PRECLINICAL ELECTROPHYSIOLOGY ASSAYS OF MITEMCINAL (GM-611), A NOVEL PROKINETIC AGENT DERIVED FROM ERYTHROMYCIN

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ABSTRACT — Mitemcinal (GM-611) is a novel erythromycin-derived prokinetic agent that acts as an agonist at the motilin receptor. Erythromycin has shown QT prolongation and torsades de pointes (TdP) in humans and cisapride, a second class of prokinetic agents typified by the 5-HT₄ receptor agonist, has been terminated due to TdP. In this study an extended series of safety pharmacology protocols and evaluations have been undertaken to assess the potential risk of mitemcinal on QT prolongation or proarrhythmic effects. Mitemcinal and its metabolites, GM-577 and GM-625, inhibited the human ether-a-go-go-related gene (HERG) tail current in a concentration-dependent manner with IC₅₀ values of 20.2, 41.7, and 55.0 μM, respectively. Administration of 10 mg/kg mitemcinal in anesthetized guinea pigs resulted in a slight prolongation of the monophasic action potential (MAP) duration during atrial pacing at the plasma concentration of mitemcinal 1.1 μM, with low maximum increases in MAPD₇₀ (6.6%) and MAPD₉₀ (4.6%) relative to vehicle. A 10-min infusion of 20 mg/kg of mitemcinal in a proarrhythmic rabbit model did not evoke TdP even when QT and corrected QT (QTc) intervals were significantly prolonged. In this study, the Cmax plasma-free concentration of mitemcinal indicates that the prolongation was more than 400-fold that of the therapeutic dose. Our findings of a wide safety margin and the absence of TdP within this margin suggest that mitemcinal may provide sufficient safety in clinical use.

KEY WORDS: Mitemcinal, Prokinetic agent, HERG assay, Monophasic action potential duration, Rabbit proarrhythmic model, QT interval prolongation

INTRODUCTION

QT interval prolongation from a noncardiac agent is a serious concern in the development of novel human pharmaceuticals and has been associated with increased risk of lethal ventricular arrhythmia such as torsades de pointes (TdP). The FDA, in the ICH S7B Guideline “Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals,” recommends the use of preclinical in vitro (single cell or multicellular preparations to assess action potential duration and/or cardiac ionic currents) and in vivo studies (conscious or anesthetized model systems) to detect drug-induced arrhythmogenic potential.

Mitemcinal ([2S,4R,5R,8R,9S,10S,11R,12R]-9-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-Hexo-pyranosyl)oxy]-5-ethyl-4-methoxy-2,4,8,10,12,14-hexamethyl-11-[3,4,6-trideoxy-3-(isopropylmethyl-amino)-β-D-xylo-hexopyranosyl]oxy]-6,15-dioxabicyclo[10.2.1]pentadec-14(1)-ene-3,7-dione(E)-2-butenedioic acid salt (2:1), Code name: GM-611) is an orally active erythromycin derivative without antibacterial activity (Fig. 1.; Koga et al.,

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Mitemcinal has shown potent gastrointestinal motility-stimulating activity via motilin receptors in rabbits, dogs and monkeys (Takanashi et al., 2007; Ozaki et al., 2007; Yogo et al., 2007). Cisapride, a piperidinyl benzamide prokinetic agent used for the treatment of gastrointestinal disorders, was withdrawn from the market in the United States in 2000 because of its propensity to prolong the QT interval, leading to life-threatening TdP arrhythmias (Wysowski and Bacsanyi, 1996). Several macrolides, such as erythromycin (Katapadi et al., 1997) and clarithromycin (Kamochi et al., 1999; Lee et al., 1998) have also been associated with QT risk interval prolongation and TdP. Although mitemcinal is also a macrolide in the same chemical class, the in vitro and in vivo QT prolonging effects have remained obscure.

In general, in vivo assessments with anesthetized and/or conscious animals and in vitro inhibition studies of the rapid component of the delayed rectifying K+ current (I_{kr}) channel with human ether-a-go-go-related gene (HERG) expressing cells are recognized as methods for evaluation of the proarrhythmic potential of novel pharmaceuticals (Friedrichs et al., 2005). For in vivo studies, the ICH S7B Guideline recognizes that an anesthetized model may also be of more value than a conscious model because, if differences in heart rate between treatment and control are great, the most useful approach is to maintain a constant heart rate using cardiac pacing, mainly used with anesthetized animals. Monophasic action potential (MAP) electrodes in anesthetized guinea pigs allowed an accurate measurement of the repolarization phase under cardiac pacing due to the exclusion of changes in heart rate (Fazekas et al., 1996).

