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ARTICLE

Comprehensive in vitro pro-arrhythmic assays demonstrate that omecamtiv mecarbil has low pro-arrhythmic risk

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Abstract

Omecamtiv mecarbil (OM) is a myosin activator (myotrope), developed as a potential therapeutic agent for heart failure with reduced ejection fraction. To characterize the potential pro-arrhythmic risk of this novel sarcomere activator, we evaluated OM in a series of International Conference on Harmonization S7B core and follow-up assays, including an in silico action potential (AP) model. OM was tested in: (i) hERG, Nav1.5 peak, and Cav1.2 channel assays; (ii) in silico computation in a human ventricular AP (hVAP) population model; (iii) AP recordings in canine cardiac Purkinje fibers (PF); and (iv) electrocardiography analysis in isolated rabbit hearts (IRHs). OM had low potency in the hERG (half-maximal inhibitory concentration [IC_{50}] = 125.5 μM) and Nav1.5 and Cav1.2 assays (IC_{50} > 300 μM). These potency values were used as inputs to investigate the occurrence of repolarization abnormalities (biomarkers of pro-arrhythmia) in an hVAP model over a wide range of OM concentrations. The outcome of hVAP analysis indicated low pro-arrhythmia risk at OM concentration up to 30 μM (100-fold the effective free therapeutic plasma concentration). In the isolated canine PF assay, OM shortened AP duration $(APD)_{60}$ and APD_{90} significantly from 3 to 30 μM. In perfused IRH, ventricular repolarization (corrected QT and corrected JT intervals) was decreased significantly at greater than or equal to 1 μM OM. In summary, the comprehensive proarrhythmic assessment in human and non-rodent cardiac models provided data indicative that OM did not delay ventricular repolarization at therapeutic relevant concentrations, consistent with clinical findings.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

A new therapeutic agent, omecamtiv mecarbil (OM), increases cardiac contractility by prolonging systolic ejection time, however, there is no published data assessing its pro-arrhythmic risks.

WHAT QUESTION DID THIS STUDY ADDRESS?

Pro-arrhythmic risk assessment of OM in in vitro and ex vivo safety pharmacology models compliant with International Conference on Harmonization S7B guideline and Comprehensive In Vitro Proarrhythmia Assay initiative.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Comprehensive in vitro pro-arrhythmic risk assays demonstrate that OM has low pro-arrhythmic risk and translate into clinical safety observations.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Low pro-arrhythmic risks consistently identified in preclinical in vitro models translate well into clinical observations (i.e., negative preclinical pro-arrhythmic findings can predict negative clinical outcomes).

INTRODUCTION

The preclinical assessment of new drug candidates for pro-arrhythmic risk (e.g., Torsades de Pointe) is outlined in the International Conference on Harmonization (ICH) S7B guideline.[1] The core in vitro model is the hERG assay, which measures a compound's potency against the function of the rapid component of delayed rectifier potassium current (IKr) encoded by a human ether-a-go-go-related gene (hERG).[2] In addition, some drug sponsors use specialized in vitro and ex vivo assays to augment pro-arrhythmia risk assessment.[3] It is well-accepted that in vitro electrophysiology studies conducted with isolated cells, myocytes, and cardiac tissues (including Purkinje fibers, ventricular tissues, and intact heart) can generate insights regarding an agent's effects on electrical activities, including ventricular repolarization and QT prolongation. The specialized ventricular repolarization assays include the measurement of action potentials (APs) in isolated cardiac tissues[4-7] and the ventricular wedge preparation[8] or electrocardiographic (ECG) parameters in isolated hearts.[6]

Since the introduction of the comprehensive in vitro pro-arrhythmic assay paradigm (Comprehensive In Vitro Proarrhythmia Assay [CiPA]),[9] much progress has been made in the methodology to perform cardiac ion channel potency determination and in silico modeling of ventricular APs. For example, to harmonize practices for improving data consistency, the protocols for testing drug potency against hERG, L-type calcium, and fast and slow inward sodium currents in patch-clamp studies have been developed by the Ion Channel Working Group and standardized protocols have been shared by the US Food and Drug Administration (FDA).[10-12] In addition, an in silico model of the human ventricular myocyte AP (e.g., O'Hara-Rudy model[13]) has been further developed by academic laboratories, industry, and regulatory agencies for application to cardiac safety evaluation.[14-19] The combination of approaches defined by ICH S7B and the new CiPA paradigm enables the use of more tools to improve pro-arrhythmic risk assessment.

Omecamtiv mecarbil (OM) is a first-in-class selective cardiac myosin activator, also called a myotrope, which directly targets the contractile mechanisms of the heart. OM increased ejection fraction in patient with heart failure (HF) and prolongs durations of myocardial systole,[20] which is decreased in patients with HF with reduced ejection fraction.[21] The clinical efficacy and safety profile of OM have been demonstrated,[20,22] however, a CiPA-based assessment for OM has not been performed. Two exploratory electrophysiological studies showed that OM (10 μM) can cause depression of the AP plateau, reduce early repolarization, and shorten ventricular APs in canine myocytes.[23,24] These observations suggest that supratherapeutic concentrations of OM do not prolong canine ventricular APs, a biomarker for delayed repolarization risk.[2] To fully characterize the pro-arrhythmic potential of OM, a battery of in vitro and in silico models were used to evaluate the cardiac safety profile of OM at clinically relevant concentrations. In addition, a major metabolite of OM (M4) was investigated in the ion channel assays to understand its pro-arrhythmic potential.

METHODS

The hERG studies performed good laboratory practice (Charles River Laboratories, Cleveland, OH)

Two GLP studies were performed with methods described in the Supplemental Methods, one tested OM, and the other tested M4, the major human metabolite of OM. Samples of the test article formulation solutions collected from the outflow of the perfusion apparatus were analyzed by high-performance liquid chromatography for concentration verification. The results from the sample analysis indicated that the measured concentrations of OM and M4 at all test concentrations were within ±15.0% of nominal concentrations and met the acceptance criteria; therefore, nominal concentrations were used to construct the concentration-response relationships and to derive the half-maximal inhibitory concentration (IC_{50}) for both agents.

