Antiarrhythmic Effects of Coronary Vasodilators on Canine Ventricular Arrhythmia Models

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Accepted February 7, 1985

Abstract—Antiarrhythmic effects of intravenous coronary vasodilators (verapamil, diltiazem, bepridil, trimetazidine and nicorandil) were evaluated using two canine ventricular arrhythmia models (halothane-adrenaline arrhythmia and digitalis arrhythmia), and the minimum effective plasma concentrations of the drugs were determined for each arrhythmia model. Verapamil (0.1 mg/kg), diltiazem (0.1 mg/kg), bepridil (1 mg/kg) and high dose trimetazidine (10 mg/kg) were effective on halothane-adrenaline arrhythmia; and the minimum effective plasma concentrations of the above drugs were less than 30±10 ng/ml, less than 18±5 ng/ml, 0.38±0.11 μg/ml and 7.0±1.5 μg/ml, respectively. Nicorandil (1 mg/kg) did not suppress halothane-adrenaline arrhythmia. Verapamil, diltiazem and bepridil must have suppressed the halothane-adrenaline arrhythmia by blocking the Ca channel. Verapamil (1 mg/kg), diltiazem (1 mg/kg), bepridil (5 mg/kg), trimetazidine (3 mg/kg) and nicorandil (3 mg/kg) were ineffective on digitalis arrhythmia, even though their maximum hypotensive doses were used.

Coronary vasodilators have been used to treat ischemic heart diseases, which are often complicated by ventricular arrhythmias. Though these arrhythmias sometimes require antiarrhythmic drug therapy using class 1 antiarrhythmic drugs (1) which block the Na channel, coronary vasodilators sometimes have been reported to suppress such ventricular arrhythmias (2, 3). The mechanisms of generation of these arrhythmias resulting from myocardial ischemia have not yet been clarified, but Ca ion related oscillatory afterpotential or slow depolarization resulting in re-entry seems to play an important role (4). Since some of the coronary vasodilators have Ca antagonistic effects, and even have additional Na antagonistic effects in high doses (5), those drugs may be effective on various arrhythmias. Therefore, we examined coronary vasodilators on two canine ventricular arrhythmia models: halothane-adrenaline and digitalis arrhythmias. Though the generation mechanisms of these arrhythmias are thought to be unrelated to myocardial ischemia, we chose adrenaline arrhythmia as a Ca channel related arrhythmia because drugs directly suppressing the Ca channel, like verapamil, or indirectly suppressing the channel, like β-blockers, were quite effective on this arrhythmia (6, 7). On the other hand, the digitalis arrhythmia seems to be Na channel related from our previous studies because class 1 antiarrhythmic drugs suppressed this arrhythmia (8–10).

We chose different types of coronary vasodilators, i.e., verapamil, diltiazem, bepridil, trimetazidine and nicorandil. Verapamil and diltiazem are typical Ca antagonists having Na channel blocking action in high concentrations in vitro cardiac tissues (11). Bepridil is a new Ca antagonist which has been reported to have Na channel blocking action in therapeutic concentrations (12, 13). Trimetazidine has been reported to have β-blocking and Ca antagonistic actions in high concentrations (14), but its beneficial effects on ischemic heart diseases have been reported to be due to the reduction of the
venous return (15). Nicorandil, 2-nicotinamidoethyl nitrate (SG-75), is a newly synthesized coronary vasodilator chemically related to nitrates and has been reported not to have a Ca antagonistic effect (16, 17).

Unlike many previous animal studies, we assayed the drug plasma concentrations simultaneously while examining their anti-arrhythmic effects in order to correlate the effectiveness of drugs on the arrhythmias to their already known concentrations of Ca or Na channel blocking or of β-blocking actions in vitro.

Materials and Methods

1) Halothane-adrenaline arrhythmia

Mongrel dogs of either sex, weighing 8 to 15 kg, were anesthetized initially with 30 mg/kg of thiopental Na. After intubation, 1.0% halothane, vaporized with 100% O₂, was administered using a volume-limited ventilator. After 30 min, adrenaline was infused for 18 min into the femoral vein using a Harvard infusion/withdrawal pump. As reported previously, ventricular arrhythmia was usually induced by 2 to 3 ng/kg/min of adrenaline infusion within 2 min, and the arrhythmia lasted for the 18 min of infusion (7). Three minutes after the start of adrenaline infusion, coronary vasodilators were injected in a bolus into the femoral vein.