Delayed ventricular repolarization, mostly due to blockade of the I_{kr} channel, favors the genesis of early afterdepolarization, which can provide the initiating beat that triggers reentrant tachyarrhythmias (Belardinelli et al., 2003); however, the precise relationship between drug-induced delayed ventricular repolarization and proarrrhythmia risk is not known. Directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval would be a logical undertaking. The in vivo experimental model of the acquired long QT syndrome developed by Carlsson et al. (1990) and widely used to examine the proarrhythmic activity of novel antiarrhythmics and other non-cardiac agents utilizes anesthetized rabbits. The ability of a test drug to evoke TdP is evaluated during co-administration of the selective α1-adrenoceptor stimulant.

The objective of the present study of mitemcinal was threefold: to determine effects on the HERG-encoded potassium channels, to assess QT prolongation in an anesthetized guinea pig model, and to assess arrhythmogenesis in an anesthetized proarrhythmic rabbit model.

**MATERIALS AND METHODS**

**Drugs**

Mitemcinal and its metabolites, GM-577 and GM-625, (Fig. 1) were synthesized in our organic chemistry laboratories. E-4031 was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and cisapride was extracted from Risamol® fine granules (Mitsubishi Pharma Corporation, Osaka, Japan).

**HERG study**

HEK293 cells stably expressing the HERG potassium channel (Zhou et al., 1998) (University of Wisconsin) were used. The cells were maintained and

![Fig. 1. Structures of mitemcinal (A) and its main metabolites, GM-577 (B) and GM-625 (C).](image-url)
Electrophysiology assays of mitemcinal.

IC\textsubscript{25} values were estimated from the sigmoidal function fit to the data where appropriate. Concentrations of mitemcinal, GM-577, and GM-625 for which there was a statistical difference from the respective vehicle-treated cells were determined.

**Effects on MAP in anesthetized guinea pigs**

This study was fundamentally conducted according to the method developed by Fazekas et al. (1996) following a protocol according to that of Carlsson et al. (1997). Twenty-eight male Dunkin-Hartley guinea pigs (body weight: 740–976 g) were used in this study. The animal handling procedures were in accordance with the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. All animals were anesthetized with pentobarbital sodium (50–60 mg/kg i.p.) and then instrumented in order to record blood pressure, blood sampling, drug infusion and ECG (lead II). A tracheotomy was performed and the animals artificially ventilated with oxygen-enriched room air. To reduce any autonomic influences, both vagi were transected in the neck and 0.5 mg/kg propranolol (Sigma Chemical Co., St. Louis, MO, USA) was given intravenously 15 min before the start of the experiment. During the procedure the body core temperature was maintained with a homeothermic blanket system, thermostatically controlled (activated at <37°C) by means of a rectal thermocouple. A catheter attached to a pressure transducer (Spectramed P23XL, Statham, CA, USA) was inserted into the carotid artery for measurement of arterial blood pressure parameters. A left thoracotomy was performed, the myocardium exposed, and a recording electrode was attached to the wall of the left ventricle for MAP measurement. A bipolar pacing electrode was attached to the left atrial appendage for pacing (2 msec duration, 1 or 2 times the threshold voltage). The pacing frequency was adjusted in each animal to pace at a heart rate approximately 10–50 bpm higher than the spontaneous baseline sinus rate. Data acquisition and analysis of mean blood pressure (MBP), heart rate (HR), MAP duration, and ECG were captured using the HEM Version 3.2 (NOTOCORD System SAS, Croissy sur Seine, France) computerized system. The MAP duration was measured at 70% of the action potential repolarization during atrial pacing and MAP\textsubscript{70(pacing)}, according to the protocol of Carlsson et al. (1997), in addition to MAP\textsubscript{90(pacing)}, the general indication of MAP duration. During pacing periods, the atrio-ventricular (A-V) conduction time (defined as the interval between the atrial pacing pulse and the start of the ventricular MAP) was measured.

