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REVIEW ARTICLE

Omecamtiv Mecarbil: A Myosin Motor Activator Agent with Promising Clinical Performance and New *in vitro* Results

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ARTICLE HISTORY

Received: May 18, 2017
Revised: September 26, 2017
Accepted: November 29, 2017

DOI:
10.2174/0929867325666171222164320

Abstract: ***Background:*** Clinical treatment of heart failure is still suffering from limited efficacy and unfavorable side effects. The recently developed group of agents, the myosin motor activators, act directly on cardiac myosin resulting in an increased force generation and prolongation of contraction. The lead molecule, omecamtiv mecarbil is now in human 3 stage. In addition to the promising clinical data published so far, there are new *in vitro* results indicating that the effect of omecamtiv mecarbil on contractility is rate-dependent. Furthermore, omecamtiv mecarbil was shown to activate cardiac ryanodine receptors, an effect that may carry proarrhythmic risk.

Methods: These new results, together with the controversial effects of the drug on cardiac oxygen consumption, are critically discussed in this review in light of the current literature on omecamtiv mecarbil.

Results: In therapeutically relevant concentrations the beneficial inotropic effect of the agent is not likely affected by these new results - in accordance with the good clinical data. At supratherapeutic concentrations, however, activation of cardiac ryanodine receptors may increase arrhythmia propensity, and the stronger effect on diastolic than systolic cell shortening, observed at higher pacing frequencies, may decrease or offset the inotropic effect of omecamtiv mecarbil.

Conclusion: Further studies with definitely supratherapeutical concentrations of omecamtiv mecarbil should be designed to map the actual risk of these potentially harmful side-effects.

Keywords: Heart failure, inotropic agents, myosin activators, Omecamtiv mecarbil, Ryanodine receptor, Cytosolic Ca^{2+}.

1. INTRODUCTION

The principles of therapy of heart failure are continuously changing, since all conventional positive inotropic strategies, including the application of cardiac glycosides, β-adrenergic agonists, phosphodiesterase inhibitors, ryanodine receptor stabilizers and Ca^{2+} sensitizers, are burdened by more or less serious side-effects. Some of these agents, like cardiac glycosides, β-adrenergic agonists and phosphodiesterase inhibitors, are strongly proarrhythmic - partially because of the concomitant Ca^{2+} overload, resulting in delayed afterdepolarizations [1-3]. Furthermore, the incidence of early afterdepolarizations is also increased by β-receptor agonists and phosphodiesterase inhibitors due to the inward shift in transmembrane ion currents in response to elevated cAMP levels [4, 5]. Another common unfavorable effect of β-adrenergic agonists and phosphodiesterase inhibitors is the increased oxygen demand of cardiac muscle, largely ascribed to the increased rate of Ca^{2+} cycling, further damaging the poor metabolic conditions of the failing heart [6]. The concept of stabilization of cardiac ryanodine receptors (RyR-2) is based on the pathologic leakiness of RyR-2 leading to diastolic Ca^{2+} leak from the sarcoplasmic reticulum (SR) [7, 8]. This may strongly compromise

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0929-8673/18 $58.00+.00

the pump function of the heart due to the increased diastolic Ca^{2+} level combined with the reduced Ca^{2+} content of the SR, resulting in diastolic and systolic failure, respectively. Ryanodine receptor stabilizers, such as Rycal, K201 and JTV519, improve the contractile performance by reducing diastolic Ca^{2+} leakage and decrease the energy demand of cardiac muscle [9, 10]. Here, it should be mentioned that the opposite strategy, *i.e.* pharmacological activation of RyR-2, has also been suggested to treat heart failure.

An alternative way to support cardiac contractility is to enhance the efficacy of the Ca^{2+} signal. In this case less Ca^{2+} is required to generate a given tension, consequently less energy has to be wasted for accumulation of Ca^{2+} into internal stores or pumping it out of the cell [11]. Ca^{2+} sensitizer drugs improve the mechanical performance of cardiac muscle by increasing the affinity of troponin C to Ca^{2+} without increasing substantially the oxygen consumption of the heart [12, 13]. In absence of increased Ca^{2+} cycling, elevated arrhythmia incidence is not anticipated with these agents [14]. The most serious side-effect of Ca^{2+} sensitizers is diastolic stiffness manifested in compromised ventricular filling as a consequence of hampered relaxation of myocardium. From this (and only this) point of view, the phosphodiesterase inhibitory action of some Ca^{2+} sensitizers, like levosimendan or pimobendan, may be beneficial [11, 15].