Lead II ECG, the atrial electrogram from the catheter tip electrode in the right atrium, and blood pressure were continuously recorded. Venous blood samples were taken from the jugular vein 1 min before and 1, 3, 5, 10, 15, 30 and 60 min after the injection of the drugs.

The doses of drugs chosen in the present experiment were determined as the minimum effective dose or the maximal tolerable hypotensive doses for ineffective drugs.

2) Digitalis arrhythmia

Mongrel dogs of either sex, weighing 8 to 15 kg, were anesthetized with 30 mg/kg pentobarbital Na. Modifying the method of Lucchesi and Hardman (18), 40 µg/kg of ouabain was injected into the femoral vein, followed by an additional dose of 10 µg/kg ouabain every 20 min until stable arrhythmia of more than 1 hr duration was produced as reported previously (9, 10). Stable arrhythmia was usually produced with a total dose of 70 to 80 µg/kg ouabain. Coronary vasodilators were injected in a bolus into the femoral vein.

Lead II ECG, the atrial electrogram from the catheter tip electrodes in the right atrium and blood pressure were continuously recorded. The venous blood samples were drawn from the jugular vein 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after the injection of the drugs.

3) Plasma drug assay

Venous blood samples were centrifuged, and the plasma was stored in a freezer at about -25°C before plasma drug concentration analysis.

Verapamil: The plasma verapamil assay was carried out at the Experimental Therapeutic Research of the Eisai Co., Ltd. (Tokyo, Japan) using a gas chromatography, mass spectrometric method similar to an assay developed by Spiegelhalder and Eichelbaum (19).

Diltiazem: Plasma diltiazem assay was carried out at the Research Laboratories of the Tanabe Seiyaku Co., Ltd. (Osaka, Japan) using a high performance liquid chromatographic method. A plasma volume of 1 ml was shaken twice with 10 ml ethylether. The aqueous phase was discarded, and the organic phase was evaporated at 40°C under a nitrogen stream. To the samples, 2 ml of 0.2 N HCl was added and shaken with 10 ml ethylether. One ml of 0.4 N NaOH and 1 ml of 0.5 N NaH₂PO₄ were added to the aqueous phase, and this was shaken twice with 8 ml ethylether. The organic phase was evaporated at 40°C under a nitrogen stream and dissolved with 0.3 ml of 500 ng/ml tipepidine hibenzate 0.02 N HCl as an internal standard solution.

Two hundred µl of this solution was injected onto the column (Hypersil 5-ODS, 4 mm i.d. × 300 mm length) of the high-performance liquid chromatograph (Hewlett Packard 1084B).

Bepridil: Plasma bepridil assay was carried out at the Nippon Organon K.K. (Tokyo, Japan) using a gas chromatographic method with nitrogen-sensitive detection according to the method of Vink and Vanhol (20).

Trimetazidine: Plasma trimetazidine assay was carried out at the Research Laboratories
of the Inabata & Co., Ltd. (Osaka, Japan) using a gas chromatographic method. To 2 ml plasma, 4 ml 0.5 N NaOH and 20 ml C₆H₆ were added. The mixture was shaken and then centrifuged at 15000 rpm for 10 min. Eighteen ml of the aqueous phase was evaporated, and 0.5 ml (CH₃CO)₂O was added. After 1 hr, 4 ml 1N HCl and 4 ml CH₂Cl₂ were added and then shaken and centrifuged at 3000 rpm for 5 min. Three grams of NaCl was added to 3 ml of the aqueous phase and the pH was adjusted to 9 with 1 N NaOH. Then 20 ml of CH₂Cl₂ was added, the samples were shaken, and they were centrifuged at 3000 rpm for 10 min. To 15 ml of the lower layer, 500 ng of trimetazidine butylester was added as an internal standard and evaporated to 0.5 ml. Five µl of this solution was injected onto the column (2.6 mm i.d. x 1.5 m length) of a gas chromatograph (Shimadzu GC-7A-FTD).

Nicorandil: The plasma nicorandil assay was carried out at the Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan) using a high performance liquid chromatographic method according to the method developed by Kamiyama et al. (21).

4) Determination of the minimum effective plasma concentrations

In order to express the severity of arrhythmia, the arrhythmic ratio, a ratio of the number of ventricular ectopic beats divided by the total number of beats counted from the lead II ECG (total heart rate), was used. The last minute of statistically significant decrease (P<0.05) in the arrhythmic ratio compared with that at zero time was determined. Then, the corresponding plasma concentration was calculated from the experimentally derived plasma concentration time equations, and this was regarded as the minimum effective plasma concentration. The concentration-time equation was derived by the two compartment open model theory in the case of digitalis arrhythmia and by the one compartment open model theory in the case of adrenaline arrhythmia.