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The animals were divided into three groups: a control group (n = 6), a mitemcinal group (n = 6) and a cisapride group (n = 6). Before administration of test substances, two basal control recordings were made 6 min apart during both sinus rhythm and atrial pacing. The mean of the two control recordings was used as the baseline. After the second control recording, phosphate buffer containing 3% D-mannitol (vehicle for mitemcinal) was administered intravenously as an infusion over 2 min (infusion volume of 3 ml/kg), followed by 3 consecutive administrations of either the vehicle or 0.1, 1, and 10 mg/kg of mitemcinal. In the case of the cisapride group, the vehicle (1% lactic acid) was administered once, followed by 0.01, 0.1, and 0.5 mg/kg of cisapride. Consecutive doses of test substances were then administered at 6-min intervals. The start of the first injection of the test substances was defined as 0 min. Data samples, other than pacing data, were taken pre-administration (~9 and ~3 min) and 4 min after the start of administration of each dose (4, 10, 16, and 22 min). Additional data samples were taken 14 and 27 min after the start of administration of the final dose (32 and 45 min). The heart was paced using a bipolar pacing catheter for 1 min pre-administration (~8 and ~2 min) and at 5 min after the start of each dose administration (5, 11, 17, and 23 min). In addition, the heart was paced at 15 and 28 min after the start of administration of the final dose (33 and 46 min). Data samplings were taken during the final 10 sec of each sampling minute. Arterial blood samples (approx. 2 ml) for analysis of test substance in plasma were collected 6 min after the start of final dose administration.

Proarrhythmic effects in anesthetized rabbits

This study was carried out in accordance with the method of Carlsson et al. (1990). Male rabbits (Kbl:NZW) weighing 2.5−3.1 kg were used in this study. All animal procedures were conducted in accordance with the ethical guidelines for animal care for the facility, and all experimental protocols were approved by the Animal Care Committee of the institution. Animals were initially anesthetized with thiopental sodium (20 mg/kg, i.v.); maintenance anesthesia was carried out by continuously administering a-chloralose for 10−20 min at a rate of 4.5 mg/kg/min using a syringe pump. After tracheotomy the animals were mechanically respirated with oxygen-enriched room air using a respirator. A cannula for recording arterial blood pressure was inserted into the jugular artery, a cannula for intravenous methoxamine (Sigma-Aldrich Inc., St. Louis, USA) and test drug administration was inserted into the jugular vein, and a cannula for blood collection was inserted into the femoral vein. MBP and HR were continuously recorded by thermal pen recorder at a recording speed of 10 mm/min. ECG output (I-III, aVR, aVL, and aVF) was passed through an electrocardiograph (ECG-8300, Nihon Kohden, Tokyo, Japan), and an electrocardiogram was recorded continuously at a speed of 50 mm/sec. The T wave of anaesthetized rabbits frequently exhibits a bimodal positive change in potential, referred to as a TU wave. The cardiac heart rate of anaesthetized rabbits is extremely fast and the potential duration of baseline between the TU wave and the following P wave is extremely short, so it is highly likely that the administration of a QT-interval-prolonging drug will cause the TU wave to be so prolonged that it overlaps the following P wave, making observation of the baseline potential impossible. The ability to find the QTU interval according to the usual method of measuring the time period from the beginning of the Q wave to the end of the TU wave was impaired; therefore the QTU interval was defined as the time period from the beginning of the Q wave to the point where the positive potential of the TU wave begins to decay.

The animals were divided into 6 groups of 6 animals each: control for mitemcinal (phosphate buffer), 5 mg/kg mitemcinal, 10 mg/kg mitemcinal, 20 mg/kg mitemcinal, control for cisapride (1% lactic acid), and 1.44 mg/kg cisapride. After all parameters had stabilized, methoxamine was intravenously injected at a rate of 70 nmol/kg/min (2 ml/kg/hr) using a syringe pump. From 10 min after the initiation of methoxamine administration, the test drug was intravenously infused for 10 min at a rate of 0.6 ml/kg/min using a syringe pump. Pre-administration of test drugs and every 1 min after administration, QTU and RR intervals were measured for five cardiac cycles from lead II; MPB was also measured. Two corrected QTU (QTUC) intervals were calculated using Bazett’s formula (QTUCB=QT/RR1/2) (Bazett, 1920) and the equation of Carlsson et al. (QTUC=QT-0.175(RR-300)) (1993). The occurrence of TdP, defined as a situation in which the QRS complex undulates for at least six consecutive beats from at least 2 of the 6 leads immediately preceded by a short-long-short sequence (in which consecutive beats have short and long or long and short RR intervals), was monitored. In the groups given mitemcinal (5, 10, and 20 mg/kg) about 1 ml of blood was collected from the femoral vein to measure the plasma concentration of mitemcinal 10 min after the start of administration.
Determination of mitemcinal concentration in plasma

Venous blood (1–2 ml) was obtained by superficial venepuncture. The samples were placed in sodium heparin blood collection vials, mixed, and stored on ice for up to 30 min until centrifugation. The samples were centrifuged at approximately 3,000 rpm at 4°C for 10 min. These samples were stored until assay in a freezer set at less than −20°C within 1 h of sampling. The plasma mitemcinal concentration was measured by LC-MS/MS.