2. CONCEPT OF MYOSIN ACTIVATION: OMECAMTIV MECARBIL AND ITS MECHANISM OF ACTION

The ideal inotropic agent (1) should enhance cardiac pumping selectively without altering other parameters of cardiac function, (2) should not increase substantially cardiac energy consumption, and (3) should not increase the incidence of cardiac arrhythmias [16]. Since these requirements can hardly be met with interactions upstream to the contractile machinery, the downstream steps came into the focus of the research. The term "positive inotropy with a downstream mechanism of action" represents an alternative approach of "Ca^{2+} sensitization", when Ca^{2+} binding affinity of troponin C in not altered; in contrast, the enhancement of the systolic force is achieved by direct modification of the actin-myosin cycle [14, 17-19]. Myosin motor activator agents differ from Ca^{2+} sensitizers, since they increase cardiac contractility by binding to the heavy chain of the cardiac myosin molecule, as the selective cardiac myosin activator, omecamtiv mecarbil (methyl 4-[(2-fluoro-3-{[N-(6-methylpyridine-3-yl) carbamoyl]amino}phenyl)methyl] piperazine-1 -carboxylate), known also as CK-1827452.

The first structure reported to enhance the ATPase activity of myosin was CK-0156636. Water solubility was improved and protein binding was reduced by substitution of the nitrate group for fluorine in the successor, CK-1032100. The first agent found to increase fractional shortening of cardiac muscle was CK-1122534, but its selectivity was insufficient due to interactions with ATP-sensitive K^+ channels. In the next step CK-1213296 was developed. This compound failed to interfere with cardiac ion channels but suppressed the activity of CYP 1A2. All these unfavorable effects were eliminated from CK-1317138, while the optimal effects were obtained with omecamtiv mecarbil (CK-1827452), which was more effective than its ancestor, by one order of magnitude [20, 21]. The chemical structures of these compounds, including omecamtiv mecarbil, are presented in Fig. (**1**), where the mentioned structural modifications are indicated by circles.

The positive inotropic effect of omecamtiv mecarbil is based on its selective association to the S1 domain of the heavy chain of cardiac β-myosin at the site where the converter domain and the relay helix converge in the vicinity of the force-generating lever arm. The consecutive conformational change in the nucleotide-binding domain of myosin results in enhanced ATPase activity and also in robust alterations of the mechanical properties of myosin head [21]. Due to the allosteric modulation of the nucleotide-binding domain, the inorganic phosphate production, which is the rate-limiting step of the actomyosin cycle, is accelerated by omecamtiv mecarbil. As a consequence, the transition rate between the weakly bound and the strongly bound configurations of the actin-myosin dimer also accelerates [22]. The resultant higher number of force-generating cross-bridges increases the amplitude and lengthens the duration of contractions [22-26]. Indeed, *in vitro* mobility assay experiments, performed with porcine and human myosins, revealed that omecamtiv mecarbil reduced unloaded shortening velocity in accordance with stabilization of the "strongly bound" configuration of myosin heads [27-29].

The ATPase stimulant effect of omecamtiv mecarbil is restricted exclusively to α and β myosin isoforms obtained from cardiac and slow skeletal muscles, independently of the origin of the thin filament. Myosins from fast skeletal or smooth muscles are not affected, but slow skeletal muscles are somewhat sensitive to the drug. Accordingly, omecamtiv mecarbil can display marked effects in healthy (containing both α and β