Exploratory cardiac ion channel (hERG, Nav1.5, and Cav1.2) studies (Nova Research Laboratories LLC, New Orleans, LA)

Studies were designed and performed according to protocols recommended by the FDA.[10]

Positive controls were verapamil (hERG and Cav1.2 assays) and flecainide (Nav1.5 assay).

HEK-293 cells transfected with human cDNA of hERG, Nav1.5, or Cav1.2 were used for the studies. The internal and external recording solutions are listed in the Supplemental Methods.

Currents were recorded with a manual patch clamp in the whole-cell mode. The electrophysiological protocols are described in Figure S1.

In silico modeling using a population model of human ventricular APs

The Virtual Assay (VA) model (software version 3.2.1119 2018, Oxford University Innovation) was used to evaluate the integrated response to multiple ion channel inputs based on the O'Hara-Rudy dynamic human endocardial ventricular AP model.[13] Based on established knowledge of ionic profiles likely to cause drug-induced repolarization abnormalities (RAs),[18] the population of models was constructed by varying nine key ionic conductances to mimic myocyte electrophysiological variability, then constrained and calibrated using authentic human AP data collected by patch-clamp recordings.[18] Nine ionic conductances were considered because they are regarded as important in the initiation, maintenance, and termination of APs. The nine ion channels include: fast and late Na+ current (GNa and GNaL, respectively), transient outward K+ current, rapid and slow delayed rectifier K+ current (GKr and GKs), inward rectified K+ current (GK1), Na+-Ca2+ exchanger (GNCX), Na+-K+ pump (GNaK), and the L-type Ca2+ current (GCaL). The ranges of variation of each conductance were optimized to maximize the number of models accepted in the population while at the same time minimizing the population size. In brief, models with severe GNa, GKr, GNaK, or GK1 down-expression often fail to produce physiological APs, whereas models with low repolarization reserve (increased GCaL, GNaL, and GNCX, reduced GNaK, GKr, and GKs) are more prone to develop drug-induced RA. Using this process, and then filtered based on experimental AP recordings, a population of 118 models was produced.[18] The VA model was validated with 69 clinically relevant compounds and found to be accurate (>80%) for predicting pro-arrhythmic risk[19] when an optimal testing concentration of 100-fold effective free therapeutic plasma concentration (EFTPC) was used[19] (i.e., lower test concentrations demonstrated less sensitivity without significant improvement of specificity). A minimal dataset of Nav1.5 (peak), Cav1.2, and hERG channel potencies was used to optimize reliable predictions with highest computational efficiency.[19]

The mean therapeutic plasma concentration of OM was 200–318 ng/ml in a randomized, placebo-controlled phase II trial,[25] which corresponds to a 0.5–0.8 μM total plasma concentration. Given the plasma protein binding of OM (82% in humans),[26] the EFTPC is ranged from 0.09 to 0.14 μM. To be on the conservative side and ease preparation of dosing solutions, 0.3 μM was used as the EFTPC. Test concentrations for OM (0.3 to 30 μM) were selected to cover multiples (1 to 100-fold) above the EFTPC. The EFTPCs of verapamil and flecainide are 0.088 and 0.753 μM, respectively,[27] and listed in Table S1. Verapamil was tested from 0.088 to 8.8 μM and flecainide was tested from 0.753 to 75.3 μM to cover 1 to 100-fold above their EFTPC.

The output of the VA model are changes in various end points indicative of pro-arrhythmic risk (e.g., the occurrence of RAs). RA includes early after depolarization (EAD) and repolarization failure (RF). EAD is identified when positive deflections were found in the membrane potential after 150 ms following the AP peak. RF is identified when the membrane potential failed to reach resting membrane potential (>−40 mV) at the end of 1 s recording. Aggregated results at the population level were presented using a scoring system (ProA score) developed and described previously.[18,19] The ProA score is calculated by integrating all RAs occurrences over multiple test article concentrations and computed with the following formula:

$$\text{score} = \frac{\sum_c (Wc * nRAc)}{N * \sum_c Wc}$$

C = the test concentration, $nRAc$ = the number of models showing RA at a tested concentration C, $Wc = \text{EFTPC}_{max}/C$: the weight inversely related to the tested concentration C, N = the total number of APs in the population.

The ProA score is a value between 0 and 1. A score of zero indicates low risk and values greater than zero indicate a high risk.

AP recordings in isolated canine cardiac Purkinje fibers performed GLP (Charles River Laboratories, Cleveland, OH)

Recordings were performed according to the procedures described in the Supplemental Methods. Concentration-response and rate-dependence were determined by the following procedure: Purkinje fibers (PFs) were paced continuously at a basic cycle length (BCL) of 2 s for at least 25 min for recovery and stabilization before obtaining control AP responses. Only fibers with resting potentials more negative than –80 mV and normal AP morphology (AP duration $[APD]_{90}$ = 250–450 ms) were used. Acceptable fibers were stimulated continuously at a BCL of 2 s for at least 20 min. At the end of this period, baseline APD rate-dependence under control conditions was measured using stimulus pulse trains consisting of ~ 50 pulses at a BCL of 2, 1, and 0.5 s. After returning to a BCL of 2 s, OM at 3 μM

was applied for at least 20 min to allow equilibration, and the stimulus trains repeated. The entire sequence (~20 min of equilibration followed by 3 cycles of stimulus trains at decreasing BCL) was repeated at cumulatively increased OM concentration. The average responses from the last five recorded APs from each stimulus train were analyzed for each test condition. DL-sotalol (100 μM) was used as a positive control in this study.

