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Effects of Cisapride and Protective Effects of Mexiletine on the QT Interval in Rats

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Summary. **This study examined the effects of cisapride, a 5-HT_4 receptor agonist, on the QT interval in rats, and evaluated whether mexiletine, a potent antiarrhythmic drug, could prevent a cisapride-induced prolongation of the QT interval. The effects of drugs on electrocardiograms in anesthetized normal Lewis and heart failure rats were evaluated. The effects of cisapride were investigated by administering three different dosages (8, 16 and 24 mg/kg) for the acute study and one dosage (24 mg/kg/day) for the chronic study (for 7 days) in control and ailing rats. After the oral administration of mexiletine at 30 mg/kg for 1 week, cisapride at 24 mg/kg was administered to the rats with heart failure (n=15 in each).**

After chronic cisapride treatment for 7 days, the QT intervals in control and ailing rats were prolonged from 68.0±1.6 (mean±s.e.m.) to 74.0±2.4 msec and from 77.6±1.9 to 88.8±2.3 msec, respectively ($p<0.05$ and $p<0.01$). A single dosage of cisapride at 8 or 16 mg/kg did not affect the QT interval in control rats. However, with a single dose of cisapride at 8, 16 and 24 mg/kg in rats with heart failure, the QT interval increased dose-dependently from 75.5±1.9 to 81.2 ±1.8 msec, 76.4±1.6 to 87.8±2.5 msec, and 78.4±2.1 to 101.4±1.2 msec, respectively (each, $p<0.01$). After the administration of mexiletine, cisapride had no such effects.

Cisapride prolonged the QT interval in rats with heart failure dose-dependently, while mexiletine prevented the effect. These results indicate that a high dose of cisapride should be used carefully during illness, but mexiletine may prevent a cisapride-induced long QT interval.

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Key words—cisapride, long QT interval, mexiletine, 5-HT_4 receptor agonist, heart failure.

INTRODUCTION

Cisapride, a 5-HT_4 receptor agonist, is a substituted benzamide compound that stimulates motor activity in all segments of the gastrointestinal tract by enhancing the release of acetylcholine from the enteric nervous system[1,2]. It has been applied toward various gastrointestinal ailments including gastroesophageal reflux, functional dyspepsia, gastroporesis, and chronic pseudobstruction syndrome[3,4]. However, cisapride is reported to prolong the QT interval, causing serious cardiac arrhythmia (torsades de pointes)[5,6], which is heightened when cisapride is used at high doses or with medications known to increase cisapride serum levels or cause prolonged QT intervals[7,8].

This study evaluates the effects of cisapride on the QT interval in rats with heart failure produced by autoimmune myocarditis (imitation trial for conditions of illness), and the protective effect of mexiletine on the cisapride-induced long QT interval.

MATERIALS AND METHODS

Experimental animals

Experiments were performed in accordance with institutional guidelines of the Niigata College of Pharmacy for animal use in research, and were

approved by the local ethics committee. Animals were housed and maintained in compliance with the Guide to the Care and Use of Experimental Animals of the college.

Nine-week-old male Lewis rats were obtained from Charles River Japan Inc. (Kanagawa, Japan).

Heart failure rats

Purified cardiac myosin from the ventricular muscle of pig hearts prepared according to a procedure described previously was dissolved at a concentration of 10 mg/ml in PBS (0.2 mol/l) containing 0.3 mol/l KCl and mixed with an equal volume of complete Freund's adjuvant supplemented with Mycobacterium tuberculosis H37Ra (Difco Laboratories, Michigan, USA.) at a concentration of 10 mg/ml[9-12]. The rats were injected in their footpads with 0.2 ml s.c. of antigen-adjuvant emulsion. The rats presenting heart failure were selected at 2 months after immunization.

Rats were divided into 9 groups of 15 rats each both in the control and ailing group. The QT interval (n=10 in each) and serum cisapride concentration (n=5 in each) were measured in each group.

