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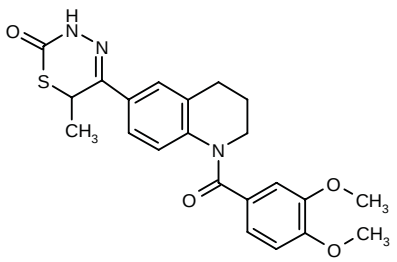
EMD-57033

Treatment of Heart Failure
Calcium Sensitizer

(+)-5-[1-(3,4-Dimethoxybenzoyl)-1,2,3,4-tetrahydroquinolin-6-yl]-6-methyl-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-one



$C_{22}H_{23}N_3O_4S$ Mol wt: 425.5100

CAS: 147527-31-9
CAS: 148714-88-9 (as [–]-enantiomer)
CAS: 120223-04-3 (as racemate)

EN: 216364

Synthesis*

Acylation of 1-acetyl-1,2,3,4-tetrahydroquinoline (I) with 2-chloropropionyl chloride (II) by means of $AlCl_3$ provides the quinoline derivative (III), which is deacetylated by treatment with HCl to give 1,2,3,4-tetrahydro-6-(2-chloropropionyl)quinoline (IV). Condensation of compound (IV) with *O*-ethyl hydrazinethioformate (V) in refluxing acetonitrile yields the thiadiazinone (VI), which is then *N*-acylated at the quinoline ring with 3,4-dimethoxybenzoyl chloride (VII) by means of Et_3N in methylene chloride to give the racemate EMD-53998 [*rac*-(VIII)] (1). Optical resolution of EMD-53998 can be performed by two different ways: a) enantioseparation via chromatography with Chiralpak AD in 100% EtOH as eluent (2) and b) acylation of EMD-53998 with (*S*)-camphanoyl chloride (IX) by means of Et_3N in methylene chloride followed by treatment with morpholine in the same solvent to afford a mixture of (–)-EMD-53998 (EMD-57439) and the camphanoyl amide (X), which are separated by chromatography. Finally, the (+)-enantiomer, EMD-57033, is isolated by further treatment of amide (X) with morpholine in methylene chloride (3). Scheme 1.

Introduction

Hypertension and ischemic heart disease lead to heart failure, defined as the inability of the heart to pump sufficient nutrients to the metabolizing cells of the body. This lethal condition is increasing in incidence, indicating that available treatments need to be further improved. Current therapeutic options include drugs to increase the force of contraction of the heart, the positive inotropic compounds. The therapeutically used positive inotropic drugs act by increasing the intracellular concentration of Ca^{2+} in cardiac myocytes by inhibiting the Na^+/K^+-ATPase (digoxin) or by cAMP-dependent mechanisms (β-adrenoceptor agonists, phosphodiesterase inhibitors) since Ca^{2+} is the critical mediator of excitation-contraction coupling. However, the elevation of cAMP and/or cytosolic Ca^{2+} is considered to be proarrhythmic and the risk of arrhythmias is already increased in the ischemic failing heart. In addition, increasing the amount of Ca^{2+} cycled with each beat will increase the energy requirements of the cell. This increased energy requirement will further reduce the efficiency of the failing heart. Thus, the use of positive inotropic compounds has declined since clinical trials have shown either no improvement in patient survival (digoxin) (4) or an increased mortality (cAMP-dependent drugs) (5, 6).