Exploratory isolated rabbit heart studies using Langendorff perfusion (Amgen)

Left ventricular contractility and ECG parameters in isolated rabbit hearts (IRHs) were recorded according to the procedures described in Supplemental Methods. Effects of OM were analyzed first by normalizing to baseline, then compared to effects with time-matched vehicle control (0.3% DMSO). Statistical analysis was performed with one-way analysis of variance followed by Dunnett's multiple comparisons test; $p < 0.05$ was considered significant.

Verapamil and flecainide were used as controls in the IRH studies.

RESULTS

The hERG studies performed GLP

The effect of OM and metabolite M4 on hERG channel function are shown in Figure 1a,b. OM inhibited hERG current in a concentration-related fashion (e.g., 0.3% at 1 μM, 5.2% at 10 μM, 17.2 at 30 μM, and 43.6% at 100 μM). Because the effect at 100 μM was close to 50%, the IC_{50} for the inhibitory effect of OM on hERG potassium current was extrapolated to be 125.5 μM (Hill coefficient = 1.1; Figure 1c).

M4 inhibited hERG current by 4.6% at 10 μM and 16.3% at 300 μM (Figure 1d), which was statistically significant at 300 μM ($p < 0.05$) compared with vehicle control values. Because the maximal inhibitory effect was less than 20%, the IC_{50} for M4 was not calculated, but estimated to be greater than 300 μM.

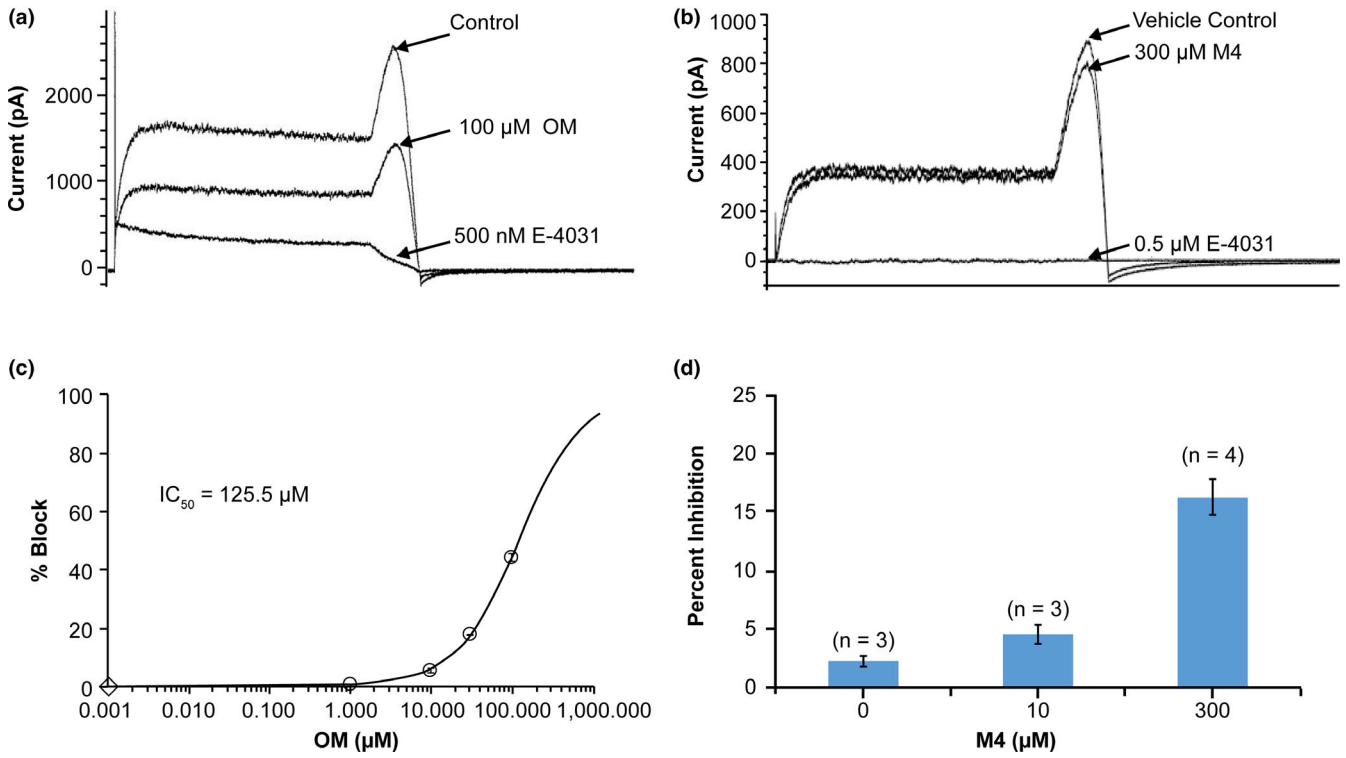

FIGURE 1 Effects of OM and its M4 metabolite in GLP-hERG assays. (a and b) Example hERG current recordings in control in the presence of 100 μM OM (a) and 300 μM M4 (b). (c) Concentration-response relationship of OM showing percent inhibition of hERG currents after application of vehicle control (diamond, $n = 3$ cells) and each concentration of OM (open circles, $n = 3$ cells). Data were fit (solid line) with a binding equation, % Block = $\{1 - 1/[1 + ([\text{test concentration}]/IC_{50})^N]\} \times 100$. N represents the hill coefficient. The derived $IC_{50} = 125.5$ μM with $N = 1.1$. (d) Percent inhibition of hERG currents (mean ± SE) in the presence of 0, 10, and 300 μM M4 ($n = 3$–4 cells). The IC_{50} for the inhibitory effect of M4 on hERG currents was estimated to be greater than 300 μM. GLP, good laboratory practice; hERG, human ether-a-go-go-related gene; IC_{50}, half-maximal inhibitory concentration; OM, omecamtiv mecarbil

Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG potassium current by 81.8% ($n = 2$) and confirms the sensitivity to detect hERG block.

