The Effects of Diltiazem and Reduced Serum Ionized Calcium on Ischemic Ventricular Fibrillation in the Dog

William T. Clusin, Michael R. Bristow, Donald S. Baim, John S. Schroeder, Patrice Jaillon, Peter Brett, and Donald C. Harrison

From the Division of Cardiology, Stanford University School of Medicine, Stanford, California

SUMMARY. Calcium influx blockers reportedly suppress ventricular arrhythmias during acute ischemia. We therefore studied the effects of diltiazem and reduced serum ionized calcium on ventricular fibrillation (VF) in a reversible ligation model. VF was produced at 15-minute intervals by simultaneous occlusion of the left anterior descending and circumflex arteries of 31 dogs. Time from coronary occlusion to onset of VF showed no significant variation during 15 consecutive trials in six dogs that received saline alone. Intravenous infusion of diltiazem (0.02 mg/kg per min) markedly delayed the onset of VF in each of 10 dogs (P < 0.0001). Mean VF latency increased from 138 to 295 seconds during a 45-minute diltiazem infusion, declined exponentially when the infusion ceased, and was strongly correlated with serum diltiazem concentration (r = 0.96, P < 10^-6). In five dogs, hemodynamic measurements, including coronary venous blood flow, were performed during each occlusion. The increase in VF latency by diltiazem was not due to increased coronary flow during occlusion or to reduction of left ventricular (LV) mechanical work. In six dogs, mean serum ionized calcium, [Ca ++], was reduced from 1.11 to 0.59 mM by infusion of sodium citrate. Citrate infusion increased mean VF latency from 155 to 243 seconds, and the increase observed in each dog was correlated (r = 0.84, P < 10^-6) with the reduction in [Ca ++]. VF latency was unaffected by lidocaine in nine dogs. The antifibrillatory effect of diltiazem during global LV ischemia may be an electrophysiological phenomenon related to reduction of cellular calcium influx. (Circ Res 50: 518-526, 1982)
the free wall of the right ventricle and connected to a WPI 800 series stimulator which delivered 2-msec current pulses at twice diastolic threshold. The proximal left anterior descending (LAD) and circumflex coronary arteries were exposed by dissection. Simultaneous reversible occlusion of these arteries was performed at 15 minute intervals using nylon snares placed within 1 cm of their origin. In rare instances, animals were sacrificed when VF could not be elicited within 240 seconds by these measures. All occlusions were performed during right ventricular pacing at a rate of 180–220/min. When VF occurred, pacing was discontinued, both snares were released, internal cardiac massage was instituted for 1 minute, and the animal was defibrillated using a 10-40 J pulse supplied by a Hewlett-Packard model 780 2C defibrillator with 5-cm paddles. The coronary snares were also released after several trials in which VF failed to occur within an arbitrary time limit of 360 seconds owing to prior infusion of diltiazem. Blood pressure and lead II of the electrocardiogram were recorded in sinus rhythm just prior to the occlusion and in paced rhythm throughout the occlusion by an optical strip chart recorder (Honeywell Visicorder 1508C) at a paper speed of 100 or 200 mm/sec.

Multiple control occlusions were performed in each dog prior to drug infusion. Diltiazem (d-3-acetoxy-cis-2,3-dihydro-5-(2-(dimethylamino)ethyl)-2-(p-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one,hydrochloride; Marion Laboratories) was diluted in 0.9% NaCl to a concentration of 0.5 to 1.5 mg/ml and administered over 45 minutes by a Harvard continuous infusion pump. Serum diltiazem concentrations were determined by the method of Rovei et al. (1977) from 5-ml arterial blood samples withdrawn at the time of coronary occlusion. Lidocaine-HCl (Astra Pharmaceuticals) was administered as a 2% solution over 1 minute beginning 5.5 minutes prior to coronary occlusion. Serum lidocaine concentrations were determined by gas chromatography using the method of Keenaghan (1968).

