Effects of NS-2, a New Class 1 Antiarrhythmic Agent, and AFD-19, Its Active Metabolite, on Ventricular Arrhythmias Induced by Coronary Artery Occlusion and Reperfusion in Anesthetized Rats: Comparison with Disopyramide and Mexiletine

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ABSTRACT—We studied the antiarrhythmic effects of NS-2 (4-diisobutylamino-1,1-diphenyl-1-butanol maleate) and AFD-19 (active metabolite of NS-2) on early stage ventricular arrhythmias induced by coronary artery occlusion and reperfusion in anesthetized male rats. These effects were compared with those of disopyramide and mexiletine. Drugs were intravenously administered either before or after coronary occlusion. When administered 5 min before occlusion, 3 mg/kg of NS-2 and AFD-19 exhibited equivalent antiarrhythmic activity to that of 5 mg/kg of disopyramide and mexiletine, as assessed by reductions in the number of premature ventricular complexes and in the incidences of ventricular tachycardia and ventricular fibrillation. In a dose of 5 mg/kg, the antiarrhythmic effects of NS-2 and AFD-19 were more pronounced. When administered 5 min after coronary artery occlusion, only NS-2 and AFD-19 (in doses of 5 mg/kg) had significant antiarrhythmic effects. None of the drugs influenced the severe ventricular arrhythmias induced by reperfusion when administered 1 min before reperfusion. In conclusion, NS-2 might be effective in reducing the severity of the life-threatening ventricular arrhythmias that occur during acute myocardial infarction.

Keywords: Arrhythmia (ischemia-induced, reperfusion-induced), NS-2, AFD-19, Antiarrhythmic drug (class 1)

NS-2 (4-diisobutylamino-1,1-diphenyl-1-butanol maleate) is a novel class 1 antiarrhythmic agent and AFD-19 (4-isobutylamino-1,1-diphenyl-1-butanol maleate) is an active metabolite of NS-2. Although there seems little doubt that class 1 antiarrhythmic agents are effective against ectopic ventricular beats occurring several hours after onset of myocardial infarction in animal experiments (1), there is some controversy concerning their efficacy during the early phase of ventricular arrhythmias; i.e., those occurring during the first 30 min following coronary occlusion (2–8). This may reflect differences in underlying electrophysiological changes occurring at different stages of ischemia. The time of administration appears to be also important. In this study, the antiarrhythmic effects of NS-2 and AFD-19 were evaluated on the early stage ventricular arrhythmia induced by coronary artery occlusion and reperfusion in anesthetized rats, and their activity was compared with those of established class 1 drugs, disopyramide and mexiletine. We also evaluated the difference in the efficacy of each drug when administered either before or after the onset of ischemia and also on reperfusion arrhythmias when administered just prior to reperfusion.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200–300 g) were anesthetized with 60 mg/kg sodium pentobarbitone administered intraperitoneally. The femoral vein was cannulated for drug administration, and the trachea was cannulated for artificial respiration. Systemic blood pressure was continuously monitored via a catheter inserted into the carotid artery. A standard limb lead 1 was continuously recorded, together with systemic blood pressure, on a Grass Model 7D recorder (Grass Instrument Co., Quincy, MA, USA). The chest was opened by a left thoracotomy, followed by
sectioning of the 4th and 5th ribs, approximately 2 mm to the left of the sternum (9). Artificial respiration was started immediately with room air, using a volume of 2 ml/100 g and a rate of 54 strokes/min in order to maintain arterial blood gases and pH within the normal range. After incising the pericardium, the heart was exteriorized using gentle pressure on the rib cage. A 6/0 braided silk suture attached to a 10-mm micropoint reverse cutting needle was placed under the coronary artery. The heart was replaced in the chest, and the rat was allowed to recover for 15 min. Rats that had arrhythmias during the recovery time and/or had a mean arterial blood pressure of less than 70 mmHg were discarded.

All experiments were performed in accordance with the Guidelines for Animal Experiments in Yamanashi Medical University.

**Studies to determine the effects of disopyramide, mexiletine, NS-2 and AFD-19 on occlusion-induced arrhythmias when administered before coronary occlusion**

Seventy-eight rats were used in this protocol. Rats were divided into 8 groups with 9–10 rats in each group. The rats received, by intravenous administration, either saline (n = 10, the vehicle for mexiletine), polyethylene glycol 200 (n = 9; the vehicle for disopyramide, NS-2 and AFD-19), disopyramide (5 mg/kg, n = 9), mexiletine (5 mg/kg, n = 10), NS-2 (either 3 or 5 mg/kg, n = 10 and 10) or AFD-19 (3 or 5 mg/kg, n = 10 and 10). The injection volume of each agent was adjusted to 0.5 ml with saline. Coronary ligation was commenced 5 min after drug administration, and the electrocardiogram and systemic arterial blood pressure were continuously recorded for 30 min after coronary ligation. Blood pressure and sinus rate were measured before injection; at 1 min, 3 min, 5 min after injection; and at 1 min, 5 min, 10 min, 15 min and 30 min after coronary artery occlusion.