Statistical analyses

Results are expressed as mean ± SEM. In the HERG study, in order to determine concentrations of mitemcinal, GM-577, and GM-625 with a statistical difference from the respective vehicle-treated cells, one-way analysis of variance (ANOVA) followed by Dunnett’s t-test (Dunnett, 1964) was used. Bartlett’s test (Bartlett, 1937) was then used, and if the variances were homogeneous, Dunnett’s multiple comparison was carried out; if not, Dunnett’s multiple comparison test was carried out using rank transformation. In the guinea pig MAP study, data are expressed as a percentage of the baseline value (mean of 2 pre-dosing control values). Comparisons were made between the vehicle and mitemcinal groups using the F test (JIS, 1965). If the variances were homogeneous, Student’s t-test (JIS, 1965) was carried out; if not, Aspin-Welch’s t-test (Welch, 1938) was carried out. Comparisons between the pre- and post-dosing values in the cisapride group were made using Student’s paired t-test. In the study in the arrhythmic rabbit model, ANOVA followed by Dunnett’s t-test (Dunnett, 1964) was used. The cisapride group and the 1%-lactate solution group were compared using ANOVA followed by Student’s t-test (JIS, 1965) or Aspin-Welch’s t-test (Welch, 1938). Statistical significance was assumed when p<0.05.

RESULTS

HERG study

The effects of mitemcinal, GM-577, and GM-625 on the HERG tail current are shown in Fig. 2. HERG tail current was significantly inhibited in a concentration-dependent manner by mitemcinal at 8.5 μM and above (p<0.01), GM-577 at 30 and 100 μM (p<0.01), and GM-625 at 30 and 100 μM (p<0.05 and p<0.01, respectively). The IC25, IC50, and IC75 values for mitemcinal inhibition of HERG tail current were 8.4, 20.2, and 49.0 μM, respectively. The IC25 and IC50 values for GM-577 and GM-625 inhibition of HERG tail current were respectively 17.0 and 41.7 μM and 23.4 and 55.0 μM. The potencies of these test drugs therefore were rank-ordered as mitemcinal > GM-577 >
GM-625.

Exposure to 0.1% DMF and 100% bath solution showed decreases in tail current of 16.2 and 19.7%, respectively. Therefore, tail currents observed in the presence of test drugs were corrected for the respective mean vehicle rundown. E-4031 produced a residual tail current of 6.3 ± 3.6%.

MAP study in the anesthetized guinea pigs

The time courses of the control for mitemcinal, mitemcinal and cisapride for RR interval at sinus rhythm, A-V conductance time, and MAP70(pacing) and MAP90(pacing) during atrial pacing are shown in Fig. 3. Following administration of 0.1 mg/kg mitemcinal, a small but significant difference in RR interval at sinus rhythm was observed when compared to the vehicle group. This was an isolated effect, did not occur following subsequent doses, and was deemed to be unrelated to mitemcinal. A-V conductance time, MAP70(pacing) and MAP90(pacing) were measured during atrial pacing. The RR interval during atrial pacing in the vehicle and mitemcinal-treated groups did not differ statistically (data not shown). There were statistically significant differences for both MAP70(pacing) and MAP90(pacing) prolongation at 15 min after the start of administration of 10 mg/kg mitemcinal (33 min, p<0.05). A significant prolongation of MAP70(pacing) was also evident during the final administration period 28 min after the start of administration of the 10 mg/kg mitemcinal (46 min, p<0.05). There were no notable differences in A-V conduction time following administration of any of the doses of mitemcinal compared to the vehicle. In six of the guinea pigs given mitemcinal, the plasma levels of mitemcinal were determined after the last dose was administered. These plasma concentrations reached 924 ± 204 ng/ml (1135 ± 251 nM).