Exploratory cardiac ion channel studies

The effects of OM and M4 were evaluated on hERG, peak Nav1.5, and Cav1.2 current amplitudes with FDA-recommended protocols[10] (Figure S1). As shown in Figure S2a, OM had an hERG IC_{50} of 229 μM, which was less than two-fold different than the GLP-hERG study (above). OM demonstrated low potency against peak Nav1.5 and Cav1.2 currents (Figures S2b and S2c); at 300 μM, OM decreased peak Nav1.5 by 40% and Cav1.2 current by 25%. The metabolite M4 had even weaker effects at the maximal test concentration of 300 μM (Figure S2).

The concentration-dependent effects of flecainide on Nav1.5 and verapamil on hERG and Cav1.2 (Figure S2 and Table S1) confirmed the sensitivities of the test systems.

In silico pro-arrhythmic assessment with a population model of human ventricular AP

The potential pro-arrhythmic risk of OM was investigated in a human ventricular AP model with a population of 118 myocytes.[18,19] The input of ion channel potencies and EFTPC are presented in Table S1. The simulated APD_{90} and EAD events are listed in Tables S2, S3, and S4 for OM, flecainide, and verapamil, respectively. Figure 2 shows 118 APs in baseline (Figure 2a), in the presence of OM (3-fold of EFTPC; Figure 2b), verapamil (3-fold EFTPC; Figure 2c), and flecainide (3-fold of EFTPC; Figure 2d). As demonstrated by the traces in Figure 2, at 3-fold of their respective EFTPC, OM and verapamil had little effect on the AP morphology; on the other hand, flecainide prolonged AP duration and elicited EAD in some myocytes.

Only at 30-fold and 100-fold the EFTPC, OM exhibited prolonged AP durations significantly (Table S2), but EAD was not elicited. The pro-arrhythmic score was zero at all test concentrations, including 100-fold EFTPC (Table S1).

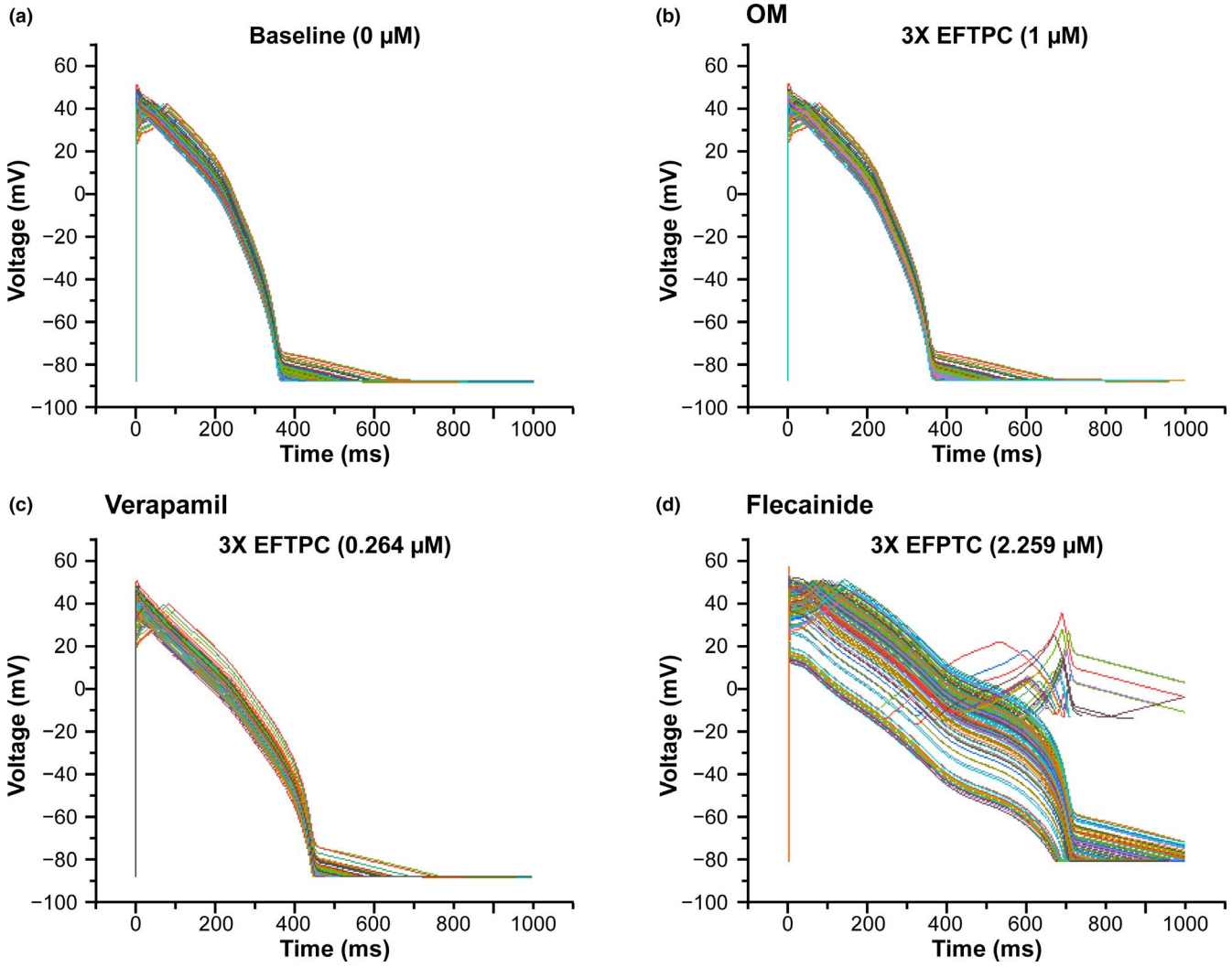

FIGURE 2 Simulated action potential traces of 118 human ventricular myocytes overlaid in control (a) and in the presence of 3 x EFTPC of OM (b), verapamil (c), and flecainide (d). EFTPC, effective free therapeutic plasma concentrations; OM, omecamtiv mecarbil

On the other hand, flecainide prolonged APD at all test concentrations and elicited EADs at threefold EFTPC and above (Table S3). The calculated pro-arrhythmic score of flecainide is 0.115, which indicated high pro-arrhythmia risk.

