American Journal of Cardiovascular Disease Research, 2014, Vol. 2, No. 1, 9-16
Available online at http://pubs.sciepub.com/ajcdr/2/1/3

DOI:10.12691/ajcdr-2-1-3

The Role of Calcium-Sensitizer Levosimendan for the Treatment of Heart Failure

Mohammad Asif*

Department of Pharmacy, GRD (PG) IMT, Dehradun, (Uttarakhand), India
*Corresponding author: aasif321@gmail.com

Received November 30, 2013; Revised March 11, 2014; Accepted March 12, 2014

Abstract In the treatment of heart failure, clinical signs are low cardiac output, therapy with positive inotropic agents in an acute cardiac care is mandatory. Three classes of inotropic drugs are currently used, including beta-adrenergic agonists (especially dobutamine), phosphodiesterase inhibitors (such as milrinone) and the recently developed calcium sensitizers such as levosimendan. The classic inotropic drugs offer short-term haemodynamic enhancement in heart failure patient and their use has been connected with poor prognosis. The inotropic drugs, the Ca^{2+}-sensitizers, may offer a choice of long-lasting result.

Keywords: *inotropic agents, heart failure, calcium-sensitizers*

Cite This Article: Mohammad Asif, "The Role of Calcium-Sensitizer Levosimendan for the Treatment of Heart Failure." *American Journal of Cardiovascular Disease Research*, vol. 2, no. 1 (2014): 9-16. doi: 10.12691/ajcdr-2-1-3.

1. Introduction

The crucial role in treating congestive heart failure (CHF) by used of inotropic and calcium sensitizer drugs. Inotropes did not always improve mortality of the patients with HF partly because of possible direct toxic effects of these drugs on myocytes; intensify arrhythmias, enhancing neurohormonal activity. The HF is a major cause of heart disease (HD). Severe dyspnea, pulmonary congestion and low cardiac output (CO) with peripheral vasoconstriction and renal hypoperfusion is a main form of clinical presentation. In patients who uses vasodilators and diuretics as first-line drugs for quick fall in dyspnea and obstruction. In patients with low CO and oliguria, inotropic agents are regularly managed to prevent extra deterioration. Beta-adrenergic blockers and phosphodiesterase (PDE) inhibitors exact the hemodynamic trouble, but also encourage arrhythmias and myocardial ischemia (MI). The use of inotropic therapy remains controversial. Conventionally, HF has been treated with a various ionotropic drugs like diuretics, catecholamine, digitalis and non catecholamines, although these drugs allowed adverse effects, like arrhythmia and even death. Some new class of drugs has recently applied, causes positive impact on the patients with HF; these drugs are kanown as Ca^{2+} sensitizers. They enhance myocardial contractility without increasing cytosolic Ca^{2+} concentrations. Levosimendan is a known Ca^{2+} sensitizers, it increase cardiac contractility by sensitizing cardiac myofibrils to Ca^{2+}, and have advantage in the management of low-cardiac output conditions, mainly in CHF.

2. Cardiac Inotropic Activity of Levosimendan and Its Analogoues

The Ca^{2+} sensitizers levosimendan has improve mortality of the CHF patients by improving cardiac contractility (CC) without a increase in intracellular calcium (Ca^{2+}) i concentrations. However, some trials disappoint these expectations, the drugs which has more specific effect of Ca^{2+} sensitizing and the choice of the patients who will receive the benefits of Ca^{2+} sensitizer drugs [1,2]. Levosimendan showed clinical and hemodynamic benefits in CHF patients, after treatment with diuretics or ultrafiltration. Diastolic, systolic, dicrotic, and mean arterial pressures (MAP); systemic vascular resistance (SVR); hemodynamic variables (cardiac output [CO], stroke volume [SV], dP/dt (max)); and cardio-vascular act (cardiac cycle efficiency [CCE], cardiac power output). The infusion of levosimendan, after an hour of treatment, significant increase in CO, CCE, SV, and dP/dt (max) and a cause significant reduction in diastolic, dicrotic arterial pressures (DAP) and SVR. Levosimendan cause reduction in signs and symptoms of HF. Most HF patients treated with diuretics or ultrafiltration receive extra clinical and hemodynamic profit from levosimendan [3]. Diaphragm muscle weakness in CHF patients and is related with exercise intolerance and improved mortality. Reduced sensitivity of diaphragm fibres to Ca^{2+} contributes to weakness of diaphragm muscle fibre in CHF. The ability of the levosimendan is to restore the reduced Ca^{2+} sensitivity of diaphragm muscle fibres with CHF. The 10 μM levosimendan, Ca^{2+} sensitivity of force generation was reduced in diaphragm muscle fibres from CHF. Levosimendan appreciably increased Ca^{2+} sensitivity of force in diaphragm muscle fibres from CHF. It enhanced the force producing ability of diaphragm fibres from CHF by rising the sensitivity of force making to Ca^{2+}

