Comparison of Cardiovascular Effects of Pirmenol with Those of Disopyramide in Isolated Canine Heart Preparations Cross-Circulated with a Donor Dog

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Abstract—To assess the cardiovascular profiles of pirmenol, a new antiarrhythmic drug, and to compare them with those of disopyramide, isolated canine sinoatrial node, papillary muscle and atrioventricular node preparations cross-circulated with a donor dog were used. Pirmenol injected intraarterially into the isolated preparations showed negative chronotropic and inotropic effects, which were comparable to those of disopyramide; and it also showed coronary vasodilator and negative dromotropic effects on atrio-His as well as His-ventricular conduction, which were significantly more potent than those of disopyramide. Similarly, pirmenol administered intravenously into the donor dog showed more potent negative dromotropic effects on the PQ interval and QRS width than disopyramide, while in the isolated preparations cross-circulated by the donor dog, pirmenol and disopyramide showed equipotent cardiodepressant effects. In the same preparation, pirmenol decreased coronary blood flow following a transient increase, while disopyramide only decreased coronary blood flow. Since the antiarrhythmic action of class I drugs is considered to result from inhibition of the fast inward current, which generates and propagates action potentials and also induces ventricular automaticity, our results suggest that pirmenol possesses an electrophysiological effect typical to an efficacious class I agent such as disopyramide.

Pirmenol (CI-845, (±)-cis-[3-(2,6-dimethyl-1-piperidinyl)propyl-alpha-phenyl-2-pyridinemethanol monohydrochloride monohydrate]) is a new synthetic antiarrhythmic agent and bears some chemical resemblance to disopyramide (Fig. 1). Among its electrophysiological and cardiovascular effects, it has been reported that it decreases the maximum rate of depolarization and action potential amplitude in canine Purkinje fibers, similar to disopyramide (1, 2), and increased the mean arterial pressure of pithed rats (1). It was also reported to be effective in a variety of experimental (3–5) and clinical arrhythmias (6–10). Since arrhythmia occurs often in diseased hearts whose cardiovascular performance is seriously impaired, the assessment of the cardiohemodynamic effect of antiarrhythmic drugs is of equal importance for the assessment of their effectiveness.

The purpose of the present study was to systematically assess the cardiovascular profiles of pirmenol in comparison with disopyramide. For this purpose, we used for the first time isolated canine sinoatrial (SA) node, papillary muscle (PM) and atrioventricular (AV) node preparations cross-circulated with a donor dog, which permit precise measurement of drug effects under both in vivo and in vitro conditions (11–13). Direct cardiac effects of drugs were examined by i.a. injections of the drugs into the nutrient artery of each preparation. Also, both the cardiohemodynamic effects in the donor dog and the direct cardiac effects in the isolated heart preparations could be simultaneously ex-
Materials and Methods

Experiments were carried out using the canine isolated SA node, PM and AV node preparations cross-circulated with heparinized arterial blood of the donor dog.

1. The isolated in vitro preparations: The hearts were obtained from mongrel dogs of either sex, weighing approximately 10 kg, which were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and given calcium heparin (500 U/kg, i.v.). They were excised after exsanguination and plunged into cold Tyrode's solution kept at about 4°C.

The SA node preparation consisted of the entire right atrium. The sinus node artery was cannulated through the right coronary artery. The spontaneous beating rate, i.e., sinoatrial rate (SAR), was measured with a cardiotachograph (San-ei Instruments, 1321) triggered by bipolar atrial electrograms sutured on the atrial epicardium close to the sinus node.

The PM preparation consisted of the anterior papillary muscle of the right ventricle attached to the interventricular septum. The anterior septal artery was directly cannulated. The PM preparation was electrically driven at a fixed rate of 120 beats/min by a stimulator (Dia Medical, DHM-226-3) and an isolation unit (Dia Medical, DPS-110) with rectangular pulses of 1–3 V (about 20% above the threshold voltage) at 5 msec duration through bipolar stimulating electrodes sutured onto the base of the papillary muscle. The developed tension (DT) of the papillary muscle preloaded with a 2 g weight was measured isometrically using a force displacement transducer (Dia Medical, DRM-1005).