Results

1) Effects on the halothane-adrenaline arrhythmia

Diltiazem (n=7): A dose of 0.1 mg/kg diltiazem was effective in suppressing the adrenaline arrhythmia. As shown in Fig. 1, the arrhythmic ratio decreased soon after injection, and this effect lasted up to 15 min. Parameters of the diltiazem concentration-time equation are shown in Table 1. The minimum plasma concentration was less than 18±5 ng/ml. This dose of diltiazem reduced the total heart rate and atrial rate without a significant change in the blood pressure.

Bepridil (n=7): A dose of 1 mg/kg bepridil transiently suppressed this arrhythmia. As shown in Fig. 2, the arrhythmic ratio was reduced soon after injection and was accompanied by a reduction in the total heart rate, atrial rate and blood pressure. The antiarrhythmic effect continued for 5 min. Parameters of the bepridil concentration-time equation are shown in Table 1. The minimum plasma concentration was 380±110 ng/ml.

Trimetazidine (n=6): A dose of 10 mg/kg trimetazidine showed a transient, 2 min, antiarrhythmic effect as shown in Fig. 3, while it markedly reduced the blood pressure and total heart rate. The atrial rate was not changed. Parameters of the trimetazidine concentration-time equation are shown in Table 1. The minimum plasma concentration was 7.0±1.5 µg/ml.

Nicorandil (n=8): A dose of 1 mg/kg nicorandil was not effective on this arrhythmia, even though this dose markedly decreased the blood pressure as shown in Fig. 4. The atrial rate transiently increased without a change in the total heart rate. Parameters of the nicorandil concentration-time equation are shown in Table 1. The maximum plasma concentration 1 min after injection reached to 2.2±0.9 µg/ml.

2) Effects on digitalis arrhythmia

Verapamil (n=7): A dose of up to 1 mg/kg verapamil, a maximal hypotensive dose, was ineffective on digitalis arrhythmia as shown in Fig. 5. The total heart rate, atrial rate and blood pressure markedly decreased for up to 60 min. Parameters of the verapamil concentration-time equation are shown in Table 2. The peak plasma concentration 1 min after injection reached 930±440 ng/ml.

Diltiazem (n=6): A dose of up to 1 mg/kg diltiazem was also ineffective on digitalis
Arrhythmia as shown in Fig. 1. The total heart rate, atrial rate and blood pressure markedly decreased. Parameters of the diltiazem concentration-time equation are shown in Table 2. The peak concentration reached 930±280 ng/ml.

**Fig. 1.** Summary of the effects of diltiazem on adrenaline arrhythmia (left) and digitalis arrhythmia (right). Diltiazem, 0.1 mg/kg, i.v., was effective on adrenaline arrhythmia, i.e., decreased the arrhythmic ratio, without significant decrease in the blood pressure. Diltiazem, 1 mg/kg, i.v., was ineffective on digitalis arrhythmia even though this dose markedly decreased the blood pressure. *P<0.05, **P<0.01 from the 0 time value. Bars represent the S.D.

**Table 1.** Pharmacokinetic parameters of vasodilators in dogs with halothane-adrenaline arrhythmia

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>A (µg/ml)</th>
<th>α (/min)</th>
<th>Minimum effective plasma concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil*</td>
<td>0.1</td>
<td>7</td>
<td>0.100±0.045</td>
<td>0.08±0.02</td>
<td>0.30±0.010</td>
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<tr>
<td>Diltiazem</td>
<td>0.1</td>
<td>7</td>
<td>0.033±0.009</td>
<td>0.04±0.03</td>
<td>0.018±0.005</td>
</tr>
<tr>
<td>Bepridil</td>
<td>1</td>
<td>7</td>
<td>0.690±0.220</td>
<td>0.12±0.05</td>
<td>0.380±0.110</td>
</tr>
<tr>
<td>Trimetazidine</td>
<td>10</td>
<td>6</td>
<td>7.900±1.800</td>
<td>0.05±0.02</td>
<td>7.000±1.500</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>1</td>
<td>8</td>
<td>2.490±0.900</td>
<td>0.06±0.03</td>
<td></td>
</tr>
</tbody>
</table>

* Data taken from the paper of Shibuya et al. (7). A: concentration at zero-time, α: time constant.

