Effects of S-1389 (711389-S), a New Antiarrhythmic Agent, on the Conduction in Perfused Guinea Pig Hearts

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Abstract—In the His bundle and ventricular electrograms of Langendorff-perfused guinea pig hearts driven at a cycle length of 450 or 700 msec, S-1389 (711389-S), a new antiarrhythmic agent, above $3 \times 10^{-7}$ or $10^{-6}$ M increased the basal conduction times in the following order: His-Purkinje system $>$ ventricular and atrial muscles $>$ atrioventricular (AV) node. Slowing of the ventricular and AV nodal conduction of extrasystoles with variable coupling intervals was also caused by S-1389. S-1389 above $10^{-6}$ or $3 \times 10^{-6}$ M significantly prolonged the functional and/or effective refractory periods of the AV node and ventricle. Disopyramide ($3 \times 10^{-6}$–$3 \times 10^{-5}$ M) also produced similar effects, but they were much less potent than those of S-1389. Although disopyramide did not produce the rate-dependent increases in the atrial and AV nodal conduction times and in the AV nodal refractory period, S-1389 increased these parameters rate-dependently.

S-1389 (711389-S), 1-[2-[(3-isopropylamino-2-hydroxypropoxy)-3,6-dichlorophenyl]vinyl]-1H-imidazole hydrochloride, is a new antiarrhythmic agent, which shows potent antiarrhythmic effects on several kinds of arrhythmias in experimental animals caused by aconitine, ouabain, epinephrine and coronary occlusion (1, 2). S-1389 also can increase the fibrillation threshold in Langendorff-perfused guinea pig hearts (3). These findings and the weak anticholinergic activity with S-1389 (4) suggest a high therapeutic potential for this drug in the treatment of various cardiac arrhythmias without urinary retention.

Electrophysiological studies demonstrated that S-1389 decreased the maximum upstroke velocity ($V_{\text{max}}$) of the action potential without changing the resting potential in isolated guinea pig papillary muscle (4), and it was classified as a class I antiarrhythmic. In crayfish giant axons, Muramatsu et al. (5) showed that selective inhibition of the sodium current with S-1389 was more clearly observed by the internal application as compared to the external one. They also suggested that S-1389 binding occurred in the resting, inactivating and open states of the sodium channels. In isolated rabbit sinoatrial node cells, Kotake et al. (6) showed that S-1389 dose-dependently depressed the slow inward current, the potassium outward current and the hyperpolarization-activated current. However, the effects of S-1389 on the cardiac conduction system have not been investigated yet.

The present study was done to examine the effects of S-1389 on the conduction and refractoriness in Langendorff-perfused guinea pig hearts driven at two different cycle lengths. These effects of S-1389 were compared with those of disopyramide.

Materials and Methods

1. Recording of His bundle electrogram: Guinea pigs weighing 0.6 to 1.0 kg were injected i.p. with heparin sulphate (500 units) about 30 min before use. The heart, quickly removed from a guinea pig which had been killed by a blow on the head, was placed in cold oxygenated high $K^+$ (27 mEq) Krebs-Ringer solution containing heparin sulphate (500 units/liter). A cannula was inserted into the aorta, and Krebs-Ringer solution equili-
brated with 95% O₂ and 5% CO₂ at 30°C was perfused at a rate of about 5 ml/min. The region of the sinoatrial node was removed. The His bundle electrogram was then recorded using epoxy-coated bipolar silver wires, with tips 1.0 mm apart, which was introduced from an opening in the right atrium and placed on the area of the His bundle. A stimulating bipolar silver electrode was attached to the right atrium near the pacemaker area. The preparation was driven at two different basic cycle lengths (CL) of 450 and 700 msec with square wave pulses of two times the threshold and of 2-msec duration. To examine the rate-dependent effect of drugs. CL was changed from 700 to 450 msec or vice versa from 450 to 700 msec, and more than 3 min was allowed for a steady state to be reached. The His bundle electrogram was amplified by a preamplifier (AB-621G, Nihon Kohden) and recorded on a Tektronix 5113 dual beam storage oscilloscope and a TEAC DR-F1 digital recorder. Atrial (SA), AV nodal (SH) and His-Purkinje system (HV) conduction times were measured from the His bundle electrogram: SA interval, from the stimulus artifact to the earliest onset of atrial excitation; SH interval, from stimulus artifact to the His signal; HV interval, from His signal to the earliest onset of ventricular excitation. The SA interval was short and its change was small throughout the experiments; therefore, a change of the SH interval represented mainly a change in AV nodal conduction time.