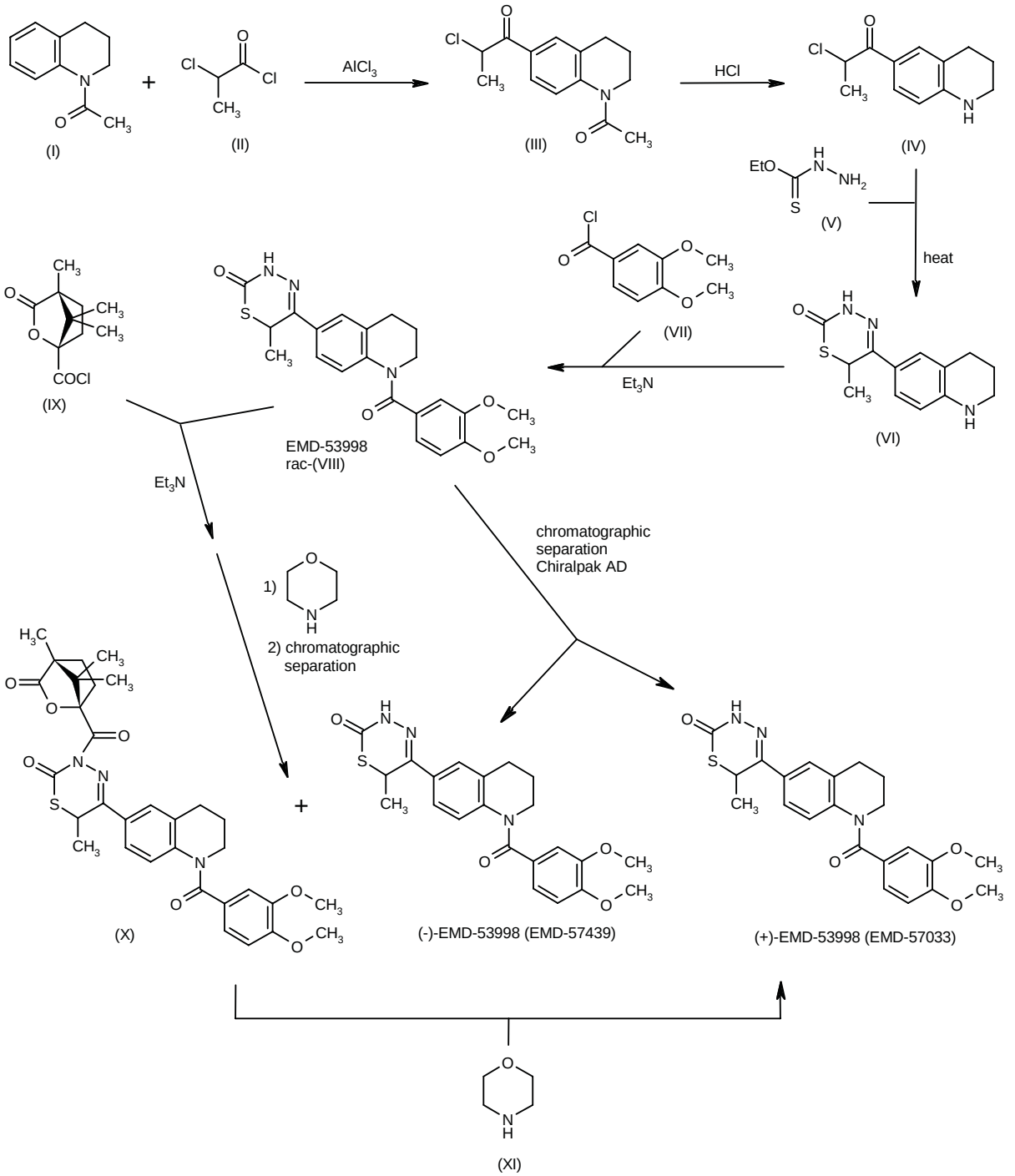
An alternative concept is to increase the binding of Ca^{2+} to the contractile proteins so as to generate more force for a given cytosolic Ca^{2+} concentration. Such Ca^{2+} sensitizers are likely to be selective positive inotropic compounds with minimal changes in myocardial energy requirements and less potential to be arrhythmogenic, as they do not increase cytosolic Ca^{2+} concentrations.

Pharmacological Actions of EMD-53998

EMD-53998, a racemic mixture of the (+)-enantiomer EMD-57033 and the (–)-enantiomer EMD-57439,

Sheila A. Doggrell[1+], Lindsay Brown[1], J. Castañer[2*]. [1]Department of Physiology and Pharmacology, School of Biomedical Sciences, The University of Queensland, Queensland, Australia; [2]Prous Science, P.O. Box 540, 08080 Barcelona, Spain. [+]Author for correspondence.

