dilution) intravenously to prevent clotting within the coronary vessels and thus permit uniform distribution of the dyes. Animals in which fibrillation did occur after reperfusion were given 5000 units of heparin via left ventricular puncture; their hearts were then hand-massaged to circulate the heparin. Immediately after heparinization all hearts were excised to allow low measurement of myocardium at risk.

The amount of myocardium at risk was assessed by simultaneous intracoronary injection of red and blue monastral dyes (I. E. DuPont). The red dye was injected at the bifurcation of the left main coronary artery and the blue dye was injected into the LAD just distal to the site of ligation. The left ventricle was then separated from the rest of the heart and the tissue dyed blue was separated from the red and both were weighed.

Data analysis. Changes in heart rate and blood pressure after drug administration and coronary occlusion were analyzed by the Wilcoxon signed-rank test. The incidences of ventricular ectopic depolarizations (VEDs) in each group were compared with the use of the Student t test. The logistic risk-regression model was used to assess any relationship between end-occlusion heart rate or blood pressure and the likelihood of fibrillation during reperfusion, and also to analyze the relationship between amount of myocardium at risk and outcome (vide infra).

Results

Of the 51 animals used in this study, one in the placebo group fibrillated during occlusion and was excluded. A dog in the bretylium-treated group developed stable ventricular tachycardia at a rate of 300 beats/min during occlusion, which persisted to the time of release. This animal had the smallest amount of myocardium at risk (23.7 g) of those dogs receiving bretylium and in which fibrillation was observed after release. Results from this animal were included in the data analysis. Thus, 25 placebo- and 25 bretylium-treated dogs survived 20 min of occlusion and were subjected to reperfusion.

Hemodynamics. Mean heart rate and blood pressure before drug administration were not significantly different in the placebo and the bretylium groups. During bretylium infusion, however, heart rate increased slightly while blood pressure increased substantially. Subsequently, both parameters returned to control levels (table 1). By the end of the occlusion period, heart rate and diastolic blood pressure were not significantly different between placebo- and bretylium-treated animals, although heart rate increased significantly only in the bretylium-treated group (table 1). Systolic blood pressure was slightly but significantly higher in bretylium-treated dogs just before reperfusion. However,
for both placebo- and bretylium-treated animals there was no correlation between outcome and either heart rate or blood pressure before release of occlusion. **Outcome.** During occlusion ventricular arrhythmias were present in both placebo- and bretylium-treated dogs. Fibrillation was observed in only one dog in the placebo group and in none in the bretylium group, so that protection from fibrillation during occlusion could not be assessed. The placebo- and bretylium-treated dogs averaged 20.8 ± 20.1 VEDs/min (mean ± SD) and 17.8 ± 32.9 VEDs/min, respectively, during occlusion; the difference was not significant. In either group animals with little myocardium at risk had few ventricular arrhythmias during occlusion; otherwise there was no relationship between VEDs and amount of myocardium at risk.

As previously reported by others, we found a clear-cut relationship between ventricular arrhythmias during occlusion and outcome after reperfusion, the more severe occlusion arrhythmias being associated with more severe reperfusion arrhythmias. In both groups animals with less than 2 VEDs/min during occlusion never had fibrillation during reperfusion, whereas those with greater than 25 VEDs/min all had fibrillation during reperfusion. After release of occlusion some animals had no arrhythmias or infrequent transient VEDs. Others developed ventricular tachycardia (≥3 ventricular ectopics in a row) that either resolved or degenerated into fibrillation. In no dogs did ventricular fibrillation develop directly from sinus rhythm. Within the three categories of reperfusion outcome (no ventricular tachycardia, transient ventricular tachycardia, or ventricular tachycardia degenerating into fibrillation) there was no difference between placebo and bretylium groups with respect to VED frequency during the 20 min occlusion period (figure 1). Although a similar number of animals in each group developed ventricular tachycardia after reperfusion (19 placebo-treated vs 21 bretylium-treated dogs), more animals treated with bretylium reverted to sinus rhythm (11 vs 5; figure 1). In fact, over the range of myocardium at risk in this study (13.9 to 37.1 g) reperfusion-induced ventricular tachycardia was almost ubiquitous in both placebo- and bretylium-treated animals, occurring in 76% of placebo-treated animals and in 84% of dogs treated with bretylium. Thus, bretylium had no effect on the incidence of reperfusion-induced ventricular tachycardia.

Fourteen of the 25 placebo-treated dogs fibrillated after reperfusion (56%) as opposed to 10 of 25 bretylium-treated animals (40%). This difference in outcome was not significant when differences in amounts of myocardium at risk were not considered. However, the mean myocardium at risk in the bretylium group was greater than that in the placebo group (26.4 vs 21.5 g). When the data were analyzed in a manner that accounted for the influence of amount of myocardium at risk, the effect of treatment with bretylium became evident.