The AV node preparation consisted of both the right atrium and interventricular septum. The right coronary and the anterior septal arteries were directly cannulated, while the posterior septal artery was cannulated through the left circumflex artery. The AV node preparation was electrically driven at a fixed rate of 150 beats/min by a stimulator (Dia Medical, DHM-226-3) and an isolation unit (Dia Medical, DPS-110) with rectangular pulses of 1–3 V (about 20% above the threshold voltage) at 5 msec duration through bipolar stimulating electrodes sutured onto the crista terminalis of the right atrium. Bipolar electrograms were recorded from the right atrium (A), His bundle (H) and the base of the anterior papillary muscle (V). These electrograms were fed to an automatic interval meter (Dia Medical, DHM-226-1), which measures AH and HV intervals individually with an analysis pitch of 1 msec. Since the posterior septal artery mainly supplies the AV node area, and the anterior septal artery mainly supplies the His-Purkinje-ventricle system, drugs selectively injected into the posterior septal artery affect the AH interval, while drugs injected into the anterior septal artery predominantly affect the HV interval. However, drugs injected into the anterior septal artery occasionally altered the AH interval in addition to the HV interval, probably due to anastomosis which exists between the anterior and posterior septal artery.

2. Donor dogs: Adult mongrel dogs of either sex, weighing 14–23 kg, were used as donor dogs. Dogs were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and supplemented with 4–5 mg/kg every hour. At the start of cross-circulation, calcium heparin (500 U/kg, i.v.) was given and 200 U/kg was supplemented every hour. Respiration was controlled using a dog respirator.
The heart rate (HR), mean arterial pressure (mAP) at the femoral artery, and lead II ECG were continuously monitored with a polygraph (San-ei Instruments, 361-6). The PQ interval and QRS width of the donor dog heart were directly measured from the lead II ECG recorded at a paper speed of 100 mm/sec every 5 min and when necessary.

3. Cross-circulation: Preparations were placed in a double-wall glass jacket maintained at 38°C by circulating warm water, and were cross-circulated through each cannulated artery with heparinized blood pumped from the carotid artery of the donor dog. The SA node and PM preparations were obtained from the same animal, while the AV node preparation was obtained from different animals. Perfusion pressure was kept at 100 mmHg with a peristaltic pump (Cole-Parmer, 7553-00) and a Starling pneumatic resistance placed parallel to the perfusion circuit. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the donor dog through the jugular vein. The rate of coronary blood flow (CBF) through each nutrient artery was continuously measured with an electromagnetic flowmeter (Nihon-Kohden, MVF-1100).

4. Direct cardiac effects in the in vitro preparations: Pirmenol (3 to 1000 µg) or disopyramide (3 to 1000 µg) was injected into each nutrient artery of the preparations over a 4-sec period with a microsyringe (Terumo). Maximal changes in each parameter were measured and expressed as a percent of their basal values before injection, and the dose-response curves for the chronotropic, inotropic, dromotropic and vasodilator effects were drawn.

5. Cardiohemodynamic effects of the donor dogs: Pirmenol (3 mg/kg) or disopyramide (3 mg/kg) in an estimated canine antiarhythmic dose (5, 14) was administered to the donor dog through the jugular vein for a period of 30 sec. Each drug caused cardiohemodynamic effects in the donor dog and also produced cardiac effects in the in vitro preparations after a lag time of 1 to 2 min. The time course of their effects on the HR, mAP, PQ and QRS of the donor dog; the SAR of the SA node preparation; the DT and CBF of the PM preparation; and the AH and HV intervals and CBF through the anterior septal artery of the AV node preparation were simultaneously recorded.

To estimate the plasma concentration of pirmenol, arterial blood was drawn from an interposed shunt in the circuit distal to the pneumatic resistance, before and at 1, 3, 5, 10, 15, 30 and 60 min after i.v. administrations. A sensitive and specific determination of pirmenol in the plasma was performed at the Nomura Research Institute of Life Science (Kanagawa, Japan) using a high performance liquid chromatographic method, which was fundamentally similar to that of Johnson and Pachla (15). The limit of quantification was 10 ng/ml.