Intravenous administration of 3 ml/kg lactic acid had no notable effect on RR interval, A-V conduction time, MAP70(pacing), or MAP90(pacing) when compared to pre-dose baseline values (Fig. 3). Administrations of 0.1 and 0.5 mg/kg cisapride caused a dose-dependent

![Fig. 3. The effects of phosphate buffer (○), phosphate buffer followed by mitemcinal (●) and 1%-lactate solution followed by cisapride (▲) on RR interval at sinus rhythm, A-V conductance time, monophasic action potential (MAP) duration at 90%, and MAP at 70% repolarization during atrial pacing in anesthetized guinea-pigs. Data presented are the mean ± SEM for all groups (n=6), except for the mitemcinal group at 45 and 46 min and the cisapride group at 11 and 17 min (n=5) and for the cisapride group at 5 min (n=3), at 23 min (n=2) and at 33 and 46 min (n=1). Doses were administered at 6 min intervals as shown with arrows. Animals received either 4 consecutive doses of 3 ml/kg phosphate buffer or 3 ml/kg phosphate buffer followed by 0.1, 1, and 10 mg/kg mitemcinal or 3 ml/kg lactic acid (1%) followed by 0.01, 0.1, and 0.5 mg/kg cisapride. *p<0.05, significantly different from control group (Student’s t-test). #p<0.05 and ## p<0.01, significantly different from initial value (paired Student’s t-test).]
Electrophysiology assays of mitemcinal.

Twenty-seven minutes after the start of administration of 0.5 mg/kg cisapride, the RR interval increased by 60.7 ± 10.4%. The RR interval during atrial pacing in the vehicle and cisapride-treated groups did not differ statistically (data not shown). Intravenous administration of cisapride caused a dose-dependent increase of $\text{MAP}_{70(y)}$ and $\text{MAP}_{90(y)}$ when compared to pre-dose baseline values. Atrial pacing was not possible in 4 animals at 5 min after administration of 0.5 mg/kg cisapride (23 min) and in 5 animals thereafter (33 and 46 min). However, in the remaining 2 animals at 23 min, $\text{MAP}_{70(y)}$ showed increases of 23.9% and 17.4% and $\text{MAP}_{90(y)}$ increased by 25.3% and 17.0%. A significant increase in A-V conduction time was noted after administration of 0.1 mg/kg cisapride when compared to baseline values (p<0.01). In these 2 animals, an increase of 20.8 ± 1.4% was observed following administration of 0.5 mg/kg cisapride.

Proarrhythmic study in the anesthetized rabbit model

Typical tracing of the ECG waveform before and after the start of 20 mg/kg of mitemcinal is depicted in Fig. 4. The results of QTU interval, QTUcB, and QTUcC for 10 min after intravenous administration of vehicle, mitemcinal and cisapride are shown in Fig. 5. For the 5- and 10-mg/kg mitemcinal groups, but there were no significant differences in QTU interval, QTUcB, or QTUcC relative to the phosphate buffer group. However, significant prolongations of these parameters relative to the phosphate buffer group were observed with 20 mg/kg mitemcinal. In the phosphate buffer group, the QTUcB and QTUcC intervals of 263 ± 8 msec and 147 ± 4 msec were prolonged by 50 ± 15 msec and 28 ± 7 msec at the end of infusion, respectively, and in the 20 mg/kg mitemcinal group, intervals of 240 ± 13 msec and 132 ± 7 msec were prolonged by 83 ± 19 msec and 49 ± 11 msec at the end of infusion, respectively. Although there were no significant differences in QTU interval between the cisapride group and the 1%-lactate solution group, significant prolongation of QTUcB and QTUcC relative to the 1%-lactate solution group was observed from dosing with cisapride. In the 1%-lactate group, the QTUcB and QTUcC intervals of 265 ± 16 msec and 147 ± 9 msec (before dosing) were prolonged by a maximum of 34 ± 13 msec and 20 ± 6 msec, respectively, and in the cisapride group, intervals of 269 ± 16 msec and 149 ± 9 msec were prolonged by a maximum of 86 ± 27 msec and 53 ± 19 msec, respectively.

The results of RR interval and MBP for 10 min after intravenous administration of vehicle, mitemcinal, and cisapride are shown in Fig. 6. For the 20-mg/kg mitemcinal group, comparison with the control group revealed significant decreases in MBP at 9 min after start of dosing. On the other hand, a comparison of the cisapride group with the 1%-lactate solution group revealed significant decreases in mean blood pressure at all time points except for 5 min after start of dosing. There were no significant differences in RR interval compared to the phosphate buffer group and the 1%-lactate solution group in all mitemcinal groups and the cisapride group, respectively.