**Studies to determine the effects of disopyramide, mexiletine, NS-2 and AFD-19 on occlusion-induced arrhythmias when administered after coronary occlusion**

Eighty-two rats were used in this protocol. Drugs and the doses were the same as above. The number of rats in each group was 10, except for group 1 (saline control) where 12 rats were used. Each drug was administered 5 min after coronary occlusion. The electrocardiogram and systemic arterial blood pressure were continuously recorded for 30 min after coronary occlusion. Blood pressure and sinus rate were estimated before occlusion and at 1 min and 5 min after occlusion (before injection), 6 min after occlusion (1 min after injection), 8 min after occlusion (3 min after injection), 10 min after occlusion (5 min after injection), 15 min after occlusion (10 min after injection) and 30 min after occlusion (25 min after injection).

**Studies to determine the effects of disopyramide, mexiletine, NS-2 and AFD-19 on reperfusion arrhythmia**

Both ends of the ligature placed under the left coronary artery were passed through a small plastic tube. Regional myocardial ischemia could be induced by pulling the suture and pressing the tube against the surface of the myocardium. The ischemia could be maintained for any desired period by clamping the tube and the suture. Reperfusion could be initiated by declamping and removing the tube.

We first evaluated the relationship between occlusion time (i.e., the duration of ischemia) and the severity of the arrhythmias resulting from reperfusion, and we ultimately used 6 min of occlusion time to determine the effects of each drug on reperfusion-induced arrhythmias. The doses of drugs were the same as above. Each drug was administered at 5 min after coronary occlusion (1 min before reperfusion). The electrocardiogram and systemic arterial blood pressure were recorded continuously for 20 min after reperfusion.

**Assessment of arrhythmias**

The total number of premature ventricular complexes (PVC), incidence of ventricular tachycardia (VT), incidence of ventricular fibrillation (VF) and mortality rate during 30 min after coronary occlusion in occlusion arrhythmias and during 20 min after reperfusion in reperfusion arrhythmias were assessed in comparison with a control group. VF is not necessarily a terminal event in this model.

The values are expressed as the mean ± standard error. Changes within each group were compared by the paired Student's t-test, whereas differences between groups were assessed by the Mann-Whitney U-test. Changes in incidences of events were analyzed by Fisher's exact probability test. Differences were regarded as significant if the P values were less than 0.05.

All drugs were kindly supplied by Nippon Shinyaku Co., Ltd. (Kyoto).

**RESULTS**

**The effects of disopyramide, mexiletine, NS-2 and AFD-19 on occlusion arrhythmias when administered before coronary artery occlusion**

In this model, premature ventricular activity commences at 4 min after the onset of ischemia, with pronounced activity over the next 10 min (mainly as VT), and this is then followed by a period of arrhythmic silence with only a few ventricular complexes (Fig. 1). Effects of class 1 antiarrhythmic drugs on the ischemia-induced arrhythmias are summarized in Fig. 2. There were no significant differences between the arrhythmias in the saline...
control group and polyethylene glycol #200 (PEG #200) vehicle control group with respect to the total number of PVC, incidences of VT, incidences of Vf or in mortality rate (10% in the saline group, 33% in the polyethylene glycol group). The total numbers of PVC in the control groups were 1185 ± 158 beats (saline) and 1160 ± 163 beats (PEG #200). Although disopyramide and mexiletine decreased the total numbers of PVC to 632 ± 204 beats and 696 ± 226 beats, respectively, these differences were not significant. Even 3 mg/kg of NS-2 and AFD-19 significantly decreased the total numbers of PVC. A 5 mg/kg dose of NS-2 and AFD-19 was more effective. The total numbers of PVC were 682 ± 121 beats (NS-2, 3 mg/kg, P < 0.05 vs. control), 480 ± 164 beats (NS-2, 5 mg/kg, P < 0.01 vs. control), 503 ± 135 beats (AFD-19, 3 mg/kg, P < 0.01 vs. control) and 429 ± 111 beats (AFD-19, 5 mg/kg, P < 0.01 vs. control), respectively. VT was significantly suppressed by only 5 mg/kg of AFD-19 (40%, P < 0.01 vs. control 100%). Incidences of Vf were

Fig. 1. Distribution of ventricular arrhythmias following acute coronary occlusion in anesthetized rats. Note that arrhythmic activity commences between 4 min and 15 min after occlusion and that there is very little ectopic activity between 15 min and 30 min. Hatched bars indicate the number of PVC occurring as VT.