Verapamil also prolonged APD at all test concentrations (Table S4), however, consistent with a profile of low pro-arrhythmic risk, verapamil did not induce EADs at any test concentrations. The calculated pro-arrhythmic score of verapamil is zero at the test concentration range, indicating a low pro-arrhythmic risk.

AP studies in isolated canine cardiac PF (GLP)

The concentration-dependent effects of OM on cardiac APs were examined in isolated canine PFs as shown in Table 1 and Figure S3. Starting at the lowest testing concentration, 3 μM (10-fold EFTPC), OM shortened APD_{60} and APD_{90} significantly compared with time-matched vehicle controls. However, the magnitude of shortening was small with maximal APD_{60} decrease of 13% at 30 μM OM under 0.5 s BCL. In addition, the effects exhibited no apparent concentration- or frequency-dependence. Consistently, the effects on APD_{60} were greater than the effects on APD_{90}, indicating effects on the plateau phase of the AP, and lack of effects on the later repolarization phase of the AP. OM did not induce significant changes in resting membrane potential, AP amplitude, and the maximum upstroke velocity up to 30 μM (Table 1).

The positive control, sotalol (100 μM), produced statistically significant ($p < 0.05$) prolongation of APD_{60} and APD_{90} at all stimulus frequencies (data not shown), confirming the sensitivity of the test system.

Exploratory isolated rabbit heart studies

The effects of OM on the cardiac ECG intervals and LVP were examined in IRHs using the Langendorff method. Figure 3a shows left ventricular pressure (LVP) and ECG traces recorded from one example heart in control and under the treatment of increasing concentrations of OM. As shown in Figure 3a, OM prolonged the duration of left ventricular (LV) contraction (left panel, black traces) due to its primary effect as a sarcomere activator; at supratherapeutic exposures (1–3 μM) a decrease in contraction amplitude was observed. The duration of LV contraction (LVPD50) was derived by subtracting the time at 50% rising phase from the time at 50% falling phase in the LVP waveform. LVPD50 was increased significantly at all test concentrations starting at 0.1 μM (Figure 3b). In contrast to classical inotropes, the maximal rate of LV pressure development (dP/dt_{max}) was not augmented at any test concentrations (Figure 3c). This is

TABLE 1 Effects of OM on canine Purkinje Fiber action potentials

2 s BCL										
OM	APD60		APD90		RMP		APA		V_{max}	
μM	% Change	SEM	% Change	SEM	% Change	SEM	% Change	SEM	% Change	SEM
3	–11*	3	–7*	3	1	1	–4*	2	1	2
10	–9*	2	–6*	3	1	1	–4	2	1	5
30	–12*	4	–7*	3	–1	2	–4	3	–2	2
1 s BCL										
OM	APD60		APD90		RMP		APA		V_{max}	
μM	% Change	SEM	% Change	SEM	% Change	SEM	% Change	SEM	% Change	SEM
3	–12*	4	–8*	3	1	1	–6	3	–2	4
10	–9*	3	–6*	3	1	0	–4	1	–4	5
30	–11*	4	–6*	3	–2	2	–3	4	–3	4
0.5 s BCL										
OM	APD60		APD90		RMP		APA		V_{max}	
μM	% Change	SEM	% Change	SEM	% Change	SEM	% Change	SEM	% Change	SEM
3	–12*	5	–7	4	1	2	–7	3	–1	2
10	–10*	4	–5*	3	1	1	–4	2	1	7
30	–13*	5	–6*	3	0	1	–4	4	0	6

Abbreviations: APA, action potential amplitude; APD60, action potential duration at 60% repolarization; APD90, action potential duration at 90% repolarization; BCL, basic cycle length; OM, omecamtiv mecarbil; RMP, resting membrane potential; V_{max}, maximal upstroke velocity.

*Statistically significant change; $p < 0.05$ compared with baseline (0 μM) with one-way analysis of variance followed by Dunnett's multiple comparisons.