Arrhythmia as shown in Fig. 1. The total heart rate, atrial rate and blood pressure markedly decreased. Parameters of the diltiazem concentration-time equation are shown in Table 2. The peak concentration reached 930±280 ng/ml.

**Bepridil (n=8):** A dose of up to 5 mg/kg bepridil was also ineffective on this arrhythmia as shown in Fig. 2. Bepridil also reduced the total heart rate, atrial rate and blood pressure. Parameters of the bepridil concentration-time equation are shown in
Table 2. The peak plasma concentration reached 2.6±1.3 µg/ml.

Fig. 2. Summary of the effects of bepridil on adrenaline arrhythmia (left) and digitalis arrhythmia (right). Bepridil, 1 mg/kg, i.v., was effective on adrenaline arrhythmia, but the higher dose of 5 mg/kg, i.v., was not effective on digitalis arrhythmia.

Table 2. Pharmacokinetic parameters of vasodilators in dogs with digitalis arrhythmia

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose mg/kg</th>
<th>n</th>
<th>A</th>
<th>α</th>
<th>B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>1</td>
<td>7</td>
<td>0.440±0.210</td>
<td>0.31±0.12</td>
<td>0.310±0.12</td>
<td>0.011±0.005</td>
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<tr>
<td>Diltiazem</td>
<td>1</td>
<td>6</td>
<td>0.320±0.140</td>
<td>0.33±0.07</td>
<td>0.360±0.070</td>
<td>0.016±0.005</td>
</tr>
<tr>
<td>Bepridil</td>
<td>5</td>
<td>8</td>
<td>1.000±0.800</td>
<td>0.27±0.06</td>
<td>2.200±1.000</td>
<td>0.014±0.006</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>3</td>
<td>6</td>
<td>4.300±2.600</td>
<td>0.25±0.11</td>
<td>4.900±0.900</td>
<td>0.018±0.004</td>
</tr>
</tbody>
</table>

A: concentration at zero-time of the distribution curve, α: time constant of the distribution curve, B: concentration at zero-time of the elimination curve, β: time constant of the elimination curve.

Table 2. The peak plasma concentration reached 2.6±1.3 µg/ml.

Trimetazidine (n=7): A dose of up to 3 mg/kg trimetazidine did not reduce the arrhythmic ratio, total heart rate and atrial rate as shown in Fig. 3. Though 10 mg/kg was used in the adrenaline arrhythmia experiment, 3 mg/kg markedly reduced the blood pressure and was regarded as the maximal hypotensive dose in this digitalis arrhythmia experiment. The pharmacokinetic parameters were not obtained for trimetazidine, because only the plasma concentrations 1 and 3 min after injection were measured. The peak plasma concentration reached 3.8±0.5 µg/ml, 1 min after injection.

Nicorandil (n=6): A dose of up to 3 mg/kg nicorandil did not suppress digitalis ar-
rhythmia as shown in Fig. 4. It only decreased the blood pressure without changing the total heart rate and atrial rate. Parameters of the nicorandil concentration-time equation are shown in Table 2. Peak plasma concentration reached $8.3 \pm 2.8 \mu g/ml$.

**Discussion**

The present study showed that the Ca antagonistic coronary vasodilators, verapamil, diltiazem and bepridil, were effective on halothane-adrenaline arrhythmia, but were ineffective on digitalis arrhythmia. Trimetazidine, which has been reported to have $\beta$-blocking and Ca antagonistic effects in high concentrations, had almost the same effects as Ca antagonists when a high dose was used. Nicorandil was not effective on both halothane-adrenaline and digitalis arrhythmias. Regardless of differences in their effectiveness on arrhythmias, all drugs markedly decreased the blood pressure as might be expected from the word "vasodilator".

We reported that $\beta$-blocking agents and verapamil were effective on halothane-adrenaline arrhythmia (7), and this was confirmed by the present study that other Ca antagonists are also quite effective. Since all the vasodilators used in the present study decreased the blood pressure, regardless of their mechanism of action, these antiarrhythmic effects of Ca antagonists might have been due to the direct or indirect blocking of Ca influx into the cell. It was also confirmed by measuring the drug plasma concentrations that the minimum effective and the clinical therapeutic plasma concentrations of the Ca antagonists were almost equal to the concentrations, which suppress the Ca channel of in vitro cardiac tissues (11, 12). Though we have reported that the
Supraventricular rate plays an important role in the genesis of the halothane adrenaline arrhythmia using the atrial stimulation technique (22), it does not seem to be the only mechanism, because the atrial rate was not changed dramatically by those Ca antagonists. Direct β-receptor stimulation on the ventricle by adrenaline through the Ca channel and its suppression must also play a role in the genesis and suppression of the adrenaline arrhythmia.