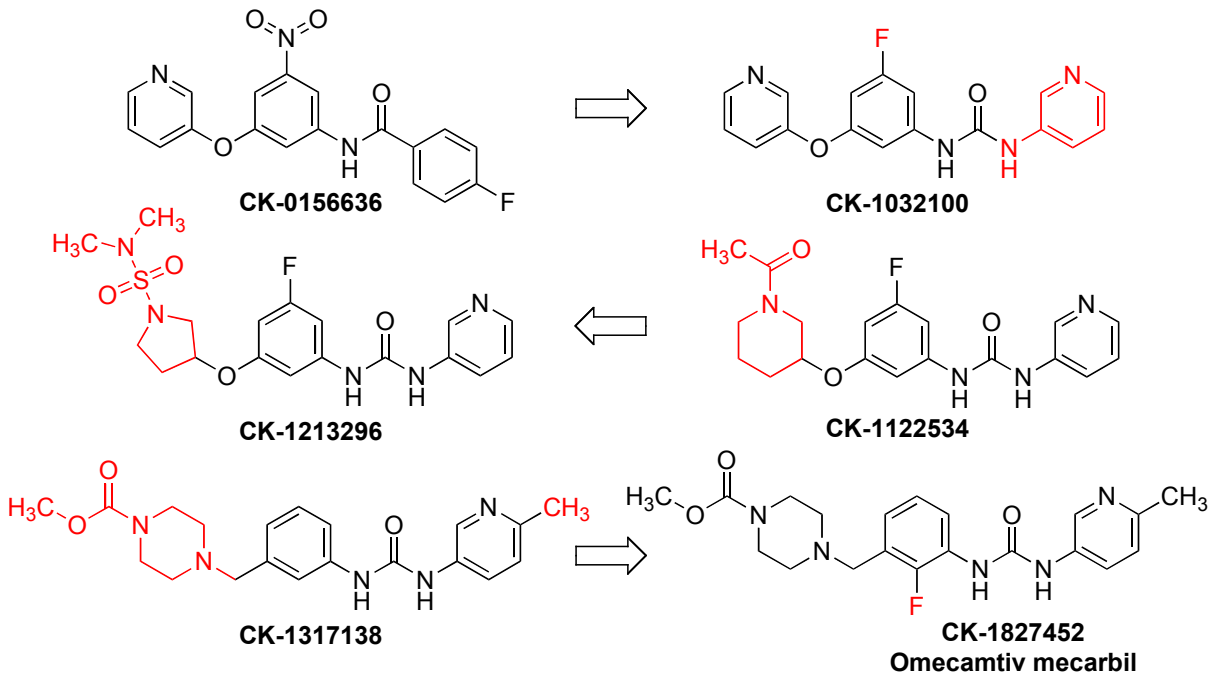

Fig. (1). Chemical structure of omecamtiv mecarbil and its ancestors showing the steps of drug development.

myosins) as well as in failing human hearts (containing almost exclusively the β isoform). Due to similar reasons, omecamtiv mecarbil is uniformly effective in atrial and ventricular myocardium [22, 23, 29-31].

3. CLINICAL STUDIES WITH OMECAMTIV MECARBIL

Based on the robust *in vitro* results and *in vivo* animal studies, omecamtiv mecarbil was tested on 34 healthy volunteers using transthoracic echocardiography in a phase 1 study, designed to evaluate safety, tolerability and pharmacokinetic properties of omecamtiv mecarbil, and to determine the maximum-tolerated dose and plasma concentration of the drug [32]. Left ventricular systolic function increased significantly by omecamtiv mecarbil in a dose-dependent manner ranging between 0.005–1 mg/kg/h doses. The correlation between systolic ejection time and plasma concentration was linear. The highest dose tolerated was 0.5 mg/kg/h. At doses less than 0.5 mg/kg/h, no adverse effect was observed. The dose-limiting toxic effect was myocardial ischemia developing on the basis of the lengthened duration of systole on the expense of diastole [32].

In another dose-ranging study, 45 patients with stable left ventricular systolic dysfunction were involved [33]. The safety and tolerability of omecamtiv mecarbil infusion, applied for 2, 24, and 72 hours, were monitored. Left ventricular ejection time was increased by up to 80 ms, stroke volume by up to 9.7 ml during omecamtiv mecarbil infusion, these changes were accompanied with a moderate reduction of heart rate. On the other hand, both end-systolic and end-diastolic volumes were compromised (by 15 and 16 ml, respectively) at supratherapeutic drug concentrations. Symptoms of myocardial ischemia developed in two patients, who were attributed to the excessive prolongation of systolic ejection time at high plasma levels of 1.35 and 1.75 μg/ml, corresponding to the extremely high concentrations of 3-4 μM [33].