Fig. 2. The effects of disopyramide (DSP, 5 mg/kg), mexiletine (MXT, 5 mg/kg), NS-2 (3 and 5 mg/kg) and AFD-19 (3 and 5 mg/kg) on ventricular arrhythmias (number of premature ventricular complexes, incidences of VT (open bar) and Vf (closed bar)) when given 5 min prior to coronary artery occlusion in anesthetized rats. Polyethylene glycol #200 (PEG #200) was used as the vehicle for DSP, NS-2 and AFD-19. NS-2 and AFD-19 significantly decreased the number of PVC. Only AFD-19 significantly suppressed the occurrence of VT and Vf. NS-2 and AFD-19 are particularly effective in reducing ischemia-induced arrhythmias. The results are given as means ± standard error. *P < 0.05, **P < 0.01 vs. vehicle control.
Effects of disopyramide, mexilentine, NS-2 and AFD-19 on occlusion arrhythmias when administered after coronary occlusion

Figure 3 shows the effects of drugs administered after occlusion. In contrast to the pretreatment, antiarrhythmic effects were attenuated when drugs were administered after occlusion. Only 5 mg/kg of NS-2 and AFD-19 significantly decreased the total numbers of PVC. None of the drugs reduced the incidence of the more serious ventricular arrhythmias (VT, Vf) or mortality (mortality in the mexiletine group, 20% vs. saline control 20%; mortality in the disopyramide, NS-2 and AFD-19 group, 10–20% vs. PEG #200 control, 10%).

Hemodynamic effects of disopyramide, mexiletine, NS-2 and AFD-19 when administered before and after coronary artery occlusion

The results are summarized in Figs. 4 and 5. The vehicles slightly decreased the heart rate and increased the mean arterial blood pressure. When administered before coronary occlusion, disopyramide decreased the heart rate from 423 ± 23 to 334 ± 15 beats/min (P < 0.01) and increased the mean arterial blood pressure from 81 ± 3 to 105 ± 7 mmHg (P < 0.01), whereas mexiletine decreased the heart rate from 447 ± 10 to 365 ± 14 beats/min (P < 0.01), but did not affect the arterial blood pressure. NS-2 significantly decreased both the heart rate and the blood pressure in a dose-dependent manner, whereas AFD-19 decreased the heart rate from 467 ± 14 to 376 ± 11 beats/min (P < 0.01) at the dose of 3 mg/kg and from 433 ± 16 to 357 ± 17 beats/min (P < 0.01) at the dose of 5 mg/kg, but did not influence the blood pressure. The hemodynamic changes induced by coronary artery occlusion were similar in all groups; there was a slight increase in the heart rate and a significant decrease in the blood pressure with some recovery over the 30-min occlusion period.

Fig. 3. The effects of disopyramide (DSP, 5 mg/kg), mexiletine (MXT, 5 mg/kg), NS-2 (3 and 5 mg/kg) and AFD-19 (3 and 5 mg/kg) on ventricular arrhythmias (number of premature ventricular complexes, incidences of VT (open bar) and Vf (closed bar)) when given 5 min after coronary artery occlusion in anesthetized rats. The results are given as means ± standard error. *P < 0.05 vs. vehicle control. The effectiveness of these drugs in reducing ischemia-induced arrhythmias is much reduced under these conditions; only the highest dose (5 mg/kg) of NS-2 and AFD-19 significantly reduced the number of premature ventricular complexes.
decreased the sinus rate and the mean blood pressure in a dose-response manner. Compared with the hemodynamic effects of drugs administered before occlusion, mexiletine and AFD-19 decreased the mean blood pressure, and the increase in blood pressure by disopyramide was attenuated, but decreases in the heart rate were accentuated.

Fig. 4. The effects of disopyramide (DSP, 5 mg/kg), mexiletine (MXT, 5 mg/kg), NS-2 (3 and 5 mg/kg) and AFD-19 (3 and 5 mg/kg) on heart rate (●) and mean arterial blood pressure (▲) when given 5 min prior to coronary artery occlusion. The effects of the vehicles (saline and polyethylene glycol #200 (PEG #200)) are also given. The results are given as means ± standard error. *P<0.05, **P<0.01 vs. values of zero min.

Effects of disopyramide, mexiletine, NS-2 and AFD-19 on reperfusion arrhythmias when administered 1 min prior to the onset of reperfusion

The severity of reperfusion arrhythmias depends on the duration of the preceding ischemic period (10, 11). Few arrhythmias occur following periods of 1 or 3 min of ischemia, but there is pronounced ectopic activity with a high
incidence of Vf, most of it might be terminal, when the myocardium is reperfused following the 5-min occlusion periods (Table 1). The severity of arrhythmias following 6-min occlusion was similar.