Drug administrations

Cisapride or mexiletine were administered orally to both the control and ailing rats. Control rats received an oral vehicle (0.5% methylcellulose). We administered cisapride at three dosages (8, 16 and 24 mg/kg) for the acute study and the dosage at 24 mg/kg/day for 7 days in the chronic study. After the oral administration of mexiletine at 30 mg/kg for 7 days, cisapride at 24 mg/kg was administered to the rats with heart failure.

ECG and measurements

Rats were anesthetized by an intraperitoneal injection of a cocktail of ketamine (10 mg/kg) and thiobutabarbital (50 mg/kg), and placed in the supine position on a thermoregulated surgical table. They were then anesthetized with thiobutabarbital (5-30 mg/kg/hr) administered intravenously. After the anesthesia, the rats were rested for an equilibration period, and baseline measurements (0 min) of ECG were obtained. After the oral administration of drugs, ECG was recorded at 30, 60, 90, 120, 150, 180 and 240 min in the acute study. In the chronic study, ECG was recorded before and at 120 min after the cisapride administration given orally. ECGs were recorded for all rats (Softron, Co. L., Tokyo, Japan) at paper speeds of 200 mm/sec (Fig. 1). Heart rates and QT intervals were measured and averaged from three cardiac cycles. Heart rates from 300 beats/min to 350 beats/min were selected for measurement of the QT interval.

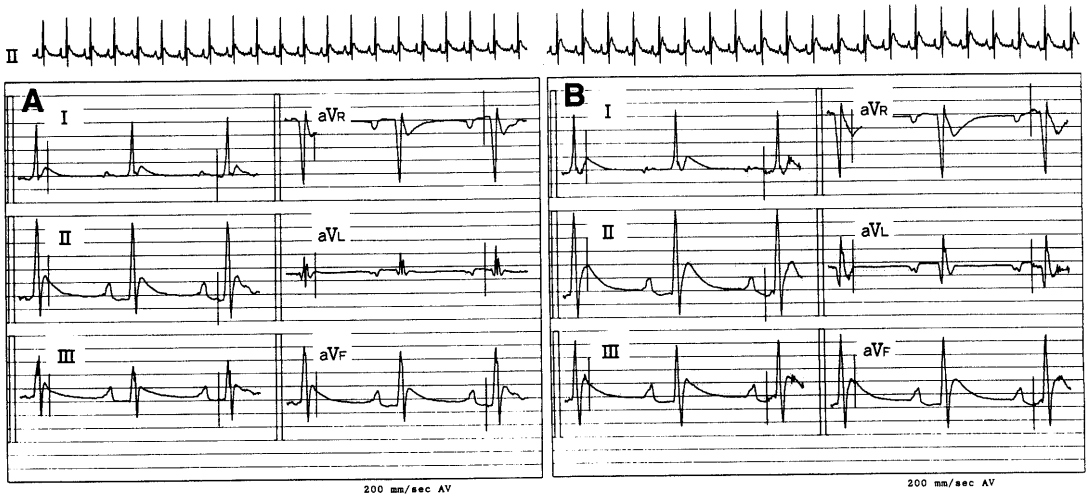

Fig. 1. Six lead (I , II, III, $_{a}V_{R}$, $_{a}V_{L}$ and $_{a}V_{F}$) electrocardiograms (ECGs).
ECG changes in response to a single administration of cisapride on rats wit heart failure. Cisapride markedly prolonged QT [(**A**) before, QT (msec) =75, HR (beats/min) =321 and QTc (msec) =173; (**B**) at 120 min after cisapride, QT=98, HR=313 and QTc=224].

Serum cisapride concentration

An improved high performance liquid chromatography was used for the determination of serum cisapride concentrations[13]. For measuring the serum cisapride concentration, 200 μl of blood was drawn at 30, 60, 90, 120, 180 and 240 min after the administration of cisapride in the acute study. In the chronic study, blood was drawn at 180 min after the oral administration.

Statistical analysis

Data were presented as the mean±s.e.m. Statistical analysis among the groups and their time course of QT interval were performed with the two-way ANOVA, followed by Tukey's method. The differences were considered significant at $p<0.05$.