Due to the ischemic complications observed with high doses of omecamtiv mecarbil, the drug was also studied in patients with ischemic heart disease in order to determine the safety and tolerability of the agent in this high risk group of patients [34]. The effect of 20 hours omecamtiv mecarbil infusion was monitored using a treadmill test. Doses of omecamtiv mecarbil producing plasma concentrations of 295 and 550 ng/ml (corresponding to 0.7 and 1.4 μM, respectively), sufficient to increase systolic function, were well tolerated during physical exercise by these patients [34].

Pharmacokinetic properties of omecamtiv mecarbil were characterized in a cohort study [35]. Oral absorption half-life of the drug was 0.62 hour, the elimination half-life 18.5 hour, and the absolute bioavailability was 90%. Plasma concentrations linearly correlated with the effects of omecamtiv mecarbil (increased stroke volume and systolic ejection time) in both healthy volunteers and heart failure patients [35].

A randomized, controlled phase 2b multicenter trial was performed on 606 patients to evaluate the safety and efficacy of omecamtiv mecarbil in individuals suffering from acute heart failure [36]. Based on the ATOMIC-AHF study, it was concluded that omecamtiv mecarbil when reaching peak plasma concentrations of 100-500 ng/ml (corresponding to 0.25-1.25 μM) is well tolerated by the patients. However, the study failed to meet its primary end point since no significant effect on dyspnea could be detected (except for the highest-dose group). In the COSMIC-HF phase-2 multicenter trial, patients with stable symptomatic chronic heart failure were orally treated with omecamtiv mecarbil for 20 weeks [37]. Patients receiving 50 mg omecamtiv mecarbil twice a day developed 318±129 ng/ml peak plasma concentrations. Their systolic ejection time was increased by 25 ms and stroke volume by 3.6 ml, while left ventricular end-systolic and end-diastolic diameters reduced by 1.8 and 1.3 mm, respectively, with the concomitant reduction of heart rate by 3 beats/min [37].

In these studies, the therapeutic plasma concentrations of omecamtiv mecarbil were typically in the submicromolar range, but - as demonstrated above - relatively high plasma concentrations ranging in the micromolar range have also been observed. It must be kept in mind, however, that 82% of omecamtiv mecarbil is bound to plasma proteins, which may make the comparison of clinical data with *in vitro* results difficult [38]. Taken all results together, omecamtiv mecarbil appears to be a promising, clinically effective compound for the treatment of systolic heart failure suggesting that the strategy of myosin motor activation may well be translated into the clinical practice - provided that the concentration of omecamtiv mecarbil can be kept at a reasonably low level. It is not fully understood, however, that what can be expected in cases of overdose, when the plasma concentration of omecamtiv mecarbil may largely exceed the micromolar range. Although phase 2 clinical studies in patients with chronic heart failure yielded quite promising results with omecamtiv mecarbil [32, 33, 36, 37], phase 3 trials are required to clarify all concerns regarding the consequences of overdose. The first phase 3 trial with omecamtiv mecarbil, the GALACTIC-HF study, started this year with 8000 patients, will be finished only in 2021 [39]. Although the efficacy, morbidity and mortality data based on the results of the completed clinical trials (including the reduction of heart size, heart rate and concentration of the circulating biomarker NT-proBNP), are very promising, the potential side effects of supratherapeutic omecamtiv mecarbil concentrations has to be handled with care before that time.

4. RECENT *IN VITRO* AND *IN VIVO* RESULTS OBTAINED WITH OMECAMTIV MECARBIL

4.1. Effect on Cell Shortening

In isolated rat cardiac cells, omecamtiv mecarbil increased fractional shortening and the duration of contraction. These effects were not associated with changes in cytosolic Ca^{2+} concentration [22]. Similar effects were obtained under *in vivo* conditions in anesthetized dogs and rats, where omecamtiv mecarbil displayed more pronounced action in the canine hearts [22]. However, the effects of omecamtiv mecarbil on cell shortening are concentration- and frequency- dependent in canine ventricular cells [40]. As demonstrated in Fig. (**2**), the shortening effect of 1 μM omecamtiv mecarbil was more pronounced on systolic than diastolic cell length, consequently the fractional shortening was increased. The opposite was observed with 10 μM omecamtiv mecarbil, where diastolic cell length shortened to a greater extent than systolic cell length by the drug, leading to the loss of the enhancement of fractional shortening at pacing frequencies of 0.5 and 1 Hz, while *reduction* of fractional shortening was observed at 2 Hz.

