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Review

Calcium sensitizers: What have we learned over the last 25 years?☆

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ABSTRACT

The use of inotropes for correcting hemodynamic dysfunction in patients with congestive heart failure has been described over many decades. Drugs such as cardiac glycosides, cathecolamines, phosphodiestherase inhibitors, and calcium sensitizers have been in turn proposed. However, the number of new chemical entities in this therapeutic field has been surprisingly low, and the current selection of drugs is limited. One of the paradigm shifts in the discovery for new inotropes was to focus on 'calcium sensitizers' instead of 'calcium mobilizers'. This was designed to lead to the development of safer inotropes, devoid of the complications that arise due to increased intracellular calcium levels. However, only three such calcium sensitizers have been fully developed over the latest 30 years. Moreover, two of these, levosimendan and pimobendan, have multiple molecular targets and other pharmacologic effects in addition to inotropy, such as peripheral vasodilation. More recently, omecamtiv mecarbil was described, which is believed to have a pure inotropy action that is devoid of pleiotropic effects. When the clinical data of these three calcium sensitizers are compared, it appears that the less pure inotropes have the cutting edge over the purer inotrope, due to additional effects during the treatment of a complex syndrome such as acute congested heart failure. This review aims to answer the question whether calcium sensitization *per se* is a sufficient strategy for bringing required clinical benefits to patients with heart failure. This review is dedicated to the memory of Heimo Haikala, a true and passionate innovator in this challenging field.

1. Historical background

In their classification of congestive heart failure, Forrester and Waters [1] recognized that two major hemodynamic dysfunctions can be manifested in this pathologic state: hypoperfusion (patient cold) and congestion (patient wet), either individually or in combination (patient wet and cold). In their seminal paper, Forrester and Waters [1] also indicated which of the treatments that were available at that time was of use according to the different hemodynamic profiles [1]. For patients with hypoperfusion and hypotension, inotropes were recommended (*albeit* with some caution). The definition of inotropy and inotropes (from Greek *in*-, fiber or sinew, plus-*trope*, turning or moving) in those years was closely linked to the regulation of cardiac contractile force *via* effects on ions [2], and especially on calcium which from 1883 was already considered a vital link in the process of contraction and relaxation [3].

The palette of inotropic drugs that were available in the clinic at the time of Forrester and Waters [1] was limited to digoxin, the peptide glucagon, and the cathecolamines (*i.e.*, isoproterenol, norepinephrine, dopamine). It was recognized that these inotropic agents have different pleiotropic hemodynamic effects, and differed predominantly in their effects on arterial pressure.

From the 1970's to the beginning of the 1980's, great efforts were made to develop new inotropic agents. The hemodynamic effects of dobutamine in man were described as early as 1973 [4], while the phosphodiesterase (PDE) inhibitor amrinone was first described in 1978 [5]. The first description of the cardiovascular properties of enoximone (originally MDL17,043) dates from 1982 [6], and one year later, the clinical effects of milrinone (originally WIN47203) were published [7].

All of these drugs, which were fully developed and became available in clinical practice, share a common feature, *i.e.* they increase contraction by mobilizing calcium. Although this is achieved by various mechanisms of action (Fig. 1), it makes these different drugs similar in terms of the reasons behind their inotropic effects. Indeed, they have recently been described collectively as the 'calcium mobilizers' [8].

This strategy of increasing contraction by increasing intracellular calcium handling, however, comes at a price:

☆ In memory of Heimo Haikala, PhD.