Scheme 1: Synthesis of EMD-57033



increases the force of contraction of cardiac muscle. For example, EMD-53998 (0.5-5 μM) concentration-dependently increased left ventricular systolic pressure in isolated rat hearts. There are two mechanisms underlying this positive inotropic response: the first is Ca^{2+} sensitization. The Ca^{2+} sensitizer action was shown in isolated left ventricular myocytes loaded with the ester derivative of the Ca^{2+} probe indo-1, where EMD-53998 (0.5-5 μM) enhanced twitch amplitude without increasing the associated Ca^{2+} transient. The Ca^{2+} concentration-response curve was shifted to lower concentrations, indicating an increased sensitivity by EMD-53998 in rat myocytes and skinned fibers from porcine ventricular muscle and human failing heart (7, 8).

The second mechanism underlying the positive inotropic effect of EMD-53998 is selective inhibition of phosphodiesterase III (PDE III). EMD-53998 inhibited guinea pig PDE III with an IC_{50} of 60 nM, but had little effect on the other PDE isoenzymes. Associated with this inhibition, there was an increase in cAMP content of rat ventricles and a potentiation of the cAMP elevating action of isoprenaline (7).

The functional response of inhibition of PDE III by EMD-53998 was shown in the isolated ferret papillary muscle. In muscles loaded with aequorin to measure intracellular Ca^{2+}, EMD-53998 at 5 μM increased tension by 240% and the Ca^{2+} transient by 85%. The increase in the Ca^{2+} transient is not due to Ca^{2+} sensitization as the transient was abolished in the presence of guanethidine to prevent noradrenaline release. This shows that EMD-53998 is acting via the sympathetic pathway, presumably by inhibiting PDE III. EMD-53998 also caused a small prolongation in time course of contraction and a small reduction in the time course of the Ca^{2+} transient. At much higher concentrations (≥ 200 μM), EMD-53998 also increased the resting tension of the ferret papillary muscle (9).

The Ca^{2+} sensitizers should have energetic advantages over other positive inotropic compounds. In 6 isolated beating blood-perfused canine hearts, infusion of $CaCl_2$ or EMD-53998 (5.4 μM) increased contraction and myocardial oxygen consumption. As no difference could be detected between $CaCl_2$ or EMD-53998 in the interrelationship between contractility and oxygen consumption, it was concluded that EMD-53998 did not provide an energetic advantage over the currently used positive inotropic drugs. However, the concentration of EMD-53998 that was used would have inhibited phosphodiesterase in addition to Ca^{2+} sensitization, and this may explain why no beneficial effect was observed on oxygen consumption (10). In contrast, another study has shown energetic advantages of EMD-57033, the Ca^{2+} sensitizing (+)-enantiomer of EMD-53988, over other positive inotropic compounds on the guinea pig trabeculae; this is discussed under EMD-57033 (11).

Although positive isotropic compounds are usually investigated in healthy cardiac tissues, these agents are being developed for use in diseased hearts. Thus, it is more appropriate and important to investigate the effects of these agents in models of cardiac disease. In hypoxic or ischemic conditions, inorganic phosphate accumulates and lowers the contractility of the myocardium by lowering the Ca^{2+} responsiveness of the myofilaments. EMD-53998 antagonized this effect of phosphate on the contractility in pig ventricular trabeculae, suggesting that EMD-53998 may be beneficial in the ischemic heart (12).

In normal ferret papillary muscle, EMD-53998 had a small but significant negative lusitropic effect that was considered unlikely to have any clinical significance. However, in a neonatal rat cell culture model of myocardial hypoxia, EMD-53998 caused a marked impairment of relaxation, possibly due to the impaired calcium sequestration and increased calcium availability exhibited by hypoxic myocytes. This suggests that Ca^{2+} sensitizers may have a deleterious effect on relaxation in the ischemic myocardium (13).