Indeed, the frequency-dependence of the action of omecamtiv mecarbil on sarcomere shortening was atypical when it was compared to that of several other inotropic agents. Butler *et al.* reported reduction of sarcomere shortening by 1-3 μM omecamtiv mecarbil at high frequencies (2-3 Hz), while augmentation was seen only at lower ones (0.5-1 Hz) [41]. This unusual frequency-profile may carry important clinical implications suggesting that tachycardic patients treated with omecamtiv mecarbil may be prone to diastolic stiffness and impaired ventricular filling in case of overdose. On the other hand, patients with normal heart rates may also develop tachycardic episodes occasionally, which may further aggravate the mechanical performance of the failing heart. Some defense against tachycardia may be provided by omecamtiv mecarbil itself, since the agent was shown to slightly decrease heart rate [33, 37].

An important advantage of omecamtiv mecarbil, compared to other inotropes, may be its limited interference with transmembrane ion currents [42], predicting a low incidence of drug-induced arrhythmias based on direct interactions with cardiac ion channels.

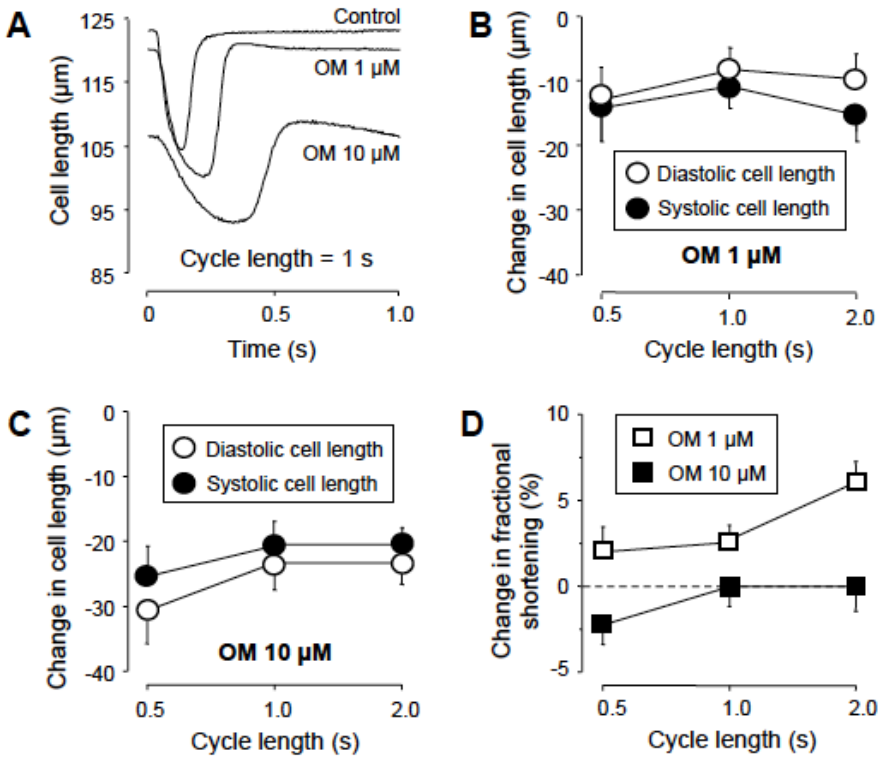

Fig. (2). Effects of 1 and 10 µM omecamtiv mecarbil (OM) on unloaded cell shortening recorded in isolated canine ventricular cardiomyocytes. **A**: Superimposed representative analogue records showing the changes in cell length during stimulation of the cells at a constant pacing cycle length of 1 s. **B, C**: Rate-dependent effects of 1 µM (**B**) and 10 µM (**C**) omecamtiv mecarbil in diastolic (open symbols) and systolic (closed symbols) cell length. Data are summarized as fractional shortening in panel **D**. Symbols and bars are mean±SEM values obtained in 5 myocytes.