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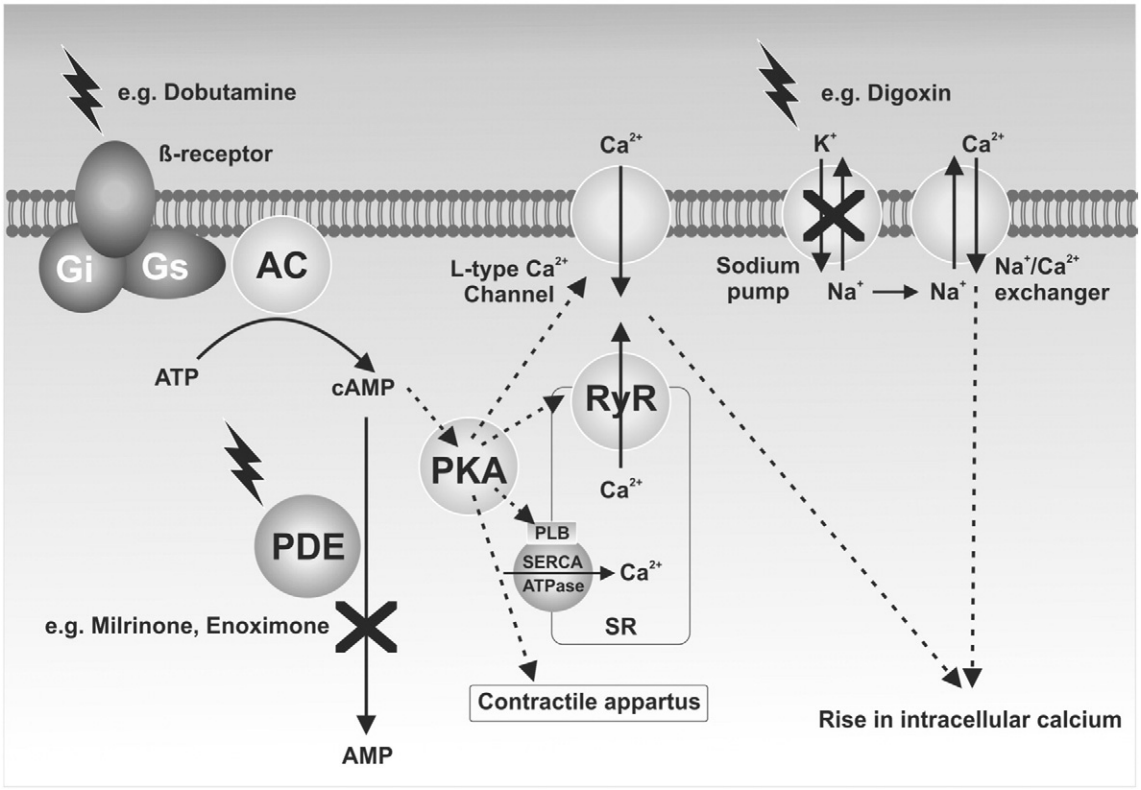

Fig. 1. Mechanisms by which calcium mobilizers increase intracellular calcium. AC, adenylate cyclase; PDE, phosphodiestherase; PKA, protein kinase A; RyR, ryanodine receptor; SERCA ATPase, sarcoplasmic endoplasmic ATP-ase; PLB, phospholamban; SR, sarcoplasmic reticulum.

(i) The increase in oxygen consumption in the myocardium that arises because of the increased need for re-internalization of calcium during diastole can lead to increased risk for ischemic patients; this may foster energy starvation in cardiac cells owing to increased ATP consumption in order to support an increased SERCA activity;
(ii) A further increase in oxygen demand is induced by the chronotropic effect induced by some of the calcium mobilizers (especially those which acts *via* an increase in intracellular cAMP level);
(iii) Drugs acting through modulation of cyclic AMP (*e.g.* cathecolamines, PDE inhibitors) also induce the phosphorylation of troponin I [9] and thus promote calcium desensitization of the contractile apparatus [10] leading to a less efficient contraction;
(iv) Disturbed intracellular calcium homeostasis can lead to ventricular arrhythmia [11] due to early and delayed afterdepolarizations, while unstable intracellular calcium dynamics can promote ventricular extrasystoles and increase the incidence of wave breaks during ventricular fibrillation [12];
(v) The increase in intracellular calcium has been associated with acceleration of myocardial remodeling, and with apoptosis [13];
(vi) Diastolic abnormalities, seen as impaired relaxation and increased diastolic wall stress, are also detrimental consequence of Ca^{2+} overload [14];
(vii) The overall worse prognosis in the mid-term to long-term, whereby the use of dobutamine and PDE inhibitors was specifically investigated in two focused meta-analyses by Tacon *et al.* [15] and by Nony *et al.* [16], respectively. Their conclusion was that these drugs do not provide any benefits in terms of patient survival.