2. Recording of ventricular electrogram:
The right atrium was dissected from the Langendorf-perfused guinea pig heart which had been prepared as described above. The stimulating bipolar silver electrode was attached to the posterior surface of the left ventricle. A bipolar recording electrode was attached to the surface of the free wall of the left ventricle about 1 cm from the stimulating electrode. The ventricle was stimulated, and the ventricular electrogram was recorded as described on the previous section. Ventricular conduction time (SV) was measured on the ventricular electrogram and is from the stimulus artifact to earliest onset of ventricular excitation.

3. Measurements of refractory periods in AV node and ventricular muscles: To examine the refractory period in the AV node or ventricular muscles, premature stimuli (S₂) were applied via the stimulating electrode on the right atrium or left ventricle at variable coupling intervals after every 9th or 15th basic stimulus (S₁) with CL of 700 msec or 450 msec. The effective refractory period of the AV node (ERPₐₙ) or ventricle (ERPᵥ) is the longest S₁-S₂ interval at which S₂ fails to conduct to the His bundle or to the ventricular muscles near the recording electrode. The conduction of extrasystoles to the His bundle (H₂) and ventricular muscle (V₂) was determined from the His bundle electrogram and ventricular electrogram, respectively. The functional refractory period of the AV node (FRPₐₙ) is the minimum interval between two His signals (H₁-H₂).

4. Ringer solution and compounds: Krebs-Ringer solution contained 120.3 mM NaCl, 4.0 mM KCl, 1.2 mM CaCl₂, 1.3 mM MgSO₄, 1.2 mM NaH₂PO₄, 24.2 mM NaHCO₃ and 5.5 mM glucose (pH 7.4). Drugs employed were S-1389 (Shionogi) and disopyramide (Nippon Roussel). S-1389 was dissolved in distilled water, and disopyramide was dissolved in ethanol and then diluted with distilled water. In all experiments, drugs were cumulatively added to the perfusate, and the preparations were exposed to each concentration of drug for 25–35 min.

5. Statistical analysis: Statistical analysis was performed using Student’s unpaired t-test, and the significant difference was established at P<0.05.

Results

1. Effects on basal conduction times through atrium, AV node and His-Purkinje system: Control values for basal conduction times through the atrium (SA), AV node (SH) and His-Purkinje system (HV) measured from the His bundle electrogram at the two different stimulating frequencies (basic CL of 450 and 700 msec) are summarized in Table 1. These values were determined from the initial conditioning periods in twelve preparations in which the effects of S-1389 and disopyramide were tested. The basal SH interval at 450 msec CL was significantly longer than that at 700 msec CL. Similar rate-
Table 1. Basal conduction times and refractory periods in Langendorff-perfused guinea pig hearts driven by two different cycle lengths

<table>
<thead>
<tr>
<th>Cycle length</th>
<th>700 msec</th>
<th>450 msec</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>11</td>
<td>14±1</td>
</tr>
<tr>
<td>SH</td>
<td>12</td>
<td>72±2</td>
</tr>
<tr>
<td>HV</td>
<td>12</td>
<td>31±1</td>
</tr>
<tr>
<td>SV</td>
<td>10</td>
<td>34±1</td>
</tr>
<tr>
<td>ERP_AV</td>
<td>12</td>
<td>235±6</td>
</tr>
<tr>
<td>FRP_AV</td>
<td>12</td>
<td>269±7</td>
</tr>
<tr>
<td>ERP_V</td>
<td>10</td>
<td>302±7</td>
</tr>
</tbody>
</table>

Values are means±S.E. SA, SH, HV and SV represent the conduction times (msec) through the atrium, atrio-ventricular node, His-Purkinje system and ventricle, respectively. ERP_AV and FRP_AV represent the effective and functional refractory periods of the atrio-ventricular node. ERP_V: Effective refractory period of ventricular muscles. *Significantly different from the value at the cycle length of 700 msec at P<0.05.