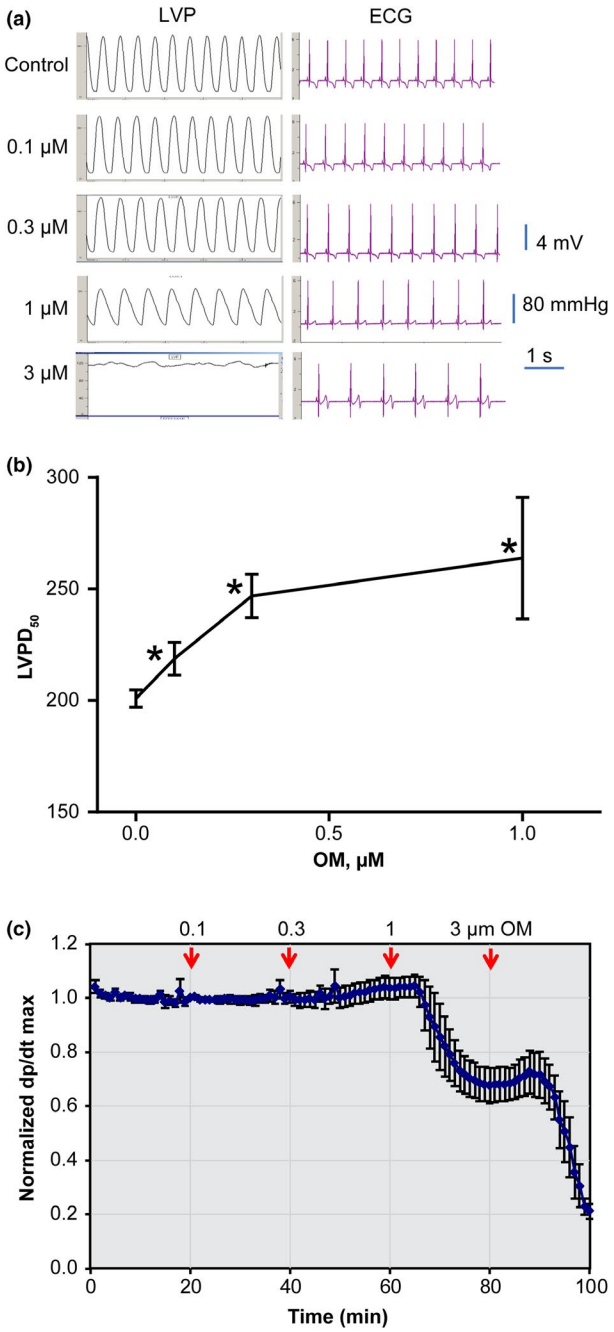

FIGURE 3 Concentration-dependent effects of OM on left ventricular pressure (LVP) and electrocardiogram (ECG) recorded from isolated rabbit hearts. (a) LVP and ECG from an example heart in control, and in the presence of 0.1, 0.3, 1, and 3 μM OM as labeled. (b) Concentration-dependent effects of OM on duration of LV contraction. $LVPD_{50}$ was derived by subtracting the time at 50% of rising phase from the time at 50% of falling phase. (c) Time- and concentration-dependent effects of OM on dP/dt_{max}. Red arrows indicate the time of application of the indicated concentrations. LV, left ventricle; OM, omecamtiv mecarbil; $LVPD_{50}$, left ventricular pressure duration 50%

consistent with clinical findings that OM increased cardiac output by increasing systolic ejection time[28] and preclinical canine studies that OM increased systolic ejection time in the absence of changes in the rate of LV pressure development (dP/dt).[20]

In the same rabbit heart, the ECG waveforms (Figure 3a, right panel, purple traces) were not affected over the entire test range. At 3 μM OM, there was no discernible LVP waveforms that could be associated with regular LV contractions; however, robust ECG waveforms showed rhythmic activities, indicating dissociation of electrical occurrences with mechanical events at supratherapeutic drug levels.

Time-matched vehicle control experiments were also performed with 0.3% DMSO ($n = 8$ hearts, data not shown). First, the effects of OM and 0.3% DMSO were calculated compared with their respective baselines. Then, OM effects were analyzed against the changes in time-matched DMSO treatment to understand if there were statistically significant effects. As shown in Table 2, JTcF and corrected QT Fridericia's formula (QTcF) intervals were decreased significantly ($p < 0.05$) at 1 and 3 μM OM in a concentration-dependent manner. In addition, QRS intervals were prolonged significantly at 3 μM, whereas PR intervals were not affected significantly at any of the test concentrations.

In the IRH model, verapamil and flecainide elicited changes in ECG parameters expected from their ion channel activities, confirming the sensitivity of the test system. Verapamil prolonged PR intervals, decreased heart rate, and shortened QTc intervals (Figure 4, right panel). Flecainide prolonged PR, QRS, QTc intervals; and decreased heart rate (data not shown).

DISCUSSION

The pro-arrhythmic risk of OM, and its primary metabolite were characterized in a series of studies based on the ICH S7B guideline and CiPA initiative. Several key findings were made: first, OM had low potency against hERG, Cav1.2, and Nav1.5 peak currents, and the calculated hERG-based safety margin was greater than 300. Likewise, the major metabolite M4 had lower potency against these cardiac ion channels. Second, the in silico modeling for pro-arrhythmia biomarkers indicated low risk over a wide exposure range. Third, AP recordings in canine PF demonstrated no prolongation of AP duration, which was corroborated by QTc interval measurement in the IRH model. To illustrate the inter-relationship of the in vitro safety pharmacology findings with the clinical exposure, an overlay plot was constructed (Figure 5), relative to the mean EFTPC derived from phase II clinical trials.[28] In addition, a recent dedicated thorough QT study of OM (50 mg) demonstrated no QTc prolongation risk at the high therapeutic plasma concentration of 800 ng/ml (~ 0.37 μM free; personal communication). The safety margin derived from the exposure data from the TQT study relative to the hERG (GLP) potency value was 339, confirming that OM has a wide safety margin in humans. The integrated findings from this battery of tests led to the conclusion that OM has

TABLE 2 Effects of OM on ECG end points in IRHs

Absolute values (% change), *n* = 8 hearts												
OM	HR		PR		QRS		QT		QTcF		JT		JTcF	
μM	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	130.3	1.2	59.1	1.4	30.9	1.2	220.7	2.3	285.9	3.5	189.8	2.7	245.8	3.9
0.1	127.4	1.1	59.7	1.5	32.0	1.1	220.8	3.5	283.7	4.5	188.8	4.2	242.6	5.2
0.3	124.2	1.8	59.3	1.6	31.4	0.9	214.4	4.9	273.1	5.7	182.9	5.0	233.0	5.7
1	119.8	3.7	59.4	1.9	32.1	1.4	208.1	6.5	261.4* (–8.9)	6.7	176.0	6.8	220.9* (–10.5)	6.9
3	116.0	6.6	62.6	2.5	34.5* (12.3)	1.2	199.8	17.6	245.7* (–14.5)	17.8	165.3	17.3	202.9* (–18.1)	17.9

Note: The absolute values (mean and standard error of the mean [SEM]) for heart rate (beats per minute) and ECG intervals (ms); *statistically significant change, $p < 0.05$ compared to time-matched DMSO treatment with one-way analysis of variance followed by Dunnett's multiple comparisons. The significant changes were also displayed by percent effects in parenthesis.