In the 1980's, Solaro [17] and Rüegg [18] suggested that molecules that can sensitize the contractile apparatus to calcium would be better inotropes. The proposed definition of a calcium sensitizer since that time has been a molecule that modulates the contractile force without inducing any changes in the calcium transient. The expectations were that such molecules would not increase oxygen consumption, would not induce arrhythmia, would not have any detrimental effects as regards remodeling or apoptosis, and would not be associated with bad outcome when used in severely decompensated patients.

Also at that time, there was convincing evidence that the cardiac myofibrillar receptor that activates the actin–myosin interaction is troponin C [19]. It was thus straightforward to select troponin as a molecular target for the development of further calcium sensitizers. However, while many such molecules were tested starting from the early 1980's [17], very few underwent complete clinical development. Two

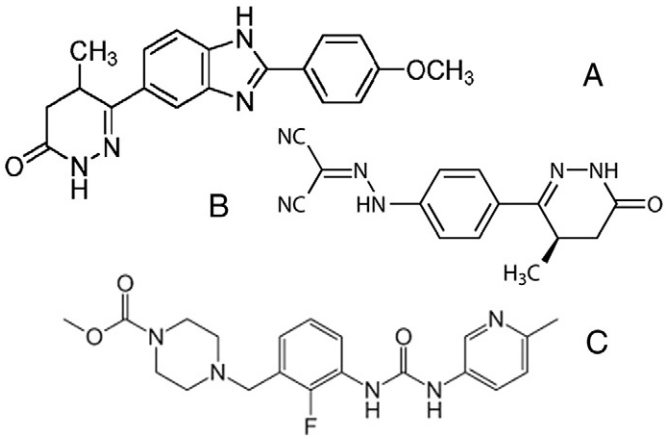

Fig. 2. Chemical structure of the 'calcium sensitizers' pimobendan (A), levosimendan (B) and omecamtiv mecarbil (C).

troponin-targeting drugs that were proposed to be calcium sensitizers in the literature were pimobendan [20] in 1984, and levosimendan [21] in 1994 (Fig. 2). These two drugs also entered into clinical practice for human use, pimobendan from the 1990's, although in Japan only [22], and levosimendan from early 2000 [23], and now across 60 countries mainly in Europe and Latin America, although not yet in the USA or Japan.

Neither pimobendan nor levosimendan, however, are pure calcium sensitizers. Pimobendan is predominantly a PDE inhibitor [24,25], while levosimendan, in addition to calcium sensitization, also has some important pleiotropic effects through the opening of ATP-dependent potassium channels, and at higher, supra-therapeutic doses, as a very selective PDEIII inhibitor [26].

Then, after almost two decades of hiatus from the first reports on levosimendan, a new molecule was discovered, omecamtiv mecarbil (originally CK-1827452) [27,28], which has now reached the late phases of development (Fig. 2) [29]. This new drug candidate was defined as a 'myosin activator', and it represents a putatively 'purer' form of sensitization of the contractile apparatus, without other additional effects.

All in all, at Oct. 20, 2015, a search for "levosimendan OR pimobendan OR omecamtiv" in PubMed provides 216 human clinical trials, of which 181 describe levosimendan, 32 pimobendan, and 3 omecamtiv mecarbil.

This review is aimed at answering three questions:

(1) What have we learnt about the calcium sensitizers from the clinical data collected over these last 25 years?
(2) Are we in a condition to understand what kind of patient will, or might, gain the greatest benefits from a calcium sensitizer?
(3) Are there some downsides in the class action of the pure calcium sensitizers that would make them inferior to molecules with additional pleiotropic effects?

This review will not describe the other calcium sensitizer compounds that did not complete the early phases of development, or for which no clinical data have been collected. It would indeed be too speculative to comment on their possible effects in human. Moreover, these other calcium sensitizers were not even fully pharmacologically characterized, which would leave us blind to their possible pleiotropic effects on other targets.