Serum ionized calcium was reduced by infusion of increasing doses of sodium citrate, as previously described (Bristow et al., 1977a). A 15% Na3 citrate solution was titrated to pH 7.4 with 1% HCl, passed through a Millipore filter, and then administered by continuous infusion beginning after the sixth control occlusion. Subsequent occlusions were performed every 15 minutes with the infusion being interrupted for 5 minutes after each resuscitation and then restarted at twice the original rate. The end point was reached when the systolic arterial blood pressure declined by 10–20 mm Hg, which generally occurred at an infusion rate between 5 and 10 mg/kg per min. The final infusion rate was defined as the “high dose,” whereas the preceding rate was defined as the “low dose.” Serum ionized calcium was measured in arterial blood samples obtained at the time of occlusion using a Clin-Ion ion-specific electrode system (Applied Medical Technology). The mean reduction in serum ionized calcium during the “high dose” infusion was 0.55 mm.

Data from the above experiments were displayed as mean ± standard deviation. Mean VF latencies were computed using a value of 360 seconds for the four trials in which VF did not occur within this arbitrary limit. Statistical significance was determined using the nonparametric Friedman test, which gives a $\chi^2$ value for each analysis (Siegel, 1956). This test was especially suitable for the VF latency data because the effects of inter-animal variation were eliminated, and because the ranking procedure permitted simple handling of the trials in which VF failed to occur.

In five dogs, hemodynamic measurements including coronary venous flow were performed during arterial occlusion in the presence and absence of diltiazem. For these experiments, a Bio-Tec BT-250 pressure transducer (Biotronix Laboratory) was inserted into the apex of the left ventricle through a stab wound. Cardiac output (C.O.) was determined by an electromagnetic flow probe (Biotronix Laboratory) placed around the ascending aorta. The output of the flow probe was recorded by a Biotronix BL613 flowmeter, and calibrated by a gravity flow method. Left ventricular minute work (W) was computed as:

$$W = (\bar{P} - LVEDP) \times C.O. \times 0.0136,$$

where LVEDP is left ventricular end-diastolic pressure, and $\bar{P}$ is the mean systolic pressure obtained by planimetry of the supravulvar aortic pressure tracing (Grossman, 1976). Coronary venous flow was measured by insertion of a specially constructed thermodilution catheter into the coronary sinus (Baim et al., 1980). The temperature of the saline injectate ($T_i$) was monitored by a thermistor at the hub of the catheter, while the baseline blood temperature ($T_0$) and the temperature of the mixed coronary sinus effluent ($T_m$) were measured by a second thermistor located 7 mm from the catheter tip. Flow was calculated as:

$$1.25 \times F_i \times (T_m - T_i)/(T_n - T_m),$$

where $F_i$ was the injectate flow rate (38–50 ml/min) and 1.25 was a correction for the different specific heats of blood and saline. Statistical significance for this portion of the study was determined by Student’s t-test, using a two-tailed probability distribution.

**Results**

**Reproducibility of VF Latency Measurements during Successive Trials**

Dogs subjected to simultaneous occlusion of the proximal LAD and circumflex coronary arteries almost always developed ventricular fibrillation within 100–240 seconds. The latency of VF was extremely reproducible in a given animal, and showed no significant variation during serial ligations over several hours. Figure 1 shows the mean VF latencies during 15 consecutive trials in six dogs that received no drug. No significant variation ($P > 0.30$) was demonstrable either by analysis of variance or by the nonparametric
Friedman test. The stability of the VF latency was particularly evident when sequential values were compared in the same animal. Table 1 shows the mean and standard deviation of VF latencies during six control trials in five dogs. The mean value of the standard deviations for the five dogs (third column) was 10.4 seconds, while the standard deviation obtained from the mean values for each animal was 24.0 seconds. Very small changes in VF latency should therefore be detectable by this model, particularly if the statistical design is insensitive to inter-animal variation.

**Effects of Diltiazem on VF Latency**

The latency of ventricular fibrillation was markedly increased by diltiazem. In Figure 2A, diltiazem was infused for 45 minutes at a rate of 0.02 mg/kg per minute after six control occlusions. Subsequent occlusions were performed at 5, 25, and 45 minutes of infusion, and at 15-minute intervals after the infusion was terminated. VF latency was markedly increased at 5 minutes of infusion. At 25 and 45 minutes, VF did not occur within the 360-second time limit, and the snares were released to prevent infarction. VF latency declined when the infusion ceased, but each value obtained during and after the infusion was greater than any of the six control values ($P < 0.001$ by the Wilcoxon rank sum test). This result was reproduced in a total of five animals that received diltiazem according to the above protocol.