concentration. The effects of Ca^{2+} sensitizers on diaphragm muscle weakness in patients with CHF [4].

The Ca^{2+} concentration dependent contact between troponin-I (cTnI) and troponin C (cTnC) activating contraction in cardiac muscle. The HF is described by a decrease in CO, and Ca^{2+} sensitizers have an therapeutic potential. The levosimendan targets cTnC. The mode of action of two fluorine containing analogs of levosimendan; 2',4'-difluoro(1,1'-biphenyl)-4-yloxy acetic acid (dfbp-o) and 2',4'-difluoro(1,1'-biphenyl)-4-yl acetic acid (dfbp). The affinities of dfbp and dfbp-o for the cTnC were calculated in the absence and presence of cTnI. The dfbp-o was found to bind more strongly than dfbp. Dfbp-o also increased the affinity of cTnI for cTnC. Dfbp-o increased the Ca^{2+}-sensitivity of cardiac trabeculae. The levosimendan work by binding to the regulatory domain of cTnC and stabilizing the pivotal cTnC-cTnI regulatory unit via a hydrophobic and electrostatic interaction [5]. The Levosimendan improve CC without increasing oxygen necessities and to make peripheral and coronary vasodilation [6]. Catecholamines (epinephrine) progress ventricular function by rising the (Ca^{2+}) i concentration and increase oxygen consumption. Since Ca^{2+}-sensitizers also give an extra therapeutic prospect.

Isolated, blood-perfused rabbit hearts, heart rate (HR), CO, left ventricular pressure (LVP), coronary blood flow (CBF), and arterio-venous oxygen difference (AVDO) were recorded during reperfusion and after administration of either levosimendan (0.75 µ mol) or epinephrine plus levosimendan. Levosimendan in postischemic hearts increased HR (32%) and improved hemodynamics in terms of CO (85%), SV (44%), W (115%), LVP(max) (95%), dP/dt(max) (133%), dP/dt(min) (121%), LVP(ed) (-63%), and CR (-17%). It altered hemodynamics in terms of AVDO (+7.0%) and MVO (+32%) and MVO/beat (+2.3%). External efficiency was increased by 307%. Additional levosimendan increased hemodynamics in terms of HR (56%), CO (159%), SV (89%), W (588%), LVP(max) (168%), dP/dt(max) (102%), dP/dt(min) (78%), LVP(ed) (-98%), and CR (-50%). It altered hemodynamics in terms of AVDO (-11%), MVO (+131%) and MVO/beat (+171%). External efficiency was increased by 212%. In contrast to epinephrine, levosimendan improves ventricular function without increasing oxygen demand, thereby considerably improving external efficiency. Even during epinephrine resistance in extremely dysfunctional hearts, levosimendan successfully improves ventricular function [7]. Levosimendan produces greater hemodynamic and symptomatic improvement in patients with acute HF than traditional inotropes. The novel biologic mechanisms explained the effects of levosimendan on the failing heart. Levosimendan has a unique dual mechanism of action by enhancing CC and peripheral vasodilatation. Immuno-modulatory and antiapoptotic properties of levosimendan may be an additional biologic mechanism that prevents further cytotoxic and hemodynamic consequences of abnormal immune and neurohormonal responses in acute HF, leads to cardioprotection, and beneficially intervenes in the progression of syndrome. The cardioprotective effects of levosimendan and its antioxidant properties is a potent inhibitor of H_2O_2-induced cardiomyocyte apoptotic cell death. The levosimendan does not raise markers of nitrosative and oxidative stress, in chronic HF patients. Levosimendan has activate mito K (ATP) channels which are main mediators of ischemic preconditioning. Pharmacological modulation of K (ATP) channels may prove valuable in patients at risk of MI, mainly those needing inotropic drugs. The effects of levosimendan have important clinical and prognostic implications in AHF and ischemic heart disease (IHD) [8].