The positive inotropic effects of EMD-53998 were preserved on isolated tissues from human failing hearts. Thus, EMD-53998 increased force of contraction on the human left ventricular papillary muscle strips from terminally failing hearts (14).

Separation of EMD-53998 into its (+)- and (–)-enantiomers, EMD-57033 and EMD-57439, respectively, led to the discovery that the calcium sensitizing action resided in EMD-57033 while EMD-57439 selectively inhibited phosphodiesterase (15-18).

Pharmacological Actions of EMD-57033

Isolated cardiac cells/trabeculae

There is compelling evidence that EMD-57033 is a Ca^{2+} sensitizer. In guinea pig myocytes loaded with the ester form of the fluorescent probe indo-1, EMD-57033 (0.5-1.5 μM) increased the twitch amplitude without altering the Ca^{2+} transient. Similarly, in canine ventricular myocytes, EMD-57033 (3 μM) increased the extent of shortening during twitch contractions without increasing the peak amplitude of the Ca^{2+} transient. In unstimulated guinea pig cells, EMD-57033 reduced the resting length and indo-fluorescence. In the presence of 2,3-butanedione monoxime (BDM), which reduces twitch amplitude by inhibiting cross-bridge mechanics, EMD-57033 restored the twitch contraction to the pre-BDM levels without augmenting the Ca^{2+} transient. EMD-57033 also caused sensitization of the Ca^{2+} concentration response curve in skinned cardiac fibers with an EC_{50} of 1.7 μM (15, 16, 18).

In isolated human nonfailing right auricular trabeculae, EMD-57033 at 30 μM increased the force of contraction, peak rate of rise of contraction and peak rate of relaxation. The effect on rate of relaxation was more marked than on the rate of increase, and the time of contraction was increased. EMD-57033 had no effect on diastolic or systolic levels of intracellular Ca^{2+} as detected by the fura 2 ratio method (19).

High concentrations of EMD-57033 inhibit phosphodiesterase. The IC_{50} for inhibition of PDE III is 1.94 μM with EMD-57033, making it about 30 times less potent than EMD-57439 (IC_{50} = 60 nM) in inhibiting this enzyme. The cAMP content of isoprenaline-stimulated rat cardiac myocytes was increased with EMD-57033 (13.6 μM) by 50% (16).

Mechanisms underlying Ca^{2+} sensitization

The most logical target for Ca^{2+} sensitizers is cardiac troponin C since this contains the Ca^{2+} binding receptor on the thin filament of cardiac muscle. Once Ca^{2+} binds, hydrophobic residues are exposed which are essential for

the binding of troponin I, transmission of the Ca^{2+} signal to other proteins in the thin filament, and ultimately to activation of the myosin-actin ATPase reaction. Recent NMR studies have shown that EMD-57033 binds stereospecifically in the hydrophobic cleft of the Ca^{2+}-saturated C-domain of human cardiac troponin C; this explains why EMD-57439, the (–)-isomer, is inactive as a Ca^{2+} sensitizer. The binding of the inhibitory region of troponin I to troponin C is a major switch between muscle contraction and relaxation by moving between troponin C and actin-tropomyosin; this interaction between troponins I and C was not inhibited by EMD-57033. EMD-57033 may compete with another epitope of troponin I to enhance the Ca^{2+}-dependent binding of troponin I to troponin C, thus increasing the apparent Ca^{2+} sensitivity of the contractile system. This direct interaction of EMD-57033 with the contractile system of the human myocardium increases the rate of cross-bridge attachment in the strongly bound force-generating state. This is not the only possible mechanism of Ca^{2+}-sensitization; the Ca^{2+}-sensitizer CGP-48506 appears to improve myofilament cooperativity in the human heart (20-24).

Effects in intact hearts

In the paced-Langendorff-perfused rat heart, both EMD-57033 (2 μM) and the β-adrenoceptor agonist, dobutamine (0.2 μM), increased developed pressure by 40%. This positive inotropic effect was associated with increased myocardial oxygen consumption with dobutamine but not EMD-57033. Lactate production and basal metabolism were unaffected by either agent (25).