2. Pimobendan: Mechanism of action and pharmacology

The pharmacology of pimobendan was reviewed by Fitton and Brodgen [30]. Pimobendan has been described as a cardiotonic vasodilator (inodilator) that derives its inotropic activity from a combination of PDE inhibition and calcium sensitization of the myocardial contractile proteins. It has an active metabolite (UD-CG 212) that contributes to its pharmacological effects *in vivo*. Both intravenous and oral formulations of pimobendan have been developed.

3. Pimobendan: Clinical data

The first clinical trial with pimobendan was described in 1988 [31]. In the clinical program for the development of pimobendan, which mainly considered oral pimobendan, improvements in New York Heart Association functional class were observed in patients with chronic heart failure who received adjunctive treatment with pimobendan. Moreover, in patients with moderate to severe chronic congestive heart failure, there was a reduction in hospitalization rates and an improvement in quality of life after 3 months and 6 months of adjunctive therapy with oral pimobendan.

Pimobendan has been tested in the settings of both acute heart failure and chronic heart failure, although data on the effects of pimobendan on mortality are scarce. Only two human clinical trials have described mortality outcomes, and of these, only the PICO trial was randomized [32]. The PICO trial included 317 patients with stable symptomatic heart failure, and oral pimobendan was well tolerated and improved exercise capacity. However, the mean mortality was 12.0% in the pimobendan arms *versus* 5.6% in the placebo arm. The clinical development of pimobendan was discontinued in 1996, although on the basis of the existing clinical data, market authorization was granted in Japan. An oral formulation is used worldwide in veterinary medicine.

4. Levosimendan: Mechanism of action and pharmacology

Levosimendan was discovered by running several compounds through an affinity chromatography column that included human recombinant troponin C, first in the presence of calcium, and then in the absence of calcium [33]. The calcium-dependent binding of levosimendan to troponin was defined by its differential elution times. Levosimendan was shown to increase cardiac contractility by calcium sensitisation of troponin C [21,33–38]. Very early on, however, it appeared that levosimendan also has molecular targets other than cardiac troponin C, and other pharmacological actions other than inotropy [26]. The additional pharmacological effects of levosimendan are: (i) vasodilation, through the opening of potassium channels on the sarcolemma of smooth muscle cells in the vasculature [39–42]; and (ii) cardioprotection, through the opening of mitochondrial potassium channels in cardiomyocytes [43–46]. Additionally, it was demonstrated *in vitro* that levosimendan is a very potent and selective PDEIII inhibitor [47,48]. However, it appears that this last mechanism of action of levosimendan contributes to the pharmacological effects during its clinical application only at higher doses [26]. Interestingly, levosimendan pretreatment can decrease infarct size in an ischemia–reperfusion model, and can improve recovery of cardiac function following global ischemia [49]. Moreover, levosimendan improved the survival rate in a healed myocardial infarction model [50]. The pharmacological and clinical effects of levosimendan are also explained by the presence of its active metabolite, OR-1896 [26]. Both intravenous and oral formulations of levosimendan have been developed, although only the i.v. formulation reached the market.

5. Levosimendan: Clinical data

The clinical effects of levosimendan were reviewed recently by Nieminen *et al.* [23]. Levosimendan has been shown to improve hemodynamics [51–53] without a significant increase in oxygen consumption [54,55], to reduce symptoms of acute heart failure [51,52,56,57], to have beneficial effects on neurohormone levels [56–59], to have sustained efficacy due to formation of an active metabolite [58,60], and not to suffer any loss of effect in patients under β-blockade [51,61] nor under amiodarone [62] or sulfunilurea [63] treatments. Levosimendan offers a predictable safety profile [51–53,57], no impairment of diastolic function [64,65], and no development of tolerance [60]. The most common adverse events of levosimendan are hypotension, headache, atrial fibrillation, hypokalemia and tachycardia [56,57]. Several meta-analyses on the effects of levosimendan on mortality have been published recently [66–70], and among these, the investigations by Landoni *et al.* [67,70] are the most comprehensive. These studies included 45–48 clinical trials with intravenous levosimendan for a total of nearly 6000 patients. Levosimendan was associated with a significant reduction in mortality, despite two large phase III studies, REVIVE [56] and SURVIVE [57], that had neutral effects on this outcome.