6. Drugs and statistics: Drugs used were pirmenol hydrochloride (Warner-Lambert K.K., Tokyo, Japan) and disopyramide phosphate (Roussel through Chugai K.K., Tokyo, Japan). The doses of drugs causing a 15% decrease in SAR, ED15 (SAR); those causing a 50% decrease in DT, ED50 (DT); those causing a 50% increase in CBF, ED50 (CBF); those causing a 50% increase in AH interval, ED50 (AH); and those causing a 50% increase in HV interval, ED50 (HV), were calculated for each experiment using the least squares method. The data were presented as the mean±S.E.M., and the statistical comparisons of mean values were evaluated by a paired t-test or unpaired t-test, and P values less than 0.05 were considered significant.

Results

1. Effects of pirmenol or disopyramide injected directly into the in vitro preparations

The SA node preparation showed spontaneous regular automaticity. When pirmenol or disopyramide was injected into the rubber tube connecting the right coronary artery, SAR decreased dose-relatedly. A typical experiment of pirmenol is shown in Fig. 2A. Dose-response curves for the negative chronotropic effects of pirmenol and disopyramide are shown in Fig. 2B. At 1 mg, sinus arrest was induced in 2 out of 7 preparations by pirmenol and 2 out of 9 by disopyramide. There was no significant difference between the ED15 (SAR) values of pirmenol and disopyramide (Table 1).
When pirmenol or disopyramide was injected into the anterior septal artery of the PM preparation, DT decreased and CBF increased, dose-relatedly. A typical experiment of pirmenol is shown in Fig. 3A. Dose-response curves for the negative inotropic effect and for the coronary vasodilator effect of pirmenol and disopyramide are shown in Fig. 3, B and C.

Table 1. Comparison of negative chronotropic, negative inotropic, coronary vasodilator, and negative dromotropic effects of pirmenol and disopyramide in the isolated canine blood-perfused sinoatrial (SA) node, papillary muscle (PM) and atrioventricular (AV) node preparations

<table>
<thead>
<tr>
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<th>Pirmenol</th>
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<th>Disopyramide</th>
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<tbody>
<tr>
<td>ED15 (SAR)</td>
<td>689±164</td>
<td>(7)</td>
<td>454±57</td>
<td>(9)</td>
</tr>
<tr>
<td>ED50 (DT)</td>
<td>922±108</td>
<td>(10)</td>
<td>800±87</td>
<td>(9)</td>
</tr>
<tr>
<td>ED50 (CBF)</td>
<td>504±111*</td>
<td>(10)</td>
<td>833±96</td>
<td>(9)</td>
</tr>
<tr>
<td>ED50 (AH)</td>
<td>234±30**</td>
<td>(8)</td>
<td>1410±479</td>
<td>(6)</td>
</tr>
<tr>
<td>ED50 (HV)</td>
<td>416±62**</td>
<td>(5)</td>
<td>1154±204</td>
<td>(5)</td>
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ED15 (SAR): The dose (µg) that caused a 15% decrease in the sinoatrial rate (SAR) of the SA node preparation. ED50 (DT): The dose (µg) that caused a 50% decrease in the developed tension of the PM preparation. ED50 (CBF): The dose (µg) that caused a 50% increase in the blood flow through the anterior septal artery of the PM preparation. ED50 (AH): The dose (µg) that caused a 50% increase in the AH interval of the AV node preparation. ED50 (HV): The dose (µg) that caused a 50% increase in the HV interval of the AV node preparation. Asterisks next to the values for pirmenol represent significant differences from the respective values of disopyramide: *P<0.05, **P<0.01.
Fig. 3. Negative inotropic and coronary vasodilator effects of pirmenol and disopyramide in the papillary muscle (PM) preparation. The basal developed tension (DT) was 6.3±0.5 g (n=19), and the basal coronary blood flow (CBF) through the anterior septal artery was 6.2±0.6 ml/min (n=19). (A) Original tracings of the inotropic (upper) and coronary vasodilator effects (lower) of pirmenol. (B) Dose-response curves for the negative inotropic effects. (C) Dose-response curves for the coronary vasodilator effects. Asterisks represent significant changes from each basal value. *P<0.05, **P<0.01.