Fig. 1. The records of His bundle electrograms obtained before (control) and 25 min after application of 10^{-6} M S-1389 in Langendorff-perfused guinea pig hearts driven by cycle length of 700 msec. S: stimulus artifact, A: atrial deflection, H: His bundle deflection, V: ventricular deflection.

dependent prolongation in AV nodal conduction time has been well-documented (7, 8). No significant differences with SA and HV intervals were seen between the two CL.

Figure 1 shows the typical records of His bundle electrograms obtained before (control) and after application of 10^{-6} M S-1389 at the basic CL of 700 msec. Figure 2 shows the percentage changes in each conduction time induced by different concentrations of S-1389 or disopyramide determined for six preparations each.

S-1389 (above 3\times10^{-7} or 3\times10^{-6} M) produced dose-dependent and significant increases in SA, SH and HV intervals, and the changes were more marked at the higher stimulating frequency. The increase in conduction time by S-1389 was most prominent in the HV interval, and the changes decreased in the order of HV>SA>SH intervals.

Disopyramide also increased SA, SH and HV intervals, but these effects were very weak compared with those induced by S-1389. The increase in the conduction time by disopyramide was more marked for the HV interval compared with the SA and SH intervals, similar to the case for S-1389. A slight rate-dependent effect was observed with the HV interval, but not with the SA and SH intervals. The SH interval tended to increase as CL was
Fig. 2. Effects of S-1389 (left) and disopyramide (right) on the basal conduction times through the atrium (SA), atrioventricular node (SH), and His-Purkinje system (HV) measured from the His bundle electrogram in Langendorff-perfused guinea pig hearts driven by different two cycle lengths. Values represent means±S.E. (n=4–7). *Significantly different from the initial value at P<0.05.

2. Effects on the refractory period of the AV node: Premature stimuli were applied from right atrium at variable coupling intervals to investigate the effects of S-1389 and disopyramide on the conduction of extrasystoles and on the refractory periods in the AV node. Experiments were done at the basic CLs of 450 and 700 msec. Control values of the ERP\textsubscript{AV} and FRP\textsubscript{AV} are summarized in Table 1, and no significant difference was observed in both between the two stimulating frequencies.

Figure 3 shows the curve relating $S_2$-$H_2$ interval as a function of $S_1$-$S_2$ interval that were obtained before and after application of different concentrations of S-1389 at the two basic CLs. At both CLs, S-1389 dose-dependently shifted the curves to the right and upwards, indicating prolonged ERP\textsubscript{AV} and slowed conduction of the extrasystoles through the AV node. In these experiments, the minimum $H_1$-$H_2$ interval was also increased by S-1389 in a dose-related manner, indicating prolonged FRP\textsubscript{AV}. The percentage changes in ERP\textsubscript{AV} and FRP\textsubscript{AV} by S-1389 and disopyramide determined in each of six preparations are shown in Figs. 4 and 5. At both stimulating frequencies, dose-dependent increases in ERP\textsubscript{AV} and FRP\textsubscript{AV} were caused by S-1389, and the changes became significant.
Fig. 5. Effects of S-1389 and disopyramide on the functional refractory period of atrioventricular node (FRPAV) in Langendorff-perfused guinea pig hearts driven by two different cycle lengths. Values are means±S.E. of six preparations for each drug. *Significantly different from the initial value at P<0.05.