VF latencies at the end of the infusion are listed for each of the five dogs in the fourth column of Table 1, and the mean VF latencies for the whole experiment are plotted in the top curve of Figure 3. VF failed to occur within the 360-second time limit in two dogs at 25 minutes of infusion, in one dog at 45 minutes of infusion, and in one dog at 15 minutes after termination of the infusion. Failure of VF to occur during these four trials prevented a parametric analysis of the data obtained for the five dogs, but a nonparametric analysis using Friedman's test showed that the changes in VF latency were highly significant ($\chi^2 = 55.9; \text{df} = 13; P < 10^{-6}$). A separate analysis of VF latencies during the six control ligations showed no significant variation ($\chi^2 = 2.7, \text{df} = 5, P > 0.70$).

The progressive increase in VF latency during the diltiazem infusion, and its subsequent decline after termination of the infusion, suggest that VF latency is related to serum diltiazem concentration. The mean serum diltiazem concentration at the time of each occlusion has been plotted in the bottom curve of Figure 3. Serum diltiazem concentration approached a steady state after 25 minutes of infusion, and declined exponentially when the infusion was terminated, as shown by the semilog plot in Figure 4A. A similar plot in Figure 4B demonstrates that VF latency also declined exponentially, with a nearly identical time constant. A close correlation between mean VF latency and serum diltiazem concentration was confirmed by the scatter plot in Fig. 5. The correlation coefficient of 0.96 was highly significant ($P < 10^{-6}$), with the largest discrepancies being observed immediately after initiation and termination of the infusion, when tissue levels were presumably changing most rapidly.

A direct relation between serum diltiazem concentration and VF latency was further suggested by the concomitant changes in PR interval (middle curve of Fig. 3). The mean PR interval increased 50% between

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Effect of diltiazem and citrate infusion on VF latency. In A, diltiazem (0.9 mg/kg) has been infused over 45 minutes after six control occlusions. The solid line represents the mean of the control latencies and the dotted line represents ± 2a. VF is delayed at 5 minutes of infusion, and does not occur within the 360-second time limit at 25 and 45 minutes of infusion. VF latency declines after cessation of the infusion. B shows the effects of sodium citrate infusion. VF latency increases after an infusion of 4.7 mg/kg per min and is further increased when the infusion rate is doubled.
the last control occlusion and the end of the diltiazem infusion, and then declined toward the control values when the infusion ceased. Although these changes in PR interval were highly significant, ($\chi^2 = 39.3$, df = 13, $P < 0.0002$), no incidence of second degree block was observed.

Failure of Diltiazem to Increase Coronary Blood Flow during Occlusion

It is possible that the increase in VF latency produced by diltiazem is related to improved coronary perfusion via arterial collaterals. To investigate this possibility, serial occlusions were performed in five instrumented dogs in which coronary venous outflow was measured by a thermodilution catheter placed in the coronary sinus. This method was chosen because venous outflow is a reflection of total myocardial blood supply, including that provided by collaterals.

In the bottom curves of Figures 6 and 7, coronary venous flow during the last of several control occlusions was compared to that recorded after a 25-minute infusion of diltiazem (0.02 mg/kg per min). Coronary artery occlusion produced an immediate, profound reduction in coronary venous flow in each of the five dogs. The small amount of residual flow (<1 ml/min)
Effects of Diltiazem on Left Ventricular Work