No occurrence of TdP was seen in the 5, 10, or 20 mg/kg mitemcinal groups, the phosphate-buffer group, or the 1%-lactate solution group. In the cisapride group, on the other hand, the development of TdP was observed in 3 out of the 6 animals. Typical tracing is shown in Fig. 7. The distribution of maximum values

![Fig. 4](https://example.com/fig4.png)  
**Fig. 4.** Typical tracing of electrocardiogram recording before and after administration of mitemcinal (14 mg/kg, 7 min after start of administration). Note the marked prolongation of the QTU interval after mitemcinal.
of QTUcB and QTUcC in each animal of each group is shown in Fig. 8. As the data shows, the mean maximum values of QTUcB and QTUcC from 20 mg/kg of mitemcinal were similar to those of the cisapride group; the distributions of QTUcB (52–158 msec) and QTUcC (31–93 msec) in this group were also similar to QTUcB (61–156 msec) and QTUcC (30–99 msec) in the cisapride group. The ranges for QTUcB and QTUcC when TdP was observed in the cisapride group were 8–142 msec and 5–89 msec, respectively.

In the groups given mitemcinal, mean maximum plasma concentration increased as the dose increased. Mitemcinal concentration immediately after the completion of dosing was 410 ng/mL (504 nM) in the 5 mg/kg group, 901 ng/mL (1107 nM) in the 10 mg/kg group, and 1774 ng/mL (2,179 nM) in the 20 mg/kg group.

![Fig. 5](image)

Fig. 5. The effects of phosphate buffer (○) and mitemcinal at 5 mg/kg (●), 10 mg/kg (▲), and 20 mg/kg (■) (A) and cisapride (●) and 1%-lactate solution (○) (B) on QTU interval (top), QTUcB (middle) and QTUcC (bottom) in the anesthetized proarrhythmic rabbit model induced by methoxamine. Data represent the mean ± SEM (n=6), exclusive of data at 3 min and 4 min for the cisapride group (n=5). *p<0.05 and **p<0.01, significantly different from the control group (Dunnett’s multiple test). #p<0.05 and ## p<0.01, significantly different from the control group (Student’s t-test).
DISCUSSION

Mitemcinal is an orally active derivative, but without the antibacterial activity, of erythromycin. It is well known that erythromycin at intravenous high dose levels has shown QT prolongation and TdP in humans (Schoenenberger et al., 1990), leading to regulatory precedents. Cisapride, another prokinetic agent and typified by a 5-HT4 receptor agonist, has been terminated due to inducing TdP (Wysowski and Bacsanyi, 1996). It has recently been reported that mitemcinal acts as a selective and full motilin receptor agonist in the smooth muscle of rabbit small intestine (Takanashi et al., 2007). Clinical trials of mitemcinal in patients with diabetic gastroparesis are currently underway (McCallum et al., 2007). Here, an extended series of safety pharmacology protocols and evaluations of mitemcinal, which was synthesized in our organic chemistry laboratories (Koga et al., 1994), have been undertaken to assess the potential risk in clinical use. In our studies, cisapride was used as a reference compound because it markedly prolongs QT interval at therapeutic dose, and cases of TdP induced by cisapride are frequently compared with erythromycin. Thus, to validate the potential of mitemcinal, a comparison with cisapride was considered valuable.

Effects on HERG and MAP duration in the anesthetized guinea-pigs

The HERG study demonstrated that the macrolides–mitemcinal, GM-577, and GM-625–inhibited the HERG tail current (IC50 values of 20.2, 41.7, and...
55.0 μM, respectively) indicating the potential to inhibit the human \(I_{Kr}\) channel. Since it has been shown that the potency of mitemcinal on the HERG channel was approximately 2- or 3-fold stronger than its metabolites, we selected mitemcinal in order to assess the effects on QT prolongation in the anesthetized guinea pig MAP study. Intravenous administration of 10 mg/kg mitemcinal, the maximum tolerance dose for an anesthetized guinea pig, significantly prolonged MAP\(_{70}(\text{pacing})\) and MAP\(_{90}(\text{pacing})\), suggesting that mitemcinal has the potential to prolong QT interval in vivo. The potential of mitemcinal, however, was very weak compared with that of cisapride, which showed significant prolongation of MAP\(_{70}(\text{pacing})\) and MAP\(_{90}(\text{pacing})\) at the dose of 0.01 mg/kg. Although we did not measure or evaluate the plasma level of cisapride in this model, an approximate 1000-fold difference between the potential of mitemcinal and cisapride to prolong MAP duration in guinea pig model at dosing levels has been indicated. The differ-