4.2. Effect on Oxygen Consumption

Cardiac oxygen consumption was reported to be close to normal in the early studies with omecamtiv mecarbil. In line with this, the actin-independent inorganic phosphate release was not elevated [22, 23]. Although this was somewhat confusing in light of the known enhancement of myosin ATPase activity by the drug (which *per se* predicts an elevated of oxygen demand), it was concluded that the energy utilization of the heart can be made more effective by omecamtiv mecarbil [43, 44]. The mammalian heart appeared to tolerate myosin motor tuning well even for a longer period of time. Indeed, transgenic rabbits containing α and β isoforms of myosin at the ratio of 1:1 had normal lifespan and failed to develop cardiomyopathy in response to overdrive - in contrast to the hearts of similarly handled normal littermates [45, 46].

However, in a recent work of Bakkehaug *et al.* [47], where the effect of omecamtiv mecarbil was studied on oxygen consumption of porcine and murine hearts, omecamtiv mecarbil significantly *increased* oxygen consumption under both *in vivo* and *ex vivo* conditions. The elevated oxygen utilization was due to the increased ATPase activity of myosin, since it could be restored by the ATPase inhibitor 2,3-butanedione monoxide. It was concluded that omecamtiv mecarbil increases the ATPase activity in the resting cardiac muscle as well, resulting in a significant waste of oxygen and impaired cardiac efficiency [47]. This might also contribute to the development of symptoms of angina reported with high, suratherapeutic doses of omecamtiv mecarbil [32, 33].

Teerlink *et al.* [47] questioned the validity of the above results of Bakkehaug *et al.* [47]. Their argumentation was based on the fact that the experiments of Bakkehaug *et al.* had been performed in anesthetized animals, without applying a placebo-control group, and under conditions that might elevate the sympathetic activity or causing ischemic damage [48]. In their response, Bakkehaug *et al.* [49] maintained that their study raised serious concerns about the energetic effects of omecamtiv mecarbil reported by the Teerlink group, and argued in support of adequacy of the Suga pig model being applied in their experiments. Clearly, further independent studies are required to elucidate this important detail.