It is also possible that diltiazem retards VF during ischemia by reducing left ventricular mechanical work. In this case, slower dissipation of metabolic energy stores could allow relative preservation of energy-dependent cellular functions such as maintenance of ionic gradients. This possibility was explored by performing hemodynamic measurements in the five instrumented dogs during occlusions before and after diltiazem infusion. As seen in Figure 7, coronary artery occlusion produced a marked decline in aortic blood pressure, cardiac output, and left ventricular minute work. The mean time required for development of complete electromechanical dissociation (defined as cessation of forward cardiac output) during the control trials was 131 ± 19 second. Diltiazem did not alter mean aortic blood pressure before or during coronary occlusion (Figure 7; top curve), but there was a moderate increase in preocclusion cardiac output (46 ± 24%; \( P < 0.005 \)), and calculated left ventricular work (56 ± 24%; \( P < 0.001 \)), probably due to a decrease in systemic vascular resistance. Despite these initial effects, there was no change in cardiac output or LV work after the first 60 seconds of coronary occlusion. Thus, diltiazem did not reduce mechanical work; neither did it delay the development of electromechanical dissociation (EMD). The increase in VF latency was due, instead, to a striking prolongation of the interval between EMD and the onset of VF (see Figure 6). The mean EMD to VF interval for the five dogs increased from 20 ± 25 seconds during the
control occlusions to 140 ± 43 seconds after diltiazem infusion (P = 0.002).

**Effect of Reduced Serum Ionized Calcium on VF Latency**

If diltiazem does not increase VF latency by improving coronary blood flow or reducing mechanical work, then this effect may be more directly related to reduction of cellular calcium influx. To investigate this possibility, we reduced the serum ionized calcium concentration by infusion of the calcium-complexing agent, sodium citrate. Figure 2B shows the effect of citrate infusion on VF latency during serial coronary occlusions. Infusion of 4.7 mg/kg per minute increased VF latency compared with the six control values, and a subsequent infusion of 9.4 mg/kg per minute produced a further increase. VF latency returned to the control range when the citrate infusion was discontinued. A similar result was obtained in five other dogs.

Average VF latencies (upper curve) and serum ionized calcium (lower curve) in the six dogs that received citrate were plotted in Figure 8. The changes in VF latency were statistically significant ($\chi^2 = 21.4, df = 8, P < 0.01$), as were the changes in serum ionized calcium ($\chi^2 = 36.4, df = 8, P < 0.0002$). The similar time course of the changes in VF latency and serum ionized calcium suggested that these two parameters were closely correlated. In Figure 9, the percent change in VF latency (relative to the mean of the control values) was plotted as a function of the change in serum ionized calcium for each dog. The resulting correlation coefficient was 0.84 ($P < 10^{-6}$).

**Failure of Lidocaine to Alter VF Latency**

To characterize further the antifibrillatory effects observed in our model, it is appropriate to determine whether antiarrhythmics that do not directly affect calcium influx also prolong VF latency. Lidocaine was chosen because it is effective against ventricular fibrillation in both clinical (Valentine et al., 1974; Lie et al., 1974) and experimental studies (Gerstenblith et al., 1972; Spear et al., 1972; Borer et al., 1975; Aravindakshan and Gettes, 1975). Figure 10A shows the mean VF latency in five dogs that received 2 mg/kg of lidocaine (mean serum level = 5.0 ± 2.3 μg/ml) after six control occlusions. Mean VF latency 5 minutes after the infusion was within the control range, and non-parametric analysis showed no significant change
six control occlusions. Mean VF latency was slightly lower than the control values but this reduction was not significant ($\chi^2 = 4.1$, df = 6, $P > 0.3$).

Discussion

The suppression of ischemic ventricular arrhythmias by calcium influx blockers has now been documented in a variety of experimental models. In their original study, Kaumann and Aramendia (1968) showed that the suppression of VF by verapamil during permanent LAD ligation is not mediated by the sympathetic nervous system because it can also be demonstrated in animals pretreated with reserpine. Verapamil does not increase the threshold for production of ventricular fibrillation by current pulses (VFT), but does attenuate the reduction in VFT normally observed during ischemia (Huang and Peng, 1977; Fondacaro et al., 1978; Brooks et al., 1980). Verapamil (Schmid and Hanna, 1967), and manganese ions (Kligfield et al., 1981) both suppress premature ventricular beats following coronary artery ligation. The effect of calcium-complexing agents has not been previously examined, but Kaumann and Serur (1975) found that infusion of calcium chloride counteracts the antifibrillatory effect of verapamil during LAD occlusion.

