The 5-HT\textsubscript{4} Agonists Cisapride, Mosapride, and CJ-033466, a Novel Potent Compound, Exhibit Different Human Ether-a-go-go-Related Gene (hERG)-Blocking Activities

Tetsuo Toga\textsuperscript{1,*}, Yumi Kohmura\textsuperscript{1,2}, and Ryoichi Kawatsu\textsuperscript{1}

\textsuperscript{1}Nagoya Laboratories, Pfizer Global Research and Development, Pfizer Japan Inc., 5-2 Taketoyo, Aichi 470-2393, Japan
\textsuperscript{2}Nagoya Branch, WDB Co., Ltd., 2-4-1 Sakae, Nagoya 460-0008, Japan

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Abstract. The blocking effect of three 5-HT\textsubscript{4} agonists, cisapride, mosapride, and the newly discovered CJ-033466 on the human ether-a-go-go-related gene (hERG) channel was studied using a whole cell patch-clamp technique in HEK293 cells. Cisapride was found to be the most potent of the hERG blockers. CJ-033466 had the widest safety margin between its hERG blocking activity and 5-HT\textsubscript{4} agonism among the tested compounds. This suggests a lower clinical risk of cardiac arrhythmia in CJ-033466 compared with the other 2 agonists. Therefore, CJ-033466 has the potential to be a drug with higher therapeutic efficacy and less cardiac risk than both cisapride and mosapride.

Keywords: human ether-a-go-go-related gene (hERG), 5-HT\textsubscript{4} agonist, patch-clamp

The serotonin 5-HT\textsubscript{4}-receptor agonist cisapride, a benzamide derivative, had been widely prescribed as a prokinetics drug for the treatment of gastrointestinal dysfunctions in patients with gastro-oesophageal reflux disease and functional dyspepsia. However, this drug has been withdrawn from the pharmaceutical market by its manufacturer due to its risk of inducing fatal cardiac arrhythmia (1).

The hERG (human ether-a-go-go-related gene) channel is the main channel responsible for the repolarization phase of cardiac action potentials (2, 3). Therefore, agents blocking the hERG channel prolong the duration of the action potential by delaying the repolarization phase. This blocking is also manifested as a prolonged QT interval in the electrocardiogram and can lead to potentially fatal arrhythmias such as Torsades de Pointes (4). In terms of cardiac safety, the ICH S7B guideline in step 4 recommends performing studies to identify the potential risk of compounds in delaying ventricular repolarization and in prolonging the QT interval for human pharmaceuticals (5).

Although cisapride has provided clinical confidence of 5-HT\textsubscript{4} agonism in the treatment of gastrointestinal dysfunction, it is a well-known potent blocker of the hERG channel (6 – 8). A serotonin 5-HT\textsubscript{4} agonist with minimal cardiac risk, as recommended in the ICH S7B guideline, could compensate for the prescription void created by the withdrawal of cisapride in the marketplace for the treatment of gastrointestinal dysfunctions.

Another benzamide derivative of mosapride (AS-4370) that possesses 5-HT\textsubscript{4} agonistic activity and gastroprokinetic efficacy has been reported (9, 10). This drug has already been launched in Japan as well as undergoing clinical studies for the treatment of gastrointestinal dysfunctions in other countries. The results have shown less effect on the hERG channel and the QT interval in preclinical studies (11 – 13).

A novel imidazopyridine derivative has also been discovered (Fig. 1). The compound, CJ-033466, possesses higher 5-HT\textsubscript{4} agonism than the benzamide derivatives cisapride and mosapride (T. Mikami et al., in preparation). CJ-033466 has the potential for the treatment of gastrointestinal dysfunction through its 5-HT\textsubscript{4} agonism and to fulfill the pharmaceutical void created from the withdrawal of cisapride, but only if the compound has less risk of QT prolongation by the hERG inhibition.

In this paper, we will report a comparative study of

*Corresponding author. toga@japan-net.ne.jp
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hERG-blocking activity among these three 5-HT₄ agonists using the whole cell patch-clamp electrophysiological technique.

HEK293 cells stably expressing hERG potassium channels (14) were licensed from Wisconsin Alumni Research Foundation (Madison, WI, USA), and used for the present experiment. Cells, in a recording chamber mounted on the stage of an inverted microscope, were superfused by a peristaltic pump with a standard external solution of the following composition: 130 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, 5 mM HEPES, adjusted to pH 7.4 with NaOH. Whole-cell recording was made using an EPC-9 patch-clamp amplifier controlled by the Pulse/PulseFit software (HEKA Elektronik, Lambrecht, Germany) and patchpipettes that had a resistance of 1 – 3 MΩ when filled with an internal solution of the following composition: 130 mM KCl, 5 mM MgATP, 1 mM MgCl₂, 10 mM HEPES, 5 mM EGTA, adjusted to pH 7.2 with KOH. Series resistance was routinely compensated up to 80% and checked repeatedly during the experiments. All experiments were carried out at room temperature (23 ± 1°C).