![Electrocardiogram](image1)

**Fig. 7.** An electrocardiogram demonstrating induction of torsades de pointes in an anesthetized rabbit given cisapride (0.14 mg/kg/min). Torsades de pointes was induced after a cumulative dose of 0.28 mg/kg cisapride.

![Graph](image2)

**Fig. 8.** The distribution of maximum values of QTUcB (●) and QTUcC (○) during infusion of test drugs in each animal of each group of anesthetized methoxamine-induced proarhythmic rabbits. Triangle figures show the incidence of torsades de pointes.
ence was similar for HERG inhibition, with cisapride inhibiting the $I_{Kr}$ channel with IC$_{50}$ values of 6.5–44.5 nM (Mohammad et al., 1997; Rampe et al., 1997). One report concluded that QT prolongation following cisapride administration is concentration-related in humans, and 2 nM free drug is associated with a 6-msec increase in QTc (Van Haarst et al., 1998). Since mitemcinal prolonged MAP duration at a plasma concentration of 924 ng/mL (1.1 μM) in the anesthetized guinea pig model, the free plasma concentration of mitemcinal was estimated to be approximately 110 nM, assuming 90% protein binding (in-house data), suggesting that the potential of mitemcinal is clearly weaker in vivo compared with cisapride.

On the other hand, concerning the effects on the $I_{Kr}$ channel, the literature describes other macrolides—erythromycin, clarithromycin and roxithromycin—exhibiting HERG tail current inhibition with IC$_{50}$ values of approximately 30–70 μM (Stanat et al., 2003; Volberg et al., 2002), suggesting that the potency of mitemcinal is slightly stronger than other macrolides. According to Wisialowski et al. (2006) in a study of erythromycin on MAP duration in a guinea pig model, erythromycin dose-dependently increased MAP$_{50}$ and MAP$_{90}$ duration at pacing, and the free drug concentration of erythromycin which prolonged MAP duration to a degree similar to mitemcinal was more than 3 μM. As mentioned above, mitemcinal prolonged MAP duration at a free plasma concentration of 110 nM in the anesthetized guinea pig model, suggesting that the potential of mitemcinal is also stronger in vivo compared with erythromycin.

**Effects on torsadogenic potential**

Contemporary preclinical in vitro and in vivo methods have been imperfect in predicting drug-induced TdP in humans. Seven antipsychotics were reviewed by Warner and Hoffmann (2002), and they estimated that the therapeutic free plasma concentration of these antipsychotics would result in approximately 10–20% inhibition of the HERG channel. This, however, translates into a different prolongation of the QTc interval, the magnitude of which does not correlate with the torsadogenic risk in humans. Therefore, directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval is a logical undertaking. In the present study using an anesthetized proarrhythmic rabbit model, it was proved that mitemcinal does not evoke torsadogenic activities even within a wide range of plasma concentrations of mitemcinal (410–1774 ng/mL) including concentrations indicating QTc interval prolongation.

Anesthetized rabbits concomitantly treated with α$_1$-adrenergic agonist methoxamine exhibit TdP-like polymorphic ventricular tachycardia in the presence of agents prolonging the repolarization process (Carlsson et al., 1993). This indicates that the anesthetized rabbit, which like humans presents a high density of α$_1$-adrenergic receptors, may be a relevant model for studies of torsadogenic compounds. However, the lack of TdP with quinidine illustrates false negative results in the applied model (Farkas et al., 2002; Lu et al., 2000). One obvious factor that may antagonize the proarrhythmic potential is the ancillary α$_1$-adrenergic receptor-blocking properties of the compounds. Quinidine has been reported to antagonize α$_1$-adrenergic receptors competitively (Motulsky et al., 1984; Muller and Noack, 1988). Cisapride also has been reported to bind to α$_1$-adrenergic receptors with high affinity as well as 5-HT$_4$ receptors (Carlsson et al., 1997), thought to be one of the reasons the incidence of TdP was low (3 of 6 animals) in spite of the high potency of cisapride in blocking the $I_{Kr}$ channel (Buchanan et al., 1993). As results show, cisapride suppressed an increase in blood pressure from methoxamine in anesthetized rabbits but, on the contrary, decreased in it immediately after infusion, suggesting indirect blocking of the α$_1$-adrenergic receptor with cisapride. One reason for the reduction in the proarhythmic potential with quinidine in the anesthetized rabbit model may be the drug’s ion channel blocking activities, such as the blockade of inwardly directed depolarizing sodium currents, which may counteract excessive repolarization delay and triggering of TdP.