The present work shows that diltiazem and reduced serum ionized calcium both retard the development of VF during acute ischemia. Our results introduce a new method of quantifying the antifibrillatory effect, which may have practical and theoretical advantages over the traditional model of permanent arterial ligation. The risk of ventricular fibrillation during a brief period of ischemia depends in part upon the amount of myocardium rendered ischemic. During isolated LAD occlusion, relatively few dogs develop VF within the first few minutes, but the incidence increases when the occlusion is maintained until permanent myocardial necrosis has occurred. The overall incidence of VF then reflects a combination of immediate and delayed events (Kaplinsky et al., 1979), the latter of which are not reproducible in a given animal because they require infarction. In contrast, simultaneous occlusion of the LAD and circumflex arteries nearly always produces VF after 2-3 minutes, with the latency of VF being particularly reproducible during serial occlusions in the same animal. Both diltiazem and reduction of serum ionized calcium produce a reversible increase in VF latency that is closely related to serum concentration, and can be demonstrated unequivocally in a small number of experiments.

There are three possible explanations for the antifibrillatory effects of calcium influx blockers. (1) These agents may increase perfusion of the ischemic zone through augmentation of collateral blood flow. (2) They may reduce myocardial work, thereby alleviating metabolic abnormalities associated with ischemia. (3) They may suppress the transmembrane currents that give rise to fibrillation.

Possible Role of Coronary Vasodilation

Pretreatment with calcium influx blocking drugs improves perfusion during ligation of single coronary arteries, as evidenced by a decrease in the size of the resulting myocardial infarct (Selwyn et al., 1979). Whereas this effect may be important in the clinical setting, it is unlikely to account for the antifibrillatory effect observed in our model. Because the right coronary artery does not supply left ventricular branches in the dog (Miller et al., 1964), proximal occlusion of both left coronary branches would be expected to produce global left ventricular ischemia. Nearly complete interruption of the left ventricular blood supply is confirmed in our experiments by the rapid cessation of forward cardiac output, and coronary venous flow. The failure of diltiazem to increase coronary flow during occlusion is therefore not surprising. Sherman et al. (1981) have found that the antifibrillatory effect of verapamil during LAD occlusion in dogs is not associated with an increase in collateral perfusion measured by the microsphere technique, while Thandroyen et al. (1981) have obtained a similar result in ischemic rat hearts treated with three different calcium influx blockers.

The effect of reduced serum ionized calcium on VF latency is also unlikely to reflect changes in coronary blood flow, because in a preliminary study (Baim and Bristow, unpublished observations) citrate infusion failed to produce a primary vasodilating effect. In these experiments, the increase in resting coronary blood flow produced by diltiazem was associated with a dose-related narrowing of myocardial A-V02 difference. Reduction of serum ionized calcium over the range achieved in Figures 8-9 increased myocardial blood flow slightly, but did not significantly reduce A-V02 difference, which is indicative of a secondary (demand-mediated) phenomenon, rather than primary vasodilation (Baim et al., 1982).

Possible Metabolic Effects

Calcium influx blockers also have direct myocardial protective effects that are independent of coronary blood flow (Higgins et al., 1980), and are manifested by a relative improvement of function during recovery from experimental hypoxia or ischemia (Henry et al., 1977; Clark et al., 1977; Weishaar and Bing, 1980; Sasayama et al., 1981). However, these beneficial effects are not necessarily due to preservation of myocardial energy stores during ischemia. In our model, diltiazem not only fails to reduce left ventricular mechanical work during ischemia, but produces a small initial increase, probably due to reduction of systemic vascular resistance. Perez et al. (1980) measured the effects of diltiazem and transient ischemia on segmental shortening in canine ventricle and found that the drug-induced improvement of contractility during reperfusion was not due to reduced contractility during ischemia. Jolly et al. (1981) measured high
energy phosphate compounds in diltiazem-treated guinea pig hearts before and after global ischemia and found that diltiazem significantly increases these compounds during reperfusion, but not during ischemia. Thadroyen et al. (1981) found that the favorable effect of diltiazem on the ventricular fibrillation threshold during ischemia is also unassociated with an increase in high energy phosphate stores. Thus, there is no evidence that the antifibrillatory effect of diltiazem is due to reduced consumption of metabolic energy during ischemia.