**TABLE 1**

<table>
<thead>
<tr>
<th>Heart rate (beats/min) and blood pressure (mm Hg) before treatment, immediately before occlusion, and at 20 min of occlusion</th>
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<tbody>
<tr>
<td><strong>Placebo</strong></td>
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<tr>
<td>Heart rate</td>
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<tr>
<td>Pretreatment</td>
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<tr>
<td>Preocclusion</td>
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<td>End-occlusion</td>
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Data are mean ± SD.

* Includes dog with ventricular tachycardia at 300 beats/min.

![Graph](http://circ.ahajournals.org/)

**FIGURE 1.** Incidence (mean ± SD) of VEDs during 20 min of occlusion. Data are grouped by treatment and by outcome after reperfusion.
Analytic model. The relationship between amount of myocardium perfused by the occluded vessel, or myocardium at risk, and the probability of ventricular fibrillation at reperfusion for placebo- and bretylium-treated dogs was analyzed with the logistic risk-regression model. For the placebo group, the model is $P(Y) = 1/[1 + e^{-a_1 + b_1 X}]$, where $P(Y)$ is the probability of ventricular fibrillation for any given amount of myocardium at risk, $X$. This equation was fitted by the method of maximum likelihood, and 95% confidence limits for the predicted probability of fibrillation were obtained. The derived values in the formula, $a_1$ and $b_1$, are intercept and slope parameters. The relationship between myocardium at risk and the likelihood of ventricular fibrillation at reperfusion for the bretylium-treated group was also determined by the logistic risk-regression model, represented in this case by the formula $P(Y) = 1/[1 + e^{-a_2 + b_2 X}]$, where $a_2$ and $b_2$ are the intercept and slope parameters derived for animals treated with bretylium. The level of correlation between amount of myocardium at risk and ventricular fibrillation was represented by the Goodman-Kruskal coefficient, $\gamma$. This is calculated by the formula $\gamma = 2(C - 0.5)$, where $C$ is the fraction of concordant pairs.

To test whether the bretylium group (treatment 2) differed significantly from the control group (treatment 1), a single slope constant for both was established ($b'$) and constants $a'_1$ and $a'_2$ were generated such that $a'_1 - a'_2$ described the overall difference in likelihood of ventricular fibrillation for a given amount of myocardium at risk. If $a'_1$ is equal to $a'_2$, then bretylium makes no difference in the outcome. A smaller value for $a'_1$ than $a'_2$ defines a shift towards the right of the relationship between the likelihood of fibrillation and myocardium at risk; in other words, at a given amount of myocardium at risk, the probability of fibrillation decreases. The amount of myocardium at risk at which 50% of the animals are expected to fibrillate (MAR$_{50}$) can be calculated either from the formula derived with the single slope constant or from individual best-fit parameters, so that setting $P(Y) = 1/2$, then $1 + e^{-(a + b X)} = 2$ and $X = -a/b$.

Effect of treatment on outcome. In both the placebo- and bretylium-treated animals outcome during reperfusion correlated significantly with myocardium at risk. The $\gamma$ value was 0.74 for the placebo group and 0.61 for the bretylium group.

The data were used to calculate the likelihood of reperfusion-induced ventricular fibrillation while controlling for myocardium at risk. The predicted probability of fibrillation upon reperfusion is described by the formula $P(Y) = 1/[1 + e^{-(a - 9.4 + 0.45X)}]$ for the placebo group and by $P(Y) = 1/[1 + e^{-(7.0 + 0.24X)}]$ for the group treated with bretylium (figure 2). When the data are combined to give an overall slope constant ($b' = 0.33$), $a'_1 = -6.7$ for placebo and $a'_2 = -9.2$ for bretylium. Since $a'_1$ is smaller than $a'_2$ ($p < .01$), the overall curve for bretylium is shifted to the right (fig-

FIGURE 2. Relationship between myocardium at risk and ventricular fibrillation after reperfusion based on the individual best-fit equations for placebo- and bretylium-treated animals. Also shown is the observed incidence of ventricular fibrillation among placebo (•) and bretylium-treated (○) dogs for each 5 g increment of myocardium at risk ($<15, 16$ to 20, 21 to 25, 26 to 30, and >30 g). In parentheses is the number of animals in each 5 g group. The MAR$_{50}$ is shown for each group.
FIGURE 3. Relationship between myocardium at risk and ventricular fibrillation after reperfusion based on equations derived from the single-slope estimate. Symbols are the same as in figure 2. Note that the MAR_{50} describes the overall relationship between myocardium at risk and outcome when either the best-fit slope or single slope estimate is used. This shift of the curve to the right is conveniently expressed as the change in MAR_{50}.

ure 3). In other words, treatment with bretylium protected animals from ventricular fibrillation caused by coronary reperfusion.

The overall treatment effect is conveniently described by the amount of myocardium at risk at which half of the animals should experience fibrillation (MAR_{50}). Using the individual best-fit parameters, MAR_{50} = -a/b = 9.4/0.45, or 20.9 g for the placebo group. Similarly, for the bretylium group, MAR_{50} = -a/b = 7.0/0.24 or 29.2 g. If the single slope parameters are used, MAR_{50} = 6.7/0.33 = 20.3 g for the placebo group and 9.2/0.33 = 27.9 g for the bretylium group.