Abbreviations: ECG, electrocardiogram; HR, heart rate; IRH, isolated rabbit heart; OM, omecamtiv mecarbil; QTcF, corrected QT Fridericia's formula.

low pro-arrhythmic risk, a profile that is supported by clinical ECG and safety data.[28]

Comparison with published studies

Previous studies reported that 1 μM OM had negligible effect on ionic currents and AP characteristics of canine cardiomyocytes, whereas small changes were observed at high OM concentrations (10–100 μM). Our findings are consistent and confirm the shortening of canine ventricular APs and decrease of early repolarization at 10 μM OM,[23,24] In the same publication,[24] OM at a single concentration of 10 μM was also tested on Ito, ICa, IKr, and IK1 currents. OM had small effects on the amplitude of Ito, ICa, and IKr; ~ 10%–30% reduction based on visual inspection of the plots. It is hard to assess the quality or relevance of these findings given the absence of positive or negative control data. Regardless, our dataset corroborates the published findings of canine AP recordings.

Consistent results from hERG assays: GLP versus exploratory

The evaluation of OM's potency against hERG function was conducted first by complying with the most recent version of the FDA Good Laboratory Practices Regulations (21 CFR Part 58) and IC_{50} estimate was 125.5 μM. Later to address an FDA request for information, OM was re-tested in a screening hERG assay (non-GLP), and found to have an IC_{50} of 229 μM. The hERG potency values were consistent (<2-fold difference) and confirmed OM to have low affinity for hERG channels. Based on the potency values, wide safety margins were calculated (418× and 763× in GLP and exploratory hERG assays, respectively) and support the same conclusion of low pro-arrhythmic risk at clinically relevant exposures. The direct comparison of hERG potencies suggest that a high quality exploratory or screening protocols are useful and effective for in vitro safety margin estimation.

Outcome of in silico modeling limited by input parameters

Computational modeling and simulation of ventricular APs can help understand an agent's pro-arrhythmic risk by classifying the risk based upon its ion channel potencies.[17-19] In the current study, OM prolonged APD at the test concentrations of 10 and 30 μM, which were 30-fold and 100-fold of the EFTPC, respectively. In ion channel function studies, OM inhibited hERG channel function by 5% at 10 μM and 17% at 30 μM, which is the mechanism responsible for the APD_{90} prolongation in the in silico human ventricular AP model.

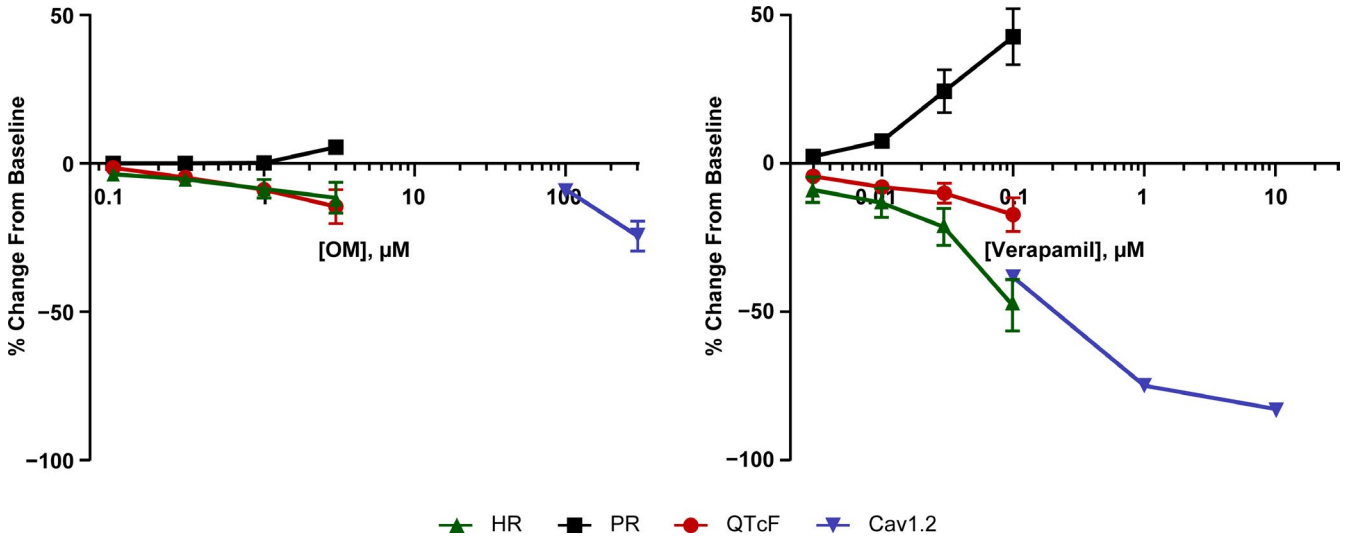

FIGURE 4 Percent changes from baseline for Cav1.2 currents, QTcF, and PR intervals in isolated rabbit hearts (IRHs) under the treatment of OM (left panel) and verapamil (right panel). For Cav1.2 currents, $n = 3–4$ cells. For PR and QTcF intervals in IRH, $n = 8$ rabbit hearts. HR, heart rate; OM, omecamtiv mecarbil; QTcF, corrected QT Fridericia's formula