Possible Electrophysiological Effects

An alternative explanation for the antifibrillatory effect is that calcium ions directly participate in the electrophysiological phenomena that give rise to fibrillation. Although the similar actions of diltiazem and reduced serum ionized calcium in our model do not directly confirm this hypothesis, it is impressive that most available methods of reducing cellular calcium influx have now been shown to suppress ischemic ventricular arrhythmias in one of various models.

There are two forms of calcium-dependent electrical activity that might be potentially important in the genesis of acute ischemic ventricular arrhythmias. First, it is possible that slowly conducted calcium-dependent action potentials are involved (see Cranefield, 1977). Such action potentials have recently been recorded from isometric canine ventricular myocardium after several minutes of fibrillation (Akiyama, 1981), although it not clear that they are present at the onset. Second, it has been proposed that ventricular fibrillation may be an extreme form of calcium-mediated abnormal automaticity (Clusin et al., 1981, 1982). In this case, the ionic currents giving rise to fibrillation would occur as a direct consequence of intracellular calcium overload, and agents that delayed the development of calcium overload by reducing influx would produce a corresponding delay in the onset of VF.

Are Changes in VF Latency Related to Antiarrhythmic Efficacy?

An important question raised by our observations is whether an increase in VF latency during global LV ischemia is indicative of potential clinical benefit. In our model, serum diltiazem levels between 30 and 50 ng/ml (6-10 x 10^-8 M) invariably delayed the onset of VF with little or no effect on blood pressure or atrioventricular conduction. All of the mean serum levels in Figure 3 are within or below the range of values normally achieved in humans during chronic oral therapy for angina. Extrapolation of these observations to the clinical setting must, however, be tempered by two important limitations that are inherent in the model. First, the production of global left ventricular ischemia is a highly artificial situation that is unlikely to occur spontaneously. Since interaction between normal and ischemic tissue across a border zone may be important in the genesis of clinical arrhythmias, some aspects of arrhythmia development might be excluded by our model. Second, since drug-induced redistribution of blood flow to the ischemic tissue is precluded a priori in our model, the potential benefit of this phenomenon cannot be studied.

We did encounter a few dogs in which global left ventricular ischemia could not be achieved because of surgically inaccessible proximal coronary branches. Although these animals were excluded from the data analysis, VF latencies obtained during control occlusions were generally longer, and the antifibrillatory effects of diltiazem were often more striking than during global LV ischemia. Thus, if persisting coronary flow had been adequate, diltiazem might have permitted survival after permanent occlusion, as was shown with verapamil by Kaumann and Aramendia (1968). Prevention of ventricular fibrillation by calcium influx blockers during regional ischemia may be due to a combination of vascular and direct myocardial effects.

References

Bairn DS, Rothman MT, Harrison DC (in press) Simultaneous measurement of coronary venous blood flow and oxygen saturation during changes in myocardial oxygen supply and demand. Am J Cardiol

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Address for reprints: Dr. William T. Clusin, Division of Cardiology, Stanford University School of Medicine, Stanford, California 94305.

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Fondacaro JD, Han J, Yoon MS (1978) Effects of verapamil on ventricular rhythm during acute coronary occlusion. Am Heart J 96: 81-86


Kaplnsky E, Ogawa S, Balke CW, Dreibus LS (1979) Two periods of early ventricular arrhythmia in the canine acute myocardial infarction model. Circulation 60: 397-403

Katz AM, Reuter H (1979) Cellular calcium and cardiac cell death. Am J Cardiol 44: 188-190


Thandroyen FT, Higginson L, Opie LH (1981) Protection against ventricular fibrillation by calcium antagonists. Am J Cardiol 47: 442(a)


The effects of diltiazem and reduced serum ionized calcium on ischemic ventricular fibrillation in the dog.
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