The positive inotropic effect of EMD-57033 is associated with a prolongation of relaxation. In isolated perfused rabbit hearts, EMD-57033 decreased time to peak isovolumic pressure and prolonged time to 50% pressure decline, and these changes were greater at slower heart rates or lower $[Ca^{2+}]_0$. The increase in developed pressure and oxygen consumption with EMD-57033 was greater for ejecting than isovolumic beats. In isovolumically beating isolated rabbit hearts, EMD-57033 at 2 μM induced positive inotropy without altering diastolic tone but caused a modest prolongation of contraction duration due both to positive inotropy and a direct lusitropic effect (26, 27).

In vivo *with or without exercise*

The positive inotropic effect of EMD-57033 is maintained in the intact animal. In anesthetized rats and dogs and conscious dogs, EMD-57033 (30 mg/kg i.p. in rats and 3 mg/kg in dogs) induced positive inotropy measured as LV first derivative of change in systolic pressure over time (dP/dt_{max}) without affecting blood pressure or heart rate. In awake pigs under resting conditions, EMD-57033 (0.2-0.8 mg/kg/min i.v.) increased LV dP/dt_{max} up to 65% and stroke volume up to 20%. There was an increase in heart rate only with the highest dose, while mean aortic blood pressure and LV dP/dt_{min} were not altered. EMD-57033 also had no effect on pulmonary vascular resistance but decreased systemic and coronary vascular resistance (28, 29).

During treadmill exercise of pigs, the positive inotropic actions of EMD-57033 gradually waned at higher levels of exercise. This waning was probably caused by the exercise-induced increase in β-adrenoceptor activity, because after pretreatment with propranolol, the positive inotropic actions of EMD-57033 were preserved at all levels of exercise. The positive inotropic effects of EMD-57033 in the presence of propranolol were not altered by the α-adrenoceptor antagonist phentolamine, and this indicated that the positive inotropism is not dependent on intact α-adrenoceptor activity. Therefore, the authors suggested that EMD-57033 might be most effective in patients with severe loss of β-adrenoceptor responsiveness, as in heart failure (29).

Energy cost

As mentioned previously, in the paced Langendorff-perfused rat heart, EMD-57033 at 2 μM induced positive inotropy without increasing myocardial oxygen consumption (25).

The rate of heat production by cardiac tissue can be used as a measure of the rate of ATP hydrolysis. In guinea pig trabeculae, both EMD-57033 (2.5, 5 and 10 μM) and the cardiac glycoside dihydro-ouabain (5, 10 and 12 μM) increased heat production and contraction. The energy cost of active tension was lower in the presence than in the absence of EMD-57033. Also, the energy cost of the positive inotropic effect of EMD-57033 was only half that of dihydro-ouabain. This shows that EMD-57033 causes a change in cross-bridge kinetics that increases the contractility of cardiac muscle and improves the economy of chemo-mechanical energy transduction (11).

In isolated, electrically stimulated rat cardiomyocytes, for a given enhancement of contractile performance (cell shortening), the increase in energetic demand after application of EMD-57033 was lower than that with elevated extracellular calcium or the beta-agonist isoproterenol. Lowering the pH to 7 decreased the economy of contraction, and this could be restored with EMD-57033 (30).

Effects in disease states

As EMD-57033 is being developed for use in disease states, it is important that it be tested in models of cardiac disease. Volume overload hypertrophy was induced in rabbits 12-15 weeks after an operation to form an arteriovenous shunt between the carotid artery and jugular vein. In myocytes isolated from the hypertrophied hearts, the cell shortening response to EMD-57033 was maintained, whereas the responses to isoprenaline and dobutamine were reduced compared to the responses in

myocytes from normal hearts. EMD-57033 at 1 μM had a similar effect on cell shortening in myocytes from normal and pacing-induced heart failure dogs and did not alter diastolic cell length or resting intracellular Ca^{2+} (31, 32).