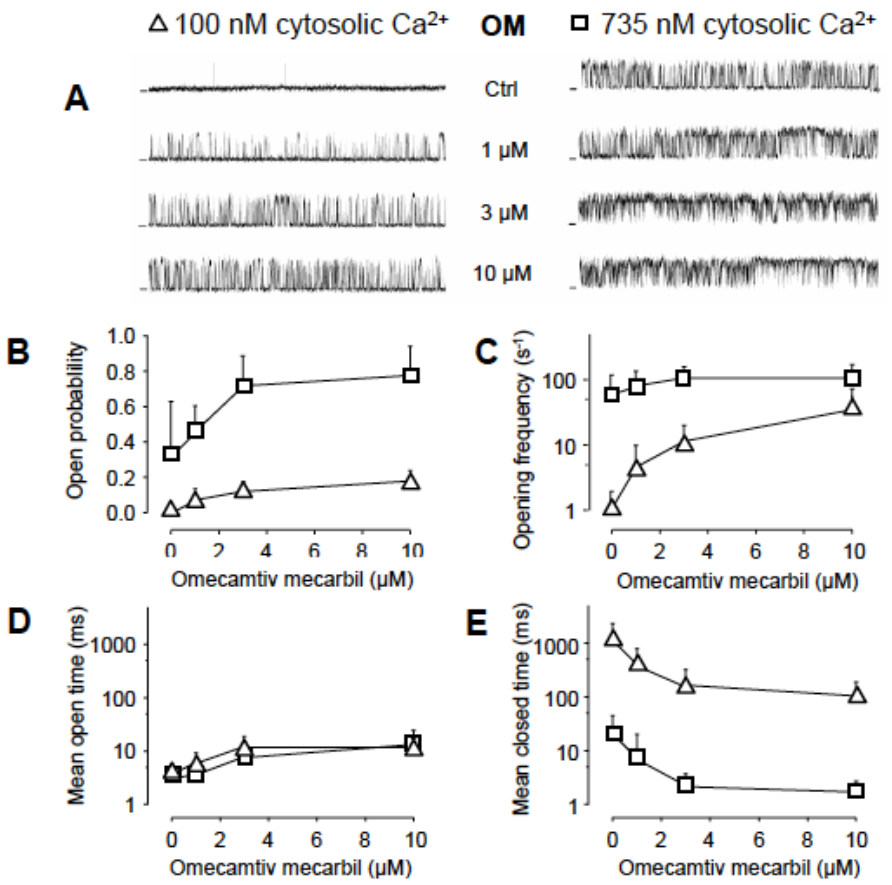

Fig. (3). Concentration dependent effects of omecamtiv mecarbil on canine single RyR-2 channels incorporated into planar lipid bilayers. **A**: Representative analogue current records displaying the activity of the channel in the presence of diastolic (100 nM) and close to systolic (735 nM) values of cytosolic calcium. Bars at the left side indicate zero current, corresponding to closed channel state. **B-E**: Concentration dependent effects of omecamtiv mecarbil on open probability (**B**), opening frequency (**C**), mean open time (**D**), and mean closed time (**E**) of RyR-2 channels. Symbols and bars indicate mean±SEM values obtained from 6-12 experiments.

4.3. Effect on Cardiac Ryanodine Receptors

The effect of omecamtiv mecarbil on canine RyR-2 was studied by monitoring the electrical activity of single RyR-2 channels incorporated into planar lipid bilayer [50]. Omecamtiv mecarbil (1-10 μM) significantly increased the open probability and opening frequency of RyR-2. Accordingly, the mean closed time was markedly reduced while the mean open time moderately increased, indicating that increased open probability was mainly due to the increased frequency of opening in the presence of omecamtiv mecarbil (Fig. **3**). These effects of omecamtiv mecarbil were fully reversible and were the greatest when 100 nM Ca^{2+} concentration, corresponding to the physiological diastolic Ca^{2+} level, was applied in the cytosolic compartment. Increasing the cytosolic Ca^{2+} to 735 nM (a value close to systolic Ca^{2+} level) resulted in an elevated baseline RyR-2 activity, leaving less room for the stimulating effect of omecamtiv mecarbil.

Theoretically, pharmacological activation of the RyR-2 might be expected to enhance the contractility - provided that this effect is more pronounced during systole than diastole. Unfortunately, according to the results shown in Fig. (**3C**), the opposite was the case, since the opening frequency increased several-fold by 1 μM omecamtiv mecarbil in the presence of 100 nM cytosolic Ca^{2+}, while only negligible effect was observed in the presence of 735 nM Ca^{2+}.

Elevation of open probability of RyR-2 is potentially proarrhythmic because the continuous leakage of Ca^{2+} of the SR may cause afterdepolarizations resulting in arrhythmias. More importantly, this Ca^{2+} leak may increase diastolic Ca^{2+} in the fuzzy space and may decrease the Ca^{2+} content of the SR. This is unfavorable in terms of pumping activity - especially in a failing heart - and may contribute to diastolic stiffness, observed occasionally with omecamtiv mecarbil under conditions of overdose. Since the direct stimulatory

action of omecamtiv mecarbil on RyR-2 was evident at the concentration of 1 μM, it has to be taken into account when discussing the mechanism of action as well as the potential side effects of the compound.

CONCLUSION

In this paper, the mechanism of action of the myosin motor activator omecamtiv mecarbil has been discussed in details. Omecamtiv mecarbil was designed to support the cardiac function of patients with heart failure by utilizing their unique ability to increase the number of force generating actomyosin cross bridges. The molecule is currently in human phase 3, and based on the previous and current preliminary clinical results, its further career is promising [51-54]. The above discussed concerns do not question the beneficial effects of omecamtiv mecarbil on the failing heart when it is applied at therapeutic concentrations. It must be kept in mind, however, that omecamtiv mecarbil is not so selective and effective as it was believed to be earlier, because it failed to decrease the oxygen demand of the heart, and it may decrease rather than increase the force of contraction at higher heart rates, and finally, activates RyR-2 receptors as well. These effects may likely develop in patients with omecamtiv mecarbil overdose. Therefore extra care has to be taken in cases of overdose if the heart rate is high, since diastolic failure due to diastolic stiffness combined with angina may be anticipated in these cases.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by a grant provided to JA from the National Research Development and Innovation Office (NKFIH PD112199). JA was also supported by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences, Lajos Szodoray Scholarship of the University of Debrecen and by the Campus Hungary Mobility Program.

We would like to say thanks to Attila Mándi (University of Debrecen, Department of Organic Chemistry) for his help in making this figure.

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