None of the drugs significantly influenced the severity of reperfusion arrhythmias when administered 1 min prior to the onset of reperfusion (Table 2).

DISCUSSION

The results of the present study indicate that NS-2 and AFD-19 are rather more effective than the standard antiarrhythmic agents, disopyramide and mexiletine in suppressing ischemia-induced arrhythmia in anesthetized rats, particularly when they are administered before occlusion. All of drugs evaluated were much less effective if given after...
the onset of occlusion. Marshall et al. (8) reviewed the antiarrhythmic effects of sodium channel blocking agents on early phase arrhythmias induced by coronary artery occlusion in anesthetized rats and concluded that most sodium channel blocking agents, when administered prior to occlusion, suppress the number of PVC and reduce the incidences of Vf. Winslow et al. (12) also reported that disopyramide administered 15 min prior to the onset of coronary occlusion supressed the early phase arrhythmias induced by coronary artery occlusion in rats.

Table 1. Reperfusion arrhythmias in anesthetized rats following occlusion times of 1, 3, 5 and 6 min

<table>
<thead>
<tr>
<th>Occlusion</th>
<th>No of PVC (beats)</th>
<th>Incidence of VT (%)</th>
<th>Incidence of Vf (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min (n=8)</td>
<td>1 ± 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 min (n=17)</td>
<td>10± 7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 min (n=24)</td>
<td>306±145</td>
<td>83</td>
<td>71</td>
<td>58</td>
</tr>
<tr>
<td>6 min (n=10)</td>
<td>208± 50</td>
<td>100</td>
<td>70</td>
<td>50</td>
</tr>
</tbody>
</table>

Only following 5 and 6 min occlusion periods, reperfusion arrhythmias are severe. PVC: premature ventricular complex, VT: ventricular tachycardia, Vf: ventricular fibrillation.

Table 2. Reperfusion arrhythmias following 6 min coronary artery occlusion period in anesthetized rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>Duration of VT/Vf (sec)</th>
<th>Incidence of Vf (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10</td>
<td>208±50</td>
<td>70</td>
<td>50</td>
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<tr>
<td>PEG #200</td>
<td>8</td>
<td>234±41</td>
<td>75</td>
<td>63</td>
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<tr>
<td>DSP 5 mg/kg</td>
<td>10</td>
<td>180±26</td>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>MXT 5 mg/kg</td>
<td>9</td>
<td>163±27</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>NS-2 3 mg/kg</td>
<td>9</td>
<td>203±31</td>
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<td>89</td>
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<td>NS-2 5 mg/kg</td>
<td>8</td>
<td>144±33</td>
<td>63</td>
<td>88</td>
</tr>
<tr>
<td>5AFD-19 3 mg/kg</td>
<td>8</td>
<td>159±28</td>
<td>100</td>
<td>75</td>
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<tr>
<td>AFD-19 5 mg/kg</td>
<td>7</td>
<td>125±22</td>
<td>100</td>
<td>71</td>
</tr>
</tbody>
</table>

Drugs and vehicles were administered 1 min prior to reperfusion (i.e., 5 min after onset of occlusion). There were no significant effects on reperfusion-induced arrhythmias. Abbreviations are the same as Table 1.

In the clinical situation, there are few opportunities to administer antiarrhythmic agents before the onset of myocardial infarction, although it has been recommended that lidocaine should be given as soon as a ventricular arrhythmia is documented after myocardial infarction (18). Many studies have confirmed the effectiveness of drugs in the treatment of arrhythmias in the coronary care unit; i.e., on the later stages of acute myocardial infarction, but there are few studies in the very early stages of acute myocardial infarction. There are several reasons for this.
The first reason is a high mortality rate in the first two hours after the onset of an acute coronary attack. The rate of mortality rapidly declines after then. The second reason is that acute myocardial infarction occurs in the large majority of patients outside of hospitals. Pantridge (19) reported that only 16% of patients reach the hospital within 4 hr of the onset of symptoms, a figure that has not been greatly improved upon over the succeeding 20 years.

Our results indicate that class I antiarrhythmic agents administered after coronary occlusion are less effective against early stage arrhythmias of myocardial infarction than those administered prior to occlusion in anesthetized rats. Moreover, the administration of drugs after occlusion lowered the blood pressure, which might result in a decrease in myocardial perfusion. They did not modify reperfusion-induced arrhythmias if given just prior to the onset of reperfusion. However, as described before, AFD-19 is an active metabolite of NS-2 and has different sodium channel blocking properties from those of NS-2. Therefore, NS-2 given orally before the onset of acute myocardial infarction is expected to be more effective. The combination therapy of different types of class I antiarrhythmic agents has already been reported to have a beneficial effect in a clinical study (20).

REFERENCES