There was no significant difference between the ED50 (DT) values of pirmenol and disopyramide (Table 1). The ED50 (CBF) of pirmenol was significantly less than that of disopyramide.

When pirmenol or disopyramide was injected into the posterior septal artery of the AV node preparation, the AH interval increased dose-relatedly, while the HV interval was little affected. A typical experiment of pirmenol is shown in Fig. 4A. Dose-response curves for the negative dromotropic effects of pirmenol and disopyramide on the AH interval are shown in Fig. 5A. The ED50 (AH) of pirmenol was significantly less than that of disopyramide.

2. Effects of intravenous pirmenol and disopyramide administered into the donor dogs

Effects of pirmenol on the donor dog: All the parameters of the donor dog changed within 30 sec after i.v. administration of 3 mg/kg of pirmenol. HR rapidly decreased for 30 sec to 3 min, followed by a transient recovery toward the basal level for 3 to 5 min, then progressively decreased again, reaching a
peak response (88% of the basal value) at 40 min, and recovered toward the basal level (Fig. 6A). MAP initially decreased for 30 sec to 1 min, followed by an increase toward the basal level for 1 to 3 min, then slowly decreased again, reaching its peak decrease (84% of the basal value) at 30 min, and gradually increased toward the basal level (Fig. 6B). PQ and QRS rapidly increased, reaching their peaks (124 and 119% of the basal values) at 1 min, and then gradually returned to the basal level (Fig. 7, A and B). The time course of the plasma concentration of pirmenol after i.v. administration is shown in Fig. 8. The plasma concentration curve of pirmenol fitted well with that predicted by the
Effects of disopyramide on the donor dog:
Similar to pirmenol, all the parameters of the donor dog changed within 30 sec after i.v. administration of 3 mg/kg of disopyramide. HR rapidly decreased, reaching a peak (83% of the basal value) at 5 min, and then gradually returned to the basal level (Fig. 6A). MAP increased, reaching a peak (105% of the basal value) at 10 min and then gradually returned to the basal level (Fig. 6B). PQ and QRS rapidly increased, reaching their peaks (119 and 111% of the basal values) at 3 min and then gradually returned to the basal level (Fig. 7, A and B).

Mean HR after i.v. pirmenol was significantly higher than that after disopyramide at 1, 3, 5 and 10 min (Fig. 6A). Mean mAP after i.v. pirmenol was significantly lower than that after disopyramide at 10 to 60 min (Fig. 6B). Mean PQ interval after i.v. pirmenol was significantly longer than that after disopyramide at 1 min (Fig. 7A). Mean QRS width after i.v. pirmenol was significantly longer than that after disopyramide at 1, 3, 10 and 20 min (Fig. 7B).

Effects of pirmenol given into the donor dog on the in vitro preparations: After i.v. administration of pirmenol, 3 mg/kg, SAR started to decrease within 1 to 2 min after injection, reaching a peak (86% of the basal value) at 15 min, and then gradually returned to the basal level (Fig. 9A). As judged by the dose-response curve of Fig. 2B, this percent
Fig. 6. Time courses of effects of 3 mg/kg of pirmenol or disopyramide administered intravenously to the donor dog on the heart rate (HR) (A) and mean arterial pressure (mAP) (B) of the donor dog. The basal HR before pirmenol was 121±6 beats/min and that before disopyramide was 106±6 beats/min. The basal mAP before pirmenol was 97±7 mmHg and that before disopyramide was 99±6 mmHg. Closed circles of pirmenol represent significant differences from the values of disopyramide observed at the same time after injection (P<0.05). Asterisks represent significant changes from the 0 time values: *P<0.05, **P<0.01.

decrease was the same as that produced by about 300 μg of pirmenol directly injected into the preparation. DT also decreased, reaching a peak (72% of the basal value) at 5 min, and then gradually returned to the basal level (Fig. 9B). As judged by the dose-response curve of Fig. 3B, this percent decrease was the same as that produced by about 300 μg of pirmenol directly injected into the preparation. CBF transiently increased, reaching a peak (116% of the basal value) at 3 min, then gradually decreased, reaching a peak decrease (85% of the basal value) at 30 min; and this decrease was maintained during the observation period (Fig. 9C). AH and HV intervals rapidly increased, reaching their maximum values (170% and 145% of the basal values) at 5 and 3 min, respectively, and then gradually returned to their respective basal levels (Fig. 10, A and B). As judged by the dose-response curves of Fig. 5, A and B, these percent increases were the same as those produced by about 270 μg and 500 μg of pirmenol directly injected into the preparation, respectively. A second degree AH conduction block occurred in 2 out of 4 preparations soon after the drug administration, but the second degree block
disappeared at 15 min, although the AH prolongation persisted.