RESULTS

Influence of chronic cisapride administration on serum cisapride levels and QT duration

In the cisapride-treated groups, the serum levels of cisapride at 120 min after administration in control and ailing rats were 149±22 and 181±25 ng/ml (mean±s.e.m), respectively. The baseline QT intervals of the control and ailing rats were 68.0±1.6 and 77.6±1.9 msec, whereas the QT intervals during cisapride administration were 74.0±2.4 and 88.8±2.3 msec, respectively. The two groups showed a significant prolongation ($p<0.05$ and $p<0.01$). The rats with heart failure had longer QT intervals than the control rats before and after cisapride administration (both, $p<0.01$).

Dose-dependent effects of cisapride on serum cisapride levels and QT intervals

The administration of cisapride increased the serum cisapride levels in all groups. The maximal serum cisapride level for the control Lewis rats at 8, 16 and 24 mg/kg was 37.2±4.0, 77.4±8.0, and 196.2±13.5, while for rats with heart failure it was 46.0±3.0, 114.0±10.4, and 221.6±20.4 ng/ml, respectively (Fig. 2).

There was a significant difference in the extent of the cisapride-induced increase in QT prolongation between the two groups (Fig. 3). Although the administration of cisapride to rats with heart failure resulted in significant increases in the QT intervals at all

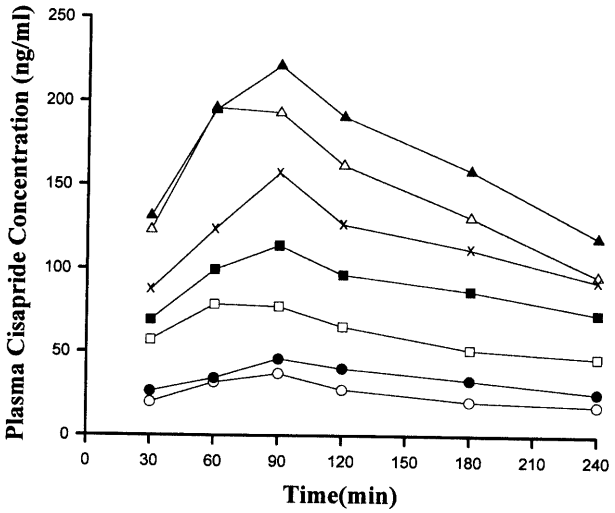

Fig. 2. Serum cisapride concentrations.
Serum cisapride level for the control Lewis (open symbols) and rats with heart failure (solid symbols) at 8 (○ and ●), 16 (□ and ■) and 24 (△ and ▲) mg/kg after cisapride administration. Although the serum cisapride levels after the administration of 24 mg/kg cisapride were lower in the group given mexiletine at 30 mg/kg for 7 days (X) than those in the non-mexiletine group (▲), the levels were higher than those in the rats administered with cisapride at 16 mg/kg (■).

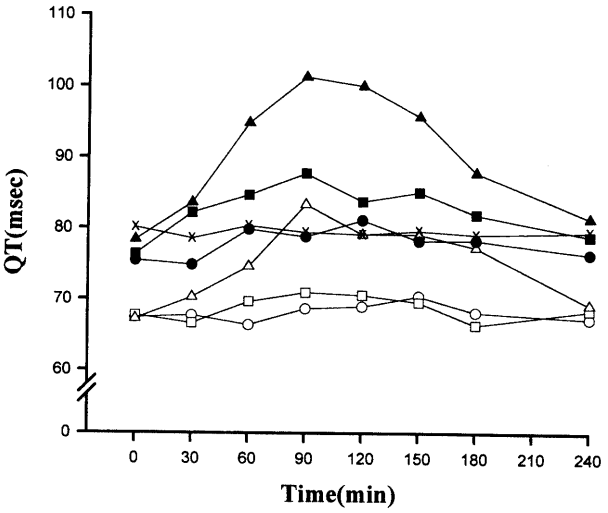

Fig. 3. QT interval before and after cisapride administration.
QT interval for the control Lewis (open symbols) and rats with heart failure (solid symbols) after cisapride administration at 8 (○ and ●), 16 (□ and ■) and 24 (△ and ▲) mg/kg. After cisapride administration, the QT interval increased dose-dependently. After mexiletine (30 mg/kg) treatment for 7 days, the QT interval was not prolonged during the administration of cisapride at 24 mg/kg in heart failure rats (X).