In isolated ferret papillary muscles, acidosis caused a shift of the pCa-tension curve to the right, indicating a desensitization of the myofilaments to Ca^{2+}, which could be reversed with EMD-57033. Hypoxia decreased maximum force, and this was not reversed by EMD-57033 (33).

Being an effective positive inotropic compound in the presence of cardiac disease in animal models and the human heart, EMD-57033 increased the force of contraction of cardiac tissues from rats with thyroid dysfunction, diabetes or hypertension. Hyperthyroidism increased the maximal ventricular response to EMD-57033 (30 μM) relative to calcium chloride, whereas hypothyroidism and streptozotocin-induced diabetes decreased these maximal responses. However, a recent study has shown the increase in force of contraction with a submaximal concentration of EMD-57033 (10 μM) was not different in nondiabetic and streptozotocin-diabetic rat papillary muscles; furthermore, responses in isolated myocytes and skinned cardiac fibers were identical in control and diabetic preparations. The slower time course of relaxation observed in diabetic papillary muscles was further prolonged in the presence of EMD-57033 to a similar extent as that observed in nondiabetic muscles. In anesthetized rats, echocardiography showed that intraduodenal administration of EMD-57033 increased left ventricular systolic function without affecting variables of diastolic filling in both groups (34, 35).

Ventricular responses were unchanged in rat models of systemic hypertension but were reduced in pulmonary hypertension. In left ventricular papillary muscles from 2K1C renal hypertensive rats, positive inotropic responses to noradrenaline were increased after 4 weeks but then decreased after 8 weeks; responses to EMD-57033 and calcium chloride were unchanged (Fig. 1). EMD-57033 was a weak positive inotrope in human isolated right atrial trabeculae, where the maximum response was only 14% that of calcium chloride. EMD-57033 produced endothelium-dependent relaxation of rat thoracic aortic rings and positive chronotropy in rat right atria at similar concentrations to those causing positive inotropy (34, 36).

EMD-57033 was also effective in the stunned myocardium or in ischemic contractile failure. In isolated rabbit hearts, stunning with depressed left ventricle function was induced by 30 min of reperfusion that followed a period of no-flow ischemia. EMD-57033 (30 μM) improved systolic and diastolic function, increased coronary blood flow and increased oxygen consumption without positive chronotropic or arrhythmogenic effects. This suggests that EMD-57033 may be useful in treating the poorly functioning reperfused myocardium (37).

Myocardial stunning was produced in porcine myocardium *in vivo* by 15 min of coronary occlusion and 30 min of reperfusion. In the stunned region, EMD-57033

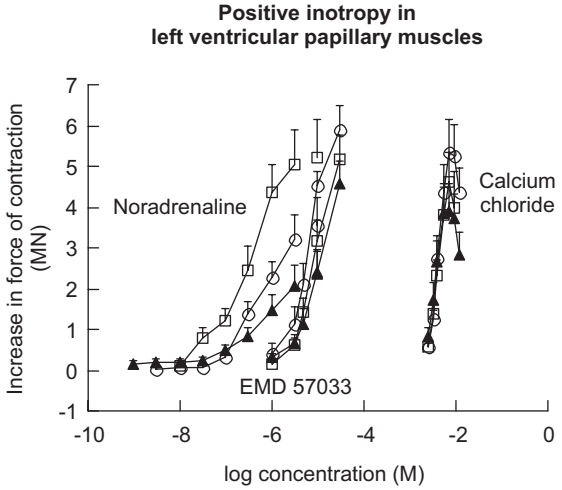


Fig. 1. Positive inotropic responses to noradrenaline, EMD-57033 and calcium chloride in left ventricular papillary muscles from untreated rats (o), 2K1C renal hypertensive rats after 4 weeks (□) and 2K1C renal hypertensive rats after 8 weeks (▲) (n = 6-10). (From Brown, unpublished results.)