6. Omecamtiv: Mechanism of action and pharmacology

Omecamtiv mecarbil was discovered [27] and characterized [28] less than 10 years ago. This new drug candidate is defined as a 'myosin activator' that facilitates action-myosin cross bridge formation, increases

the number of myosin heads involved into the force generation, and stimulates myosin ATPase. It was described also as a calcium sensitizer [71], and a putatively purer inotrope, without additional effects. The calcium sensitivity of force production by omecamtiv mecarbil was described in detail by Nagy *et al.* [71]. Interestingly, the omecamtiv mecarbil evoked calcium sensitization was also demonstrated in skeletal muscle fibers of the diaphragm with intrinsically slow kinetics [71]. Omecamtiv mecarbil has not yet been fully characterized, although in a recent report, it was associated to both increased myocardial oxygen consumption and impaired cardiac efficiency [72].

7. Omecamtiv: Clinical data

Only three clinical trials involving omecamtiv mecarbil have been published to date. A preliminary study on 34 healthy subjects showed highly dose-dependent augmentation of left ventricular systolic function in response to omecamtiv mecarbil [73]. A proof-of-concept study by Cleland *et al.* [29] on 45 patients with stable heart failure and left ventricular systolic dysfunction showed that omecamtiv mecarbil improves cardiac function. Neither of these studies described mortality. A recent 600-patient phase II clinical trial (ATOMIC-AHF) has shown an increase in plasma troponin I levels upon omecamtiv mecarbil administration, nevertheless a clear relationship between plasma troponin I and omecamtiv mecarbil concentrations could not be established [74]. This might represent just chance, or it might be somehow related to the signs of cardiac ischemia described by the previous trials [29,73]. It appeared thus critical to evaluate omecamtiv mecarbil in patients with ischemic cardiomyopathy and angina during exercise. On the bright side, data from a recent study [75] in which omecamtiv was administered at first intravenously and then orally in patients with ischemic cardiomyopathy and angina do not show additional risk of development of ischemia by the drug. The study, however, was underpowered.

8. Is there a pattern?

Any inotropic effects through calcium mobilization are achieved by increasing the calcium load in cardiomyocytes, which has been shown to worsen ischemia and to increase the risk for arrhythmia, apoptosis, and remodeling, and to worsen patient outcome. Calcium sensitization was proposed as an alternative way for a safer inotropic effect. There is indeed a physiological mechanism for the regulation of the calcium sensitivity of the contractile apparatus, *i.e.* the cAMP-protein kinase A-dependent phosphorylation/dephosphorylation of troponin I, which in turn regulates the calcium binding affinity of troponin C. The calcium sensitivity of troponin C can also be modified by drugs that stabilize its active form, or that interact with other components of the contractile apparatus.

Very few calcium sensitizers, however, have been fully characterized and developed. Both preclinical and clinical data are described in the literature only for pimobendan, levosimendan and omecamtiv mecarbil. These three drugs differ profoundly not only in their molecular targets and mechanisms of action, but also in their clinical effects and adverse events (Table 1). Neither pimobendan nor levosimendan can be described as pure calcium sensitizers, with pimobendan showing a dominant PDE inhibitory activity, and levosimendan showing two additional molecular targets, which results in a three-fold mechanism of action and its relatively unique pharmacological effects. At the moment, omecamtiv mecarbil is the only one of these drugs that appears to have a pure inotropic effect.

We are thus clearly not confronted by a single family of drugs. We are neither, therefore, in the position to describe these 'calcium sensitizers' according to their clinical and therapeutic effects. Indeed, the therapeutic efficacies and benefits, and the adverse events, that have been collected in clinical trials for pimobendan and levosimendan probably derive from the combination of their multiple pharmacological effects. We could thus potentially compare the effects of pimobendan and levosimendan with those of omecamtiv mecarbil to isolate the effects of a 'pure' calcium sensitizer. This exercise would be valid, however, only if we assume that omecamtiv mecarbil has been fully characterized as regards its pharmacology. However, since omecamtiv mecarbil is relatively new, it is possible that this molecule has not been fully characterized yet.