In the present study, Ca antagonists were ineffective on the digitalis arrhythmia. Digitalis arrhythmia has been proposed to be due to increased intracellular Ca, resulting in oscillatory afterpotential (23). It was reported that Ca antagonists suppressed the oscillatory afterpotential of an in vitro preparation (23), so Ca antagonists are thought to suppress in vivo digitalis arrhythmia. Also in ischemic conditions, where the resting potential of the ventricular muscle is thought to elevate, the slow inward current may become important in generating action potential. So Ca antagonists can also be expected to suppress ischemia induced ventricular arrhythmias. However, as previously reported (24), verapamil (0.3 mg/kg) did not suppress two-stage coronary ligation arrhythmias, even the less severe 48 hr arrhythmia. Contrary to our results on model arrhythmias, there are reports that Ca antagonists suppressed experimental arrhythmias (25–27) and clinical ventricular arrhythmias not directly related to adrenaline (3). Whether all those arrhythmias were produced by an abnormality in the Ca channel such as supraventricular arrhythmias related either to A-V node or S-A node (3, 28–30) would be difficult to clarify.

Fig. 4. Summary of the effects of nicorandil on adrenaline arrhythmia (left) and digitalis arrhythmia (right). Nicorandil at 1 and 3 mg/kg, i.v., was ineffective on both adrenaline and digitalis arrhythmias, respectively. Blood pressure was decreased significantly by both doses.
Fig. 5. Summary of the effects of verapamil, 0.1 mg/kg, i.v., on digitalis arrhythmia. Verapamil decreased the total heart rate, atrial rate and blood pressure, but the number of conducted beats and arrhythmic ratio were not changed.

According to our previous experiments (8), all class 1 antiarrhythmic agents are effective on digitalis arrhythmia at concentrations blocking the Na channel. The Na channel blocking concentrations in vitro are reported to be about 10 \( \mu \)g/ml, 10 \( \mu \)g/ml and 4 \( \mu \)g/ml for verapamil, diltiazem and bepridil, respectively (11, 12). Since we could not increase the doses of Ca antagonists beyond the dose which induced profound hypotensive effects, the maximum doses used did not raise the plasma concentration of the drug to Na channel blocking concentrations. This must be the reason for their ineffectiveness on digitalis arrhythmia. The fact that the blocking of the Ca channel did not suppress digitalis arrhythmia may indicate that either generation of digitalis arrhythmia is not related to the slow inward current or antiarrhythmic drugs do not suppress the arrhythmia by directly acting on abnormal electrophysiological phenomena. Na channel blockers may act on the normal myocardium to suppress the response to abnormal impulse generation, whatever the cause of its electrophysiological mechanisms.

The effects of a high dose of trimetazidine were the same as those of Ca antagonists, but the duration of its effect was brief. The minimum effective plasma concentration of trimetazidine was 6.9 \( \mu \)g/ml, which is markedly lower than the reported concentration for the Ca antagonistic effect and the \( \beta \)-blocking effect in vascular smooth muscle (14). There is no report of a Ca-antagonistic concentration of trimetazidine using cardiac preparations.

Nicorandil suppressed neither halothane adrenaline arrhythmia nor digitalis arrhythmia. This was to be expected because nicorandil had no effect on Na and Ca channels (16, 17). As a vasodilator, nicorandil reduced the blood pressure as markedly as other coronary vasodilators.

Applying our present study to clinical use of vasodilators in patients with ventricular arrhythmia, Ca antagonists may have therapeutic value only in those related to sympathetic drive, but may be of limited value in ventricular arrhythmias when compared to Na channel blocking drugs because verapamil (24), diltiazem (26) and trimetazidine (our unpublished observation) were not effective on two-stage coronary ligation arrhythmia. However, there are possibilities that Ca antagonists may be effective for prophylaxis of ventricular arrhythmias because pretreatments of experimental animals with verapamil were reported to increase the toxic doses of digitalis (31) and prevent fibrillation following acute coronary occlusion (32).

Acknowledgements: The authors thank Eisai Co., Ltd.; Tanabe Seiyaku Co., Ltd.; Nippon Organon Co., Ltd.; Inabata & Co., Ltd. and Chugai Pharmaceutical Co., Ltd for plasma drug assays, and we also thank Mr. J. Sendoda and Mrs. A. Tezuka for preparing the manuscript.

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