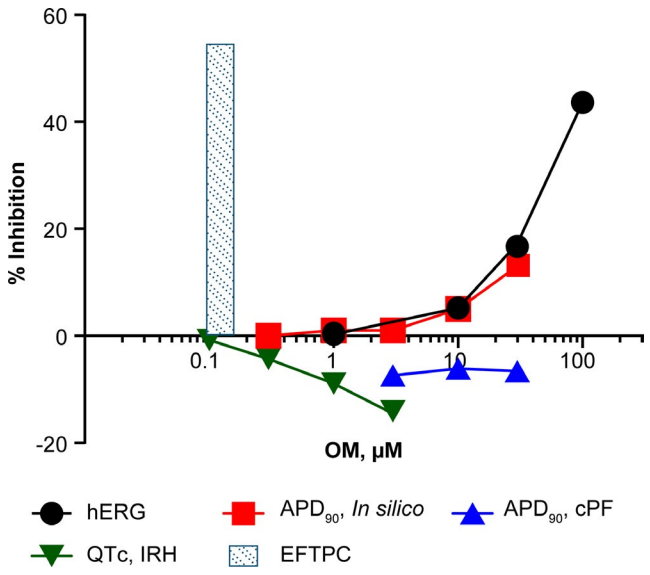

FIGURE 5 Concentration-dependent effects of OM on hERG, APD_{90} from in silico model and canine Purkinje Fiber, QTc from isolated rabbit heart (IRH) overlaid with the mean effective free therapeutic plasma concentration (EFTPC) in phase 2 clinical trial. APD, action potential duration; QTc, corrected QT

Despite the APD_{90} prolongation, OM did not induce EAD events at any test concentrations, which reflects low pro-arrhythmic risk.

The positive control in this study, flecainide, is a well-known agent with pro-arrhythmic risk. It inhibits hERG and Na^+ channels (Table S1). In the in silico human ventricular AP model, flecainide prolonged action potential duration and induced EAD, which indicates high pro-arrhythmic risk. The negative control in this study, verapamil, is recognized clinically as having low pro-arrhythmic risk. Verapamil is a potent hERG blocker, but it also inhibits Ca^{2+} channels (Table S1). In the in silico human ventricular AP model, verapamil prolonged APD, consistent with hERG inhibition. However, it did not induce EAD at any test concentrations, due to compensation from Ca^{2+} current reduction. This mixed channel profile leads to this agent having low pro-arrhythmic risk.

The low-risk score derived for OM (0) directly reflects its wide hERG safety margin, inability to delay ventricular repolarization in the canine PF, and IRH models at therapeutically relevant test concentrations.

However, in contrast to the results of APD shortening in canine PF and QTc shortening in IRH, the in silico model indicated that OM could cause prolongation of APD_{90} in human ventricular myocytes at concentrations associated with hERG-blockade (Table S2). The lack of concordance between the in silico model and experimental data from nonrodent cardiac models highlights a potential limitation: the output of any in silico model will be based on the input parameters, in this case, hERG, Nav1.5, and Cav1.2 potency values. Mechanisms that are not included in the input, which alter cardiac electrical activities through direct (other channels) or indirect mechanisms, will not manifest as a change in any output parameters, including APD, in the VA.

QT shortening by OM and its potential mechanisms

In the canine cardiac PF study, OM shortened AP duration; QTc shortening was also observed in the rabbit heart model. Our observations are consistent with prior reports in canine ventricular myocytes.[24] These preclinical in vitro studies

demonstrate translational relevance to the clinic, as QTc shortening was also observed in a phase II clinical trial.[28]

In the current clinical and regulatory environment, there is a lack of a better understanding of the clinical significance of QT shortening. Accordingly, there are less concerns regarding the pro-arrhythmic risk of drugs causing QT shortening.[29] On one hand, suggestions were made to further substantiate or confirm the safety of QT-shortening drugs.[30] On the other hand, arguments were presented to suggest that drug-induced QT shortening might not be a significant safety issue.[31] Consistent with the latter argument, there are no regulatory guidelines for the approval of QT-shortening drugs.

A well-known mechanism for QT shortening is inhibition of L-type Ca channels, which also leads to conduction delay from atria to ventricle (PR interval prolongation) and reduces heart rate. We investigated this potential mechanism by comparing OM with verapamil, a prototypic phenylalkylamine L-type Ca channel blocker.[32] Analysis of their effects on ion channel function and ECG parameters in IRHs strongly argued against this possibility. In our hands, verapamil inhibited Cav1.2 channels with an IC_{50} of ~ 0.2 μM, prolonged PR, decreased heart rate, and shortened QTc intervals as expected (Figure 4, right panel). In contrast, OM had no effect on Cav1.2 channel function up to 100 μM. In the IRH, OM shortened QTc, but it did not prolong PR intervals, nor decreased heart rate significantly (Figure 4, left panel). This side-by-side comparison of OM with verapamil excludes L-type Ca channel blockade as the mechanism by which OM shortens ventricular repolarization. This conclusion is also supported by the in silico simulation of human ventricular AP based on potencies against hERG, Nav1.5, and Cav1.2. As shown in Table S2, OM is predicted to cause prolongation of APD_{90} by integrating ion channel inhibitions, suggesting that shortening of APD_{90} and QTc by OM observed experimentally is not driven by its effects on the above-mentioned ion channels.

Cardiac glycosides (digoxin, etc.) also shorten QTc intervals,[33] but the effect is mediated by increased intracellular Ca^{2+},[34] which regulate various sarcolemmal ionic channels that affect AP configuration.[35] It has been well-established that OM increases cardiac contractility without enhancing intracellular Ca^{2+}.[20,36] Therefore, the possibility of any Ca-dependent changes in ion channel or transport functions that could shorten AP duration is not plausible. The exact mechanism of QTc shortening following OM administration is unknown, but is likely related to its ability to stimulate cardiac sarcomere shortening, given that cardiac stretch and compression can modify the electrical, hemodynamic, metabolic, and structural properties of the heart.[37]

CONCLUSION

The pro-arrhythmic risk of OM was evaluated in a series of high quality in vitro, ex vivo, and in silico safety pharmacology models. The integrated risk assessment demonstrates that OM has low pro-arrhythmic risk in the models tested. This preclinical outcome is consistent with human cardiac safety data collected in clinical studies.

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CONFLICT OF INTEREST

All authors are employees and shareholders of Amgen Inc.

AUTHOR CONTRIBUTIONS

Y.Q. and H.M.V. wrote the manuscript. Y.Q. and H.M.V. designed the research. B.G., Z.A., and M.F. performed the research. Y.Q., B.G., Z.A., and M.F. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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