Effects of disopyramide given into the donor dog on the in vitro preparations: SAR gradually decreased, reaching a peak (83% of the basal value) at 20 min, and then gradually returned to the basal level (Fig. 9A). As judged by the dose-response curve of Fig. 2B, this percent decrease was the same as that produced by about 400 μg of disopyramide directly injected into the preparation. DT gradually decreased, reaching the peak decrease (82% of the basal value) at 10 min and then gradually returned to the basal level (Fig. 9B). As judged by the dose-response curve of Fig. 3B, this percent decrease was the same as that produced by about 180 μg of disopyramide directly injected into the preparation. CBF through the anterior septal artery of the PM and AV node preparations gradually decreased, reaching a peak (77% of the basal value) at 40 min; and this decrease was maintained during the observation period (Fig. 9C). AH and HV rapidly increased, reaching their maximum (142% and 133% of the basal values) at 5 and 10 min, respectively, and then gradually returned to their respective basal levels (Fig. 10, A and B). As judged by the
dose-response curves of Fig. 5, A and B, these percent increases were the same as those produced by about 600 μg and 700 μg of disopyramide directly injected into the preparation, respectively. A second degree AH conduction block occurred in 1 of 4 preparations.

There was no significant difference between the mean SAR after i.v. pirmenol and disopyramide (Fig. 9A). There was also no significant difference between the mean DT after i.v. pirmenol and disopyramide (Fig. 9B). The values of mean CBF through the anterior septal artery at 1 and 3 min after i.v. pirmenol were significantly higher than those after i.v. disopyramide (Fig. 9C). The mean AH interval at 3 min after i.v. pirmenol was significantly longer than that after i.v. disopyramide (Fig. 10A). There was no significant difference between the mean HV intervals after i.v. pirmenol and disopyramide (Fig. 10B).

Discussion

The present study using the blood-perfused isolated preparations revealed effects of pirmenol and disopyramide in arterial blood and provided new information about their direct or indirect effects on cardiac properties, which has not been reported except for the data on disopyramide by Chiba and co-workers (16, 17) using the SA node preparation and those by Satoh et al. (13) using the AV node preparation. Pirmenol injected directly into the in vitro preparations showed negative chronotropic and inotropic effects, which were comparable to those of disopyramide. We did not find that pirmenol had a less potent negative inotropic effect as reported in the isolated rabbit atrium (1). Pirmenol also showed coronary vasodilator and negative dromotropic effects, increasing AH and HV intervals, which were significantly more potent than those of disopyramide. On the other hand, intravenous pirmenol administered into the donor dog showed a lower bradycardic effect but more potent negative dromotropic effects on PQ interval and QRS width than disopyramide. Pirmenol induced modest hypotension, while disopyramide increased the blood pressure. In the in vitro preparations cross-circulated by arterial blood of the donor dog given pirmenol or disopyramide, i.v., cardiodepressant effects similar to those observed by i.a. injection were observed except that pirmenol decreased CBF following a transient increase and disopyramide only decreased CBF.

Pirmenol injected into the right coronary artery decreased the SAR of the SA node preparation in a dose-dependent manner. Pirmenol i.v.-administered into the donor dog also caused bradycardia in the donor dog and decreased the SAR of the cross-circulated SA node preparation. These results are consistent with an earlier report that pirmenol caused bradycardia in isolated rabbit SA node preparations.

Fig. 8. Time course of the plasma concentration of pirmenol after i.v. administration. Each value was obtained from 6 experiments.
nodes and in pithed rats (1). Dukes et al. (1) indicated that the former effect caused by pirmenol was partially due to a slowing of repolarization from the peak of the sinus node potential to the maximum diastolic potential without significant change in the slope of the slow diastolic potential or maximum rate of depolarization. In the present study, there was a gradual decrease in the HR by pirmenol but a rapid one by disopyramide. As the direct negative chronotropic effects of the two drugs were almost the same, intravenous pirmenol might possess an indirect action to counteract its direct negative chronotropic effect.