Discussion

In this study we characterize a method for assessing the effect of interventions on outcome after coronary reperfusion. This approach is based on the observation that reperfusion-induced ventricular fibrillation is related to the mass of myocardium perfused by the occluded vessel, or myocardium at risk. The results of our study confirm this relationship and demonstrate a similar relationship for animals treated with bretylium, namely that fibrillation is uniformly unlikely in dogs with small amounts of myocardium at risk and uniformly likely in those with large amounts of myocardium at risk, with a steep positive correlation at mid-range values.

The nature of this relationship implies that a given treatment effect will result in a variable change in incidence of ventricular fibrillation after reperfusion, which depends on the amount of myocardium at risk (figures 2 and 3). In the most obvious case, animals with small amounts of myocardium at risk will have a low incidence of ventricular fibrillation after reperfusion when given placebo and there will be little change when they are treated with an effective drug. On the other hand, animals that have values for myocardium at risk on the steep portion of the curve may show a substantial decrease in the incidence of ventricular fibrillation during treatment with the same drug. Finally, for animals with very large amounts of myocardium at risk, little change in incidence of ventricular fibrillation may result from treatment with the same drug, despite its efficacy.

The fact that a given drug effect can result in a variable reduction in incidence of ventricular fibrillation after reperfusion, depending on the amount of myocardium at risk, makes it difficult to quantitate drug effect when overall incidence of fibrillation is used as the major assessment parameter. In addition, both positive and negative assessments of treatment interventions might be explained by subtle differences in amount of myocardium at risk, rather than by the presence or absence of a treatment effect. We avoid these problems by controlling for myocardium at risk using the logistic risk-regression model and by quantitating overall drug effect using MAR_{50}. This value is
readily calculated and allows outcome to be expressed as an amount of myocardium at risk required to cause half the animals to fibrillate after reperfusion — a clinically understandable expression. This method of analysis also permits comparison of different drugs or dosage regimens, and can be extended to evaluation of the effects of other interventions on outcome in the occlusion-reperfusion preparation.

To apply this evaluation format to interventions designed to manipulate infarct size, a more direct measure of ischemic myocardium than the monastral dye technique would be required. We chose the dye perfusion method to measure myocardium at risk because it is simple and has been shown to correlate with infarct size, as assessed by histologic techniques, and also with amount of ischemic myocardium, as assessed by epicardial mapping of the ST segment. When occlusion is limited to 20 min extensive collateral development does not occur; thus, the demarcation between regions dyed blue and those dyed red is distinct and corresponds to the epicardial arterial distribution.

Using this method we were able to show a significant difference in outcome between the control and treated groups that was not detected when myocardium at risk was not taken into account. Although the difference in the percentage of animals surviving between the bretylium- and the placebo-treated groups was small (40% vs 56%), this can be explained by the difference noted in the amounts of myocardium at risk in the two groups. This difference in myocardium at risk may in part be due to the fact that the placebo-treated animals were a nonconcurrent control group. Although it would clearly have been preferable to randomly assign animals to placebo or active treatment on a concurrent basis, such randomization of small groups does not guarantee equal distribution into groups of animals with the same amounts of myocardium at risk. By measuring myocardium at risk we were able to control for this disparity and show a highly significant difference in outcome. This difference represents a 37% increase in the amount of myocardium at risk at which half the dogs are expected to fibrillate (MAR = 20.3 g for the placebo group vs 27.9 g for the bretylium group). In short, our data support the findings of Kniffin et al. that bretylium decreases the incidence of ventricular fibrillation upon reperfusion.

We administered bretylium 90 min before the time of release to allow for accumulation of drug in myocardium, and also to avoid the catecholamine-release phase of bretylium administration. While heart rate and diastolic blood pressure were not significantly different between the placebo- and bretylium-treated groups, systolic blood pressure was higher in bretylium-treated animals just before reperfusion. This difference in systolic pressure is probably related to the slightly higher control systolic blood pressure in this group of animals (175 vs 164 mm Hg, table 1) rather than to a continued catecholamine effect. Our findings are consistent with the position that the antifibrillatory effects of bretylium can be dissociated from its catecholamine-releasing effects.

Although neither heart rate nor blood pressure contributed significantly to the prediction of outcome, these findings do not imply that hemodynamic factors will not affect outcome when varied over a wider range than that observed in the present study. The analysis does suggest, however, that these parameters did not, in our study, substantially alter the relationship observed between outcome and amount of myocardium at risk in either the placebo- or bretylium-treated animals.

In this study, bretylium did not significantly affect the incidence of VEDs during occlusion or the incidence of ventricular tachycardia after reperfusion. Thus, the data offer support for the theory that bretylium is predominately antifibrillatory.

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