dosages ($p<0.01$) and the maximal levels were reached at 90 min. The QT interval at lower dosages (8 and 16 mg/kg) of cisapride did not increase significantly in control rats. The baseline QT intervals of the control Lewis rats at 8, 16 and 24 mg/kg were 67.4 ± 1.5, 67.7 ± 1.3 and 67.2 ± 1.5, and those of the rats with heart failure were 75.5 ± 1.9, 76.4 ± 1.6 and 78.4 ± 2.1 msec, respectively. The QT intervals of the control rats after cisapride at 8, 16 and 24 mg/kg were 70.5 ± 1.6, 71.0 ± 2.6 and 83.4 ± 4.3, and those of the rats with heart failure were 81.2 ± 1.8, 87.8 ± 2.5 and 101.4 ± 1.2 msec, respectively. At each dosage, there was a difference between the group with heart failure and the control group ($p<0.01$, Fig. 3). The increase in the QT interval during the cisapride administration at 24 mg/kg was slightly greater in rats with heart failure (24.6 ± 1.9) than in control rats (20.7 ± 3.5 msec, NS).

Protective effect of mexiletine on cisapride-induced long QT interval

Although the serum cisapride level after the administration of 24 mg/kg cisapride was lower in the mexiletine group than the non-mexiletine group, it was higher than that in the 16 mg/kg cisapride group (Fig. 2). After mexiletine-treatment for 7 days, the QT interval was not prolonged at 24 mg/kg cisapride in rats with heart failure (Fig. 3).

DISCUSSION

We found that the cisapride-induced lengthening of the QT interval in rats with heart failure was associated with a high dose of cisapride. Although cisapride can be used safely in most humans, its use in some patients or premature infants should be avoided; or, when used, monitoring of the QT interval should be considered. In one report, a prolonged QT was found in infants who received cisapride at a dose higher than 1 mg/kg/day (mean dosage, 1.34 mg/kg/day)[14]. One study demonstrating an association between high dose cisapride and prolonged QT interval points to the use of the lowest effective dose[15].

Electrocardiographic QT intervals are the estimate of the interval between two cardiac events – earliest ventricular depolarization and latest ventricular depolarization – and consistent with the action potential duration in cardiac ventricular muscles. Excess prolongation of QT intervals may lead to the development of serious ventricular arrhythmia, including torsade de pointes. Puisieux et al. reported that cisapride caused the prolongation of action potential duration and early afterdepolarizations in rabbit isolated Purkinje fibers.[16] These abnormalities may increase under conditions of heart failure or other diseases.

The QT interval is "indexed" for the heart rate, which is why the corrected QT interval (QTc) for human has been reported. Because the heart rate of rats is about 350 beats/min, we did not use QTc in this study[17]. This study cannot show the degree of cisapride-induced QT prolongation that is associated with a significantly increased risk for cardiac arrhythmia. The degree of QT prolongation and torsade de pointes associated with cisapride therapy would appear to depend on serum concentrations of the drug, on the individual bio-transformation capacity, and on its co-administration with other drugs causing pharmacokinetic and/or pharmacodynamic interactions. Our practice has been to recommend decreasing the dose of cisapride or eliminating other possible risk factors for prolonged QT in patients with heart failure or other diseases.

Although the serum cisapride level after the administration of 24 mg/kg cisapride was lower in the mexiletine group than the non-mexiletine group, it was higher than that in the 16 mg/kg cisapride group. The protective effect of mexiletine on a cisapride-induced long QT interval may involve not only a low level of serum cisapride but other mechanisms as well. Mexiletine, a class Ib antiarrhythmic agent, shows rapid dissociation kinetics from the sodium channel. Like many sodium channel blockers, at slow rates and at relatively low concentrations, the drug is thought to be capable of blocking late I_{NA} with little or no effect on the fast sodium current[18,19]. Another report shows that mexiletine increases the activation of K_{ATP} channels[20]. Further studies delineating the mechanism of cisapride and mexiletine effects on the transcriptional or post-transcriptional regulation of ion channels should provide a better understanding of the effects of cisapride in the heart.

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