Fig. 6. Effects of S-1389 and disopyramide on the basal conduction time through ventricular muscles (SV) measured from the ventricular electrogram in Langendorff-perfused guinea pig hearts driven by two different cycle lengths. Values are means±S.E. of five preparations for each drug. *Significantly different from the control at P<0.05.

at 3×10^{-6} M. These changes were somewhat remarkable at the higher stimulation frequency. Disopyramide also increased both ERP_{AV} and FRP_{AV} in a dose-related manner, but these actions of disopyramide were less potent than those of S-1389. In comparison with S-1389, the changes in ERP_{AV} and FRP_{AV} induced by disopyramide were greater at the lower stimulation frequency.

3. Effect on basal conduction time through ventricular muscles: The effects of S-1389 and disopyramide on the basal conduction time through ventricular muscles were examined on the ventricular electrogram in each of five preparations driven at a CL of 450 or 700 msec. The control value of the basal ventricular conduction time (SV) is summarized in Table 1. No significant difference was observed between the two stimulating frequencies. The averaged percent change in the SV interval induced by each drug concentration is illustrated in Fig. 6. S-1389 above 10^{-6} M as well as disopyramide above 10^{-5} M caused a dose-dependent increase in the SV interval. The relative potency of S-1389 to disopyramide was more than ten times greater. With both drugs, the changes were somewhat greater at the higher stimulation frequency.

4. Effect on refractory period of ventricular muscles: The control value of ERP_{V} measured at the two different CLs are summarized in Table 1. ERP_{V} at 450 msec of CL was significantly shorter than that at 700 msec. This rate-induced shortening of ERP_{V} should be mainly due to the rate-induced shortening of APD in ventricular muscles (9), which in our experiments was estimated from the shift of the small deflection in the ventricular electrogram that corresponded to the repolarizing phase of the action potential.

The changes of the curves relating S_{2}-V_{2} as a function of S_{1}-S_{2} induced by S-1389 are shown in Fig. 7. S-1389 caused a dose-dependent shift of the curve to the right and upwards, which indicates prolonged ERP_{V} and slowed conduction of the extrasystoles through ventricular muscles. Figure 8 shows the averaged percentage changes of ERP_{V} induced by S-1389 and disopyramide in five experiments. Both drugs prolonged ERP_{V} in a dose-related manner, and these effects were slightly greater at the higher stimulating frequency. The relative potency of S-1389 to disopyramide in the prolongation effect on ERP_{V} was about ten times greater.
Discussion

In Langendorff-perfused guinea pig hearts, S-1389 above $3 \times 10^{-7}$ or $10^{-6}$ M caused significant slowing of basal conduction times through the atrium, His-Purkinje system and ventricle, as reflected by the increases of SA, HV and SV intervals. The excitation and conduction in these cardiac muscles are mediated by the fast sodium channels (10). In our previous (4) studies, we confirmed that S-1389 above $10^{-6}$ M inhibited the sodium channels in ventricular muscles by the decrease in the $V_{\text{max}}$ of their action potentials. These actions with S-1389 are enough to explain the slowing effects on the conduction times through the ventricle. The slowings of the His-Purkinje system and atrial conduction times are likely to be explained by similar actions.

S-1389 also produced slowing of the AV nodal conduction time, as reflected by an increase in the SH interval, although its relative increase was less than those in SA, HV and SV intervals. The effect of S-1389 on AV nodal action potentials has not been studied yet. However, Kotake et al. (6) demonstrated that a relatively high concentration of S-1389 (above $4 \times 10^{-6}$ M) decreased the $V_{\text{max}}$ and amplitude of the action potential in isolated rabbit sinus node cells. Since the electrical activity of the AV node as well as the sinus node is mediated by the slow calcium channels (10–12), the drug-induced slowing of AV nodal conduction is most likely explained by its inhibitory action on the slow calcium channels in the AV node.