(0.2 mg/kg/min for 60 min) increased systolic shortening, end-systolic elastance and maximum rate of fall of myocardial elastance. EMD-57033 also decreased the time constant of the decay of elastance, while end-diastolic elastance remained unchanged. EMD-57033 had similar effects in normal myocardium. EMD-57033 increased cardiac output by up to 27% and LV dP/dt_{max} by 86%. It was concluded that EMD-57033 was a prime candidate for use in acute states of heart failure, because it was a powerful enhancer of systolic performance in vivo at doses that do not exert adverse effects on diastolic function. Further studies showed that the reduction in contractile responses in open-chested anesthetized pigs to intracoronary Ca^{2+} infusion was reduced by EMD-57033 (38, 39).

EMD-57033 (0.6 mg/min/kg) exerted a positive inotropic effect in the canine pacing-induced model of dilated heart failure. Thus, there was an increased peak LV dP/dt with a subsequent increase in cardiac output at the same mean arterial pressure. Another study using the canine pacing model of heart failure confirmed this positive inotropism with the agent (41). Thus, in contrast to the blunted dobutamine responses in heart failure, low-dose EMD-57033 (0.4 mg/kg/min for 20 min) markedly enhanced contractility, doubling end-systolic elastance and raising fractional shortening in conscious dogs with normal and failing hearts. EMD-57033 also improved diastolic function, lowering LV end-diastolic pressure and increasing the filling rate. At equipotent inotropic doses and matched preload, EMD-57033 lowered the oxygen cost of contractility by 11%, whereas it rose 64% with dobutamine and 28% with the phosphodiesterase inhibitor milrinone. Doubling the EMD-57033 dose further augmented the positive inotropy in nonfailing and failing

hearts, accompanied by vasodilatation, increased heart rate and other changes consistent with inhibition of PDE III, but the oxygen cost of contractility remained improved compared with the use of dobutamine (40, 41).

Although EMD-57033 was not arrhythmogenic in the stunned rabbit myocardium, and many other studies of EMD-57033 have not reported proarrhythmia, it may be arrhythmogenic under some circumstances. EMD-57033 was arrhythmogenic in the isolated working heart, where arrhythmias can be induced by increases in ventricular afterload. In this model, EMD-57033 at 2 μM increased the incidence of both ventricular ectopics and complex arrhythmias such as ventricular tachycardia. The mechanism underlying this arrhythmia remains unknown. However, because of this unwanted effect, it may be appropriate to evaluate EMD-57033 in other models of arrhythmia (42, 43).

Relaxation is impaired in heart failure because of abnormal $[Ca^{2+}]_i$ handling; therefore Ca^{2+} sensitizers may potentially increase diastolic force further in failing hearts. In severely failing isolated human heart tissue taken from explanted hearts, EMD-57033 produced positive inotropic effects. In nonfailing human myocardium, EMD-57033 at 50 μM increased the force of contraction by 280%, time to 80% relaxation by 278% and diastolic force by 28%. In trabeculae from failing human myocardium, the positive inotropic effect was similar but the effects on relaxation (378%) and diastolic force (65%) were more pronounced. Although EMD-57033 at 50 μM did not change the peak of the $[Ca^{2+}]_i$ transient, it prolonged the transient and increased the diastolic $[Ca^{2+}]_i$ in both failing and nonfailing heart. Thus, this study suggests that EMD-57033 will have detrimental effects on relaxation and diastolic $[Ca^{2+}]_i$ in the failing human heart. However, the study used high concentrations of EMD-57033 which may have inhibited phosphodiesterase, and this could underlie the detrimental effect. However, in guinea pig myocytes, a concentration of EMD-57033 (3 μM) which probably produces minimal inhibition of phosphodiesterase lengthened the relaxation time by 34% without changing the time to peak contraction. The effects of lower concentrations of EMD-57033 on relaxation and diastolic $[Ca^{2+}]_i$ in human failing heart should be assessed (34, 44-46).

Conclusions

The development of Ca^{2+} sensitizers for use as positive inotropic agents in the treatment of heart failure has been relatively slow. As EMD-57033 has favorable effects on energetics and the positive inotropic effects are maintained in disease, it possesses properties that should be important in the treatment of heart failure.

Manufacturer

Merck KGaA (DE).

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