Table 1
Molecular targets, mechanisms of action, and pharmacological and clinical effects of the calcium sensitizers pimobendan, levosimendan and omecamtiv mecarbil.

Effects		Pimobendan	Levosimendan	Omecamtiv mecarbil
Molecular targets	Cardiac troponin C	×	×	
	Cardiac myosin β-heavy chain			×
	PDEIII	×	×	
	PDEIV	×		
	K_{ATP} channels		×	
Pharmacological effects	Inotropy	↑	↑	↑
	Lusitropy		↑	
	Vasodilation	↑	↑	
	Peripheral perfusion		↑	
	Cardio-protection		↑	
	Chronotropy	↑	↔↑	↓
Clinical effects	Cardiac output	↑	↑	↑
	Pulmonary capillary wedge pressure		↓	
	Symptoms (dyspnoea, fatigue)		↓	↓↔
	Neurohormones		↓[a]	
Adverse clinical events	Hypotension	↑	↑	↔
	Atrial arrhythmia	↑	↑↔	
	Ventricular arrhythmia	↑	↔	↔
Effects on mortality		↑	↔↓	

[a] Atrial natriuretic peptide, brain natriuretic peptide [56–59].

From a qualitative analysis of the available pharmacological data, a possible drawback of 'pure' calcium sensitization appears to include prolongation of systolic ejection time on the expense of relaxation time, which has been observed for omecamtiv mecarbil but not for levosimendan. This reduction of relaxation time might be detrimental in ischemic hearts in the presence of an increased heart rate. For levosimendan, the absence of prolongation of systole is probably due to either the presence of other effects on top of its binding to troponin C, or the strictly calcium-dependent binding to this target molecule. Additionally, levosimendan has been shown to improve ventriculo-arterial coupling and cardiovascular performance in coronary patients with left ventricular dysfunction, by both enhancing myocardial contractility and reducing arterial elastance [76,77]. These effects on ventriculo-arterial coupling might be more important than the mere inotropic effects related to calcium sensitization.

9. Conclusions

The selection of targets such as troponin C and myosin for the actions of inotropic drugs still appears to be fascinating some 50 years from the discovery of calcium-dependent regulation of striated muscle contraction [78,79].

The question remains whether a 'safe inotrope' needs to have pleiotropic effects in addition to calcium sensitization to compensate for the potential drawbacks of pure calcium sensitizers. Are such 'spurious' calcium sensitizers such as levosimendan actually better? Has levosimendan been relatively successful due to its unique combination of mechanisms of action? As we still lack mortality data on omecamtiv mecarbil, we remain essentially in the still.

Further clinical trials will help to decide whether this hypothesis can be translated into favorable clinical outcomes. At the same time, as levosimendan has shown evidence of short-term benefits without adverse events in the long term, we believe that this represents the minimum standard for any future inotropic or inodilator drug that is developed for treatment of a failing heart.

An additional issue, however, is the selection of the patient groups that will benefit from the use of calcium sensitizers. Clinical trials need to be more targeted and less comprehensive, as it might remain difficult to find a cure-all drug for all of the patients in need of inotropic support. There is no realistic expectation of a blockbuster in such a multidimensional condition. In the era of personalized therapies, we should search for drugs that are targeted to more focused patient groups (*e.g.* either cardiogenic shock, or acute heart failure with low ejection fraction, or low cardiac output syndromes after cardiac surgery, or septic-shock-related cardiac depression, among others), and that are not used as panaceas.

Striving after this magic bullet has been the downfall of much research in the past. As an example, levosimendan was developed as an inotrope and it was meant to be used independently of what was the systolic blood pressure of the patient. However, its vasodilatory effects became evident very soon, and now the use of a loading dose is not recommended for patients with systolic blood pressure <100 mmHg [23]. A successful strategy for new effective and safe inotropes has still to be defined, and we cannot be sure at present that we are looking at the correct patient groups [80].

Author contributions

All of the authors independently performed the preliminary searches for relevant publications. All of the authors contributed substantially to discussions of the existing literature, and reviewed the manuscript before submission.

Conflict of interest

This project did not receive any financial support. PP is an employee of Orion Pharma. The other authors declare that they do not have any conflicts of interest to disclose.

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