The increases of SA, SH and HV intervals on the His bundle electrogram and SV intervals on the ventricular electrogram were also observed after application of disopyramide. A relative increase in SH interval was less than those in SA, HV and SV intervals, similar to the results with S-1389. These effects of disopyramide, which were very weak compared with those induced by S-1389, can also be caused by its relatively lesser inhibitory actions on the sodium or calcium channels (4, 13–15).

As can be seen in the plot of $S_2-V_2$ vs. $S_1-S_2$ (Fig. 7), S-1389 induced the slowing of the ventricular conduction of extrasystoles at all coupling intervals. This mode of action was similar to those of class Ia and Ic antiarrhythmics such as disopyramide (16) and SUN 1165 (17), but differed from that of class Ib antiarrhythmics such as lidocaine and...
tocainide (16). These class lb antiarrhythmics caused the slowing of only mid-range (250–400 msec) extrasystoles. S-1389 also caused slowing of the conduction of extrasystoles through the AV node at all coupling intervals (Fig. 3). This mode of action was similar to those of disopyramide (data not shown) and encainide (18).

In the present study, S-1389 was demonstrated to prolong the refractory periods of the AV node and ventricular muscles. Relative prolongation by S-1389 on ERPv was marked in comparison with ERP_{AV} and FRP_{AV}. In addition to the inhibitory actions on V_{max}, S-1389 increased the duration of the action potentials in both isolated ventricular muscles (4) and sinus node cells (6). These actions of S-1389 on the fast and slow action potentials might explain its prolongation of refractoriness in ventricular muscles and the AV node. Disopyramide also prolonged ERP_{AV}, FRP_{AV} and ERPv; and these actions were less potent than those of S-1389.

Rate-dependent effects on the slowing of the conduction and prolongation of refractoriness in various cardiac muscles were observed with S-1389, but were not very marked within the range of frequencies tested. The inhibitory action of S-1389 on the V_{max} of the ventricular action potential was rate- and voltage-dependent, and these findings may support the rate-dependent drug effects on the conduction and refractoriness in ventricular muscles. Unlike S-1389, disopyramide did not produce the rate-dependent increases of atrial and AV nodal conduction times and of AV nodal refractory periods. With in vivo findings, Birkhead and Vaughan Williams (19) indicated that disopyramide prolonged AV nodal conduction time and increased AV nodal and atrial refractory periods under cholinergic blockade, and they suggested that these effects might often be masked by its anticholinergic action, especially under high vagal tone. This is one possible explanation for the lack of rate dependency on disopyramide: the direct ionic actions of disopyramide at the higher stimulating frequency were more masked by its potent anticholinergic activity. Some support comes for this explanation as we observed a rate-dependent increase of conduction times and refractory period in the His-Purkinje system and ventricular muscles, which were not innervated by the parasympathetic nervous system.

In our previous report (4), S-1389 was classified as a class Ia antiarrhythmic based on the characteristics of its V_{max} inhibition in isolated guinea pig ventricular muscles. The class I antiarrhythmic agents were recently subclassified into three groups (a, b and c) according to the speed of onset and/or recovery kinetics of V_{max} inhibition (20–22). The rate constant on V_{max} inhibition with S-1389 (4) was similar to those with class Ia antiarrhythmics such as disopyramide (20), but the recovery time constant on V_{max} inhibition was rather similar to those with class lc antiarrhythmics such as flecainide, encainide and SUN 1165 (23, 24). In the present study, S-1389 has been shown to potently slow the conduction time through the His-Purkinje system as well as ventricular muscles. In addition to these effects, S-1389 also shortened the action potential of the Purkinje fibers (M. Ninomiya et al., unpublished data). These findings suggest that it may be better to group S-1389 as a class lc antiarrhythmic instead of a class Ia one.

In the present study, it was shown that S-1389 slowed the cardiac conduction and prolonged the refractory period in the ventricle and AV node. These effects with S-1389 were qualitatively similar to those with disopyramide; however, different rate-dependent effects were observed between S-1389 and disopyramide in the atrial and AV nodal conduction times and in the AV nodal refractory period.

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