Disopyramide slightly increased the blood pressure. This pressor effect of disopyramide must be due to the increase in the peripheral resistance as reported elsewhere (18), which appears to be one of the disadvantages of disopyramide. On the other hand, pirmenol caused modest decrease in blood pressure, although in the pithed rat, blood pressure was reported to be increased by pirmenol (1). As the direct negative inotropic effect of pirmenol was almost the same as that of disopyramide,
pirmenol must possess a lower vasoconstrictor effect on canine peripheral vasculature as compared to disopyramide. Whether this effect of pirmenol on the vasculature is favorable clinically or not must be determined by further studies.

It has been reported that almost all the class I antiarrhythmic agents transiently increased CBF when they were directly injected into the isolated canine blood-perfused heart preparation (13). In our study, i.e. pirmenol also transiently increased CBF, and this coronary vasodilator effect was more potent than that of disopyramide, as judged by the ED50 (CBF) values. Taking into account that hypoxia is one of the causes of arrhythmias, the increase in CBF may be a favorable effect in the treatment of arrhythmia in such cases. However, in contrast to the direct effect on the in vitro preparation, i.v.-administered pirmenol decreased the CBF of the in vitro preparation following a transient increase, and i.v. disopyramide only decreased CBF. Whether these different coronary effects of both drugs are clinically significant remains to be clarified.

In the AV node preparation, pirmenol prolonged the AH as well as the HV intervals. However, it has been reported that pirmenol showed no direct slowing of conduction within the AV node itself (1). Therefore, the delay might have occurred in the atrial to AV nodal sections, and in the nodal to His-Purkinje-ventricle sections, which could not be determined by our present method.

The antiarrhythmic action of class I drugs

Fig. 10. Time courses of effects of 3 mg/kg of pirmenol or disopyramide administered intravenously to the donor dog on the AH (A) and HV (B) intervals of the AV node preparation. The basal AH and HV intervals before pirmenol were 99±11 and 45±4 msec, and those before disopyramide were 105±1 msec and 36±2 msec. Closed circles of pirmenol represent significant difference from the values of disopyramide observed at the same time after injection (P<0.05). Asterisks represent significant changes from the 0 time values. *P<0.05, **P<0.01.
is considered to result from their membrane effects: namely an inhibition of the fast inward current, because we showed in the previous studies that the antiarrhythmic effect of class I drugs on canine coronary ligation and digitalis induced arrhythmias occurred at concentrations decreasing the Na current (5, 14). Since Na current is the most important depolarizing current responsible for the intraatrial and His-Purkinje-ventricle conduction, our results that the negative dromotropic effects of pirmenol were more potent than those of disopyramide suggest that pirmenol at least possesses an electrophysiologic profile, like the well-established class I agent disopyramide.

The plasma concentration curve of pirmenol fitted well with that predicted by the two compartment theory of pharmacokinetics in the present study and also in our previous report (5). The relationship between the plasma concentrations of pirmenol and cardiac effects in the donor dog and the isolated preparations seems to indicate that the absolute values of the plasma concentration are helpful in predicting direct depressant effects on the automaticity, contraction and conduction, but less helpful in predicting the effects on CBF, HR and mAP. If this is also true in clinical practice, therapeutic plasma concentration measurements will be helpful in predicting its direct cardiodepressant effects, especially when the cardiac function is impaired and the drug pharmacokinetics is abnormal.

In summary, pirmenol has negative chronotropic and inotropic effects, which are comparable to those of disopyramide, and more potent negative dromotropic effects than those of disopyramide, which may be related to the antiarrhythmic activity of both drugs. The results therefore support our previous report that pirmenol is a more potent antiarrhythmic drug on canine coronary ligation-induced arrhythmia than disopyramide (5) and also suggest that pirmenol may be an effective class I antiarrhythmic agent as has already been shown in studies using a limited number of patients (6–10).

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References