A large outward potassium current was elicited by a 1-s duration stepped depolarization pulse from a holding potential of −80 to +40 mV, followed by a descending repolarization ramp (0.5 mV·ms⁻¹) back to the holding potential.
potential (Fig. 2A). Amplitude of the peak current elicited around –40 mV during the descending ramp was measured.

Once stably evoked currents were obtained in the external solution, a vehicle of external solution containing 0.5% dimethyl sulfoxide (DMSO) was applied for 10 – 20 min followed by drug application for 10 min. After the drug application, the cells were washed out with the vehicle and a high dose of dofetilide (5 µM), a hERG-channel-specific blocker, was applied to confirm complete suppression of the current (Fig. 2: A and B).

Test compounds were dissolved in DMSO and stocked as frozen aliquots. An aliquot was diluted with the external solution 200-fold to obtain the desired test concentration, and then it was applied to the cells. Cisapride and dofetilide were synthesized at Pfizer Sandwich, Kent, UK. Mosapride and CJ-033466 were synthesized at Pfizer Nagoya, Aichi. Only one concentration of a test drug was applied to a single cell in an experiment.

A total of 400 – 600 recordings of current values were obtained in a series of experiments from a single cell because the voltage pulse for eliciting the hERG current was applied to a cell continuously throughout the experiment every 4 s (Fig. 2B). The last 10 consecutive current values were taken during the periods of control (vehicle perfusion) and drug application. Arithmetic means of each of the 10 values were calculated. The percent decrease in each separate experiment was obtained by the normalized current value using the following formula: % decrease = (1 – I₀ / Ic) × 100, where I₀ is the mean value of drug responses and Ic is the mean value of the control currents. Data were expressed as the mean ± S.E.M. from independent experiments. Curve fitting and IC₅₀ calculations were carried out with GraphPad Prism® software (GraphPad software, San Diego, CA, USA).

A total of 45 cells were tested by the whole-cell patch-clamp technique. The hERG potassium current was decreased by the peak-to-peak amplitudes following application of any drugs (Fig. 2B).

The three tested compounds showed relative peak reduction in a dose-dependent manner (Fig. 2C).

Cisapride showed higher inhibitory effects on a hERG current, as indicated by its IC₅₀ of 9.4 × 10⁻⁹ M, while both CJ-033466 and mosapride showed lower blocking potency, as indicated by their IC₅₀ values of 2.6 × 10⁻⁶ and 4.8 × 10⁻⁶ M, respectively (Table 1). There is a previous report on an electrophysiological study about hERG-blocking activity by cisapride using hERG-transfected HEK293 cells (6). The IC₅₀ value in the present study (9.4 × 10⁻⁹ M) is similar to that reported in the previous study (6.5 × 10⁻⁹ M), in spite of some differences in voltage protocols used in whole cell patch-clamp and data analysis. The consistency in the reported effects of cisapride in both studies validates the present results in the hERG-current-blocking assay using this cell-line, although cisapride possesses voltage dependency in hERG blocking (6).

The IC₅₀ of CJ-033466 was higher than that of cisapride (Table 1). This demonstrates that CJ-033466 is a safer 5-HT₄ agonist in terms of the cardiac risk of QT prolongation than cisapride. Mosapride showed weaker hERG blocking activity than CJ-033466. However, the 5-HT₄ agonistic activity of CJ-033466 is about 1000 times more potent than that of mosapride (Table 1). This high agonism potency of CJ-033466 may produce a lower effective plasma concentration than mosapride when CJ-033466 is systemically administered for the treatment of gastrointestinal dysfunctions. This would produce full range safety margin between hERG-blocking activity and the treatment of gastrointestinal dysfunction with CJ-033466.

These results show that CJ-033466 possesses lower hERG-blocking activity and has the widest margin for cardiac safety out of the three drugs tested. This suggests that CJ-033466 would be the best-in-class drug for the treatment of gastrointestinal dysfunctions with high 5-HT₄ agonism yet without the QT cardiac risks and could meet the medical demand that cisapride had previously occupied in the clinical field. Detailed further hERG-blocking studies on the 5-HT₄ agonists by the patch-clamp technique, for example, determining their time- and voltage-dependency, would help to address the

<table>
<thead>
<tr>
<th>Compounds</th>
<th>hERG IC₅₀ (M)</th>
<th>5-HT₄ EC₅₀ (M)*</th>
<th>Therapeutic window (hERG IC₅₀/5-HT₄ EC₅₀)</th>
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</thead>
<tbody>
<tr>
<td>CJ-033466</td>
<td>2.6 × 10⁻⁶</td>
<td>0.9 × 10⁻⁹</td>
<td>2889</td>
</tr>
<tr>
<td>Cisapride</td>
<td>9.4 × 10⁻⁹</td>
<td>1.4 × 10⁻⁷</td>
<td>0.067</td>
</tr>
<tr>
<td>Mosapride</td>
<td>4.8 × 10⁻⁶</td>
<td>9.8 × 10⁻⁷</td>
<td>4.898</td>
</tr>
</tbody>
</table>

*data from T. Mikami et al., in preparation (2007).
clinical potential of CJ-033466 as a safe prokinetics drug for the treatment of gastrointestinal dysfunctions.

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