In the case of mitemcinal, TdP was not evoked even at the highest dose with QTc interval prolongation. Mitemcinal affected blood pressure only slightly at the high dose, although hypotension has been associated with the response of motilin agonists via a motilin receptor presented in the endothelium (Iwai et al., 1998; Mangoni et al., 2004). Moreover, there is no evidence that mitemcinal acts on sodium channels because mitemcinal did not increase A-V conductance time, which solely depends on the sodium current (Sugiyama et al., 1994), in the guinea pig model even at the high dose of 10 mg/kg. Thus, the assessment of mitemcinal for torsadogenic activities was conducted under suitable conditions, and it was found that mitemcinal does not evoke TdP in an anesthetized proarrhythmic rabbit model. Interestingly, although the prolongations of QT and QTc intervals with mitemcinal were the same levels as those observed with cisapride,
the results were quite different, suggesting that QT and QTc prolongation are not the only contributing factors to TdP generation. In the cisapride group, the fact that the QTc intervals of animals with induced TdP did not always show increased prolongation also supports this theory. Recent experiments show that a set of repolarization disturbances characterized by triangulation, reverse use dependence, instability and dispersion (TRiAD) rather than simple changes in the duration of the QT interval provides the proarhythmic substratum (Hoffmann and Warner, 2006). Thus, it may be difficult to discuss this phenomenon with only an increased prolongation of QTc interval. In the case of the cisapride group, Tdp occurred under a condition that did not prolong the QT interval. The cause may be inconsistency in the measurement of the points between QT interval prolongation and the incidence of TdP in spite of the combination of such complicated factors.

The results of the current report clarify the safety margin of mitemcinal in the clinical field. In order to provide further indications of its proarhythmic potential in clinical use, we calculated the safety margin of mitemcinal between the IC$_{50}$ values of HERG and the therapeutic concentration in a clinical study (free concentration of 0.25 nM, in-house data) using the method proposed by Redfern et al. (2003). Mitemcinal showed a 80,800-fold safety margin, greatly exceeding the acceptable ratio of 30-fold, determined from the fact that the majority of compounds having a >30-fold difference between the IC$_{50}$ value of HERG and the free plasma concentration of therapeutic range are free from TdP in human (Redfern et al., 2003). Furthermore, the safety margins between the free concentration level affecting QTc interval prolongation in the guinea pig model (110 nM) or in the rabbit model (220 nM) and the maximum concentration of the patient population in a clinical study were approximately 440-fold and 880-fold, respectively. Clinical concentrations of erythromycin and cisapride show levels close to those that induce TdP and therefore, mitemcinal has a wider safety margin for clinical use.

In conclusion, after investigation of the electrophysiological effects of mitemcinal to evaluate QT prolongation and TdP potential, it was proven that mitemcinal has a blocking effect on HERG currents in a dose-dependent manner (IC$_{50}$ of 20.2 μM) and prolongs MAP duration or QTc interval in anesthetized guinea pig and proarrhythmic rabbit models at a high plasma concentration (more than 1 μM). TdP, a major clinical concern in relation to QT prolongation, was not evoked in the proarrhythmic rabbit model even when QT and QTc interval prolongation were induced by intravenous administration of mitemcinal. Moreover, the Cmax free concentrations of mitemcinal which induced QT-prolonging effects were more than 400-fold those of the therapeutic dose. These findings suggest that mitemcinal, a novel prokinetic agent, may be sufficient for clinical use due to a wide margin of safety and due to the absence of TdP in the proarrhythmic rabbit model. Further studies in specialized electrophysiology such as effective refractory periods and terminal repolarization periods are required to clarify the mechanism of the absence of TdP in this study.

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REFERENCES


Electrophysiology assays of mitemcinal.


Welch, B.L. (1938): The significance of the difference between two means when the population variances are unequal. Biometrika, 29, 350-362.


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