3. Levosimendan in Heart Failure Patients

The Ca^{2+} sensitizers, act by a combination of the upstream and central/downstream mechanism. The upstream and central mechanisms are markedly suppressed in failing myocytes and under acidotic conditions; Ca^{2+} sensitizers can induce cardiotonic effects. Ca^{2+} sensitizers have therapeutic potential for contractile dysfunction in congestive HF and IHD, because they have advantages and less risk of Ca^{2+} overload and can retain efficiency under pathological condition [9]. The Ca^{2+} sensitizers that act directly on contractile proteins are free from the risk of Ca^{2+} overload and they could improve haemodynamic parameters, including acidosis and stunned myocardium. Beneficial effects of levosimendan, it acts by combination of Ca^{2+} sensitization and PDE inhibition on CHF due to hypertensive cardiomyopathy. Since chronic CHF is much more complex, careful analysis of clinical outcomes will be required to establish the therapeutic significance of Ca^{2+} sensitizers in the treatment of chronic CHF [10]. Levosimendan offers haemodynamic and symptomatic improvement by combining a positive inotropic action via Ca^{2+}-sensitization and a vasodilatory effect via adenosine triphosphate (ATP)-sensitive K^+ (K (ATP)), Ca^{2+}-activated $K(^+)$ (K (Ca^{2+})) and voltage-dependent K^+ (K (V)) channels activation. Levosimendan also induce a prolonged haemodynamic improvement in patients with HF as a result of the long half-life of its active metabolite, OR-1896. Furthermore, levosimendan may have additional antiinflammatory and antiapoptotic properties, affecting main pathways in the pathophysiology of HF. Despite the benefit of levosimendan on short- and long-term mortality in patients with severe HF, the results from the clinical trials are rather disappointing, and it is still unclear whether it is superior to dobutamine in affecting survival of patients with severe HF [11]. The Ca^{2+}-sensitizers improve cardiac function by increasing the contraction of the myocardium without significantly increasing (Ca^{2+}) i concentrations. The levosimendan also affects myocardial especially systolic waves of ventricle [12]. The effect of levosimendan, and milrinone, a PDE inhibitor, on ventricular arrhythmias was compared in a model of MI and reperfusion. Levosimendan, but not milrinone, significantly attenuated the pronounced increase in the number of ventricular premature beats (-63%), tachycardia (-50%), fibrillation (-70%), and inhomogeneity of ventricular electrical activation. Levosimendan significantly improved the survival rate. Levosimendan has a more beneficial profile than milrinone regarding the development of ventricular arrhythmias during and after regional MI [13]. Levosimendan, besides increasing contractility, has a vasodilating effect due to the activation of K (ATP)

channels, being both mechanisms responsible for an advantageous therapeutic option. The levosimendan is a real and safe alternative treatment for patients with acute or chronic ventricular failure that need pharmacological support [14].

Levosimendan is a most potent Ca^{2+} sensitizer, exhibiting a unique dual mechanism of action that combines a positive inotropic action mediated via Ca^{2+} sensitization and a vasodilator property via ATP-dependent K^+ channels. The Ca^{2+} sensitizer agents represent a promising class of inotropic agents [15]. Levosimendan, acting through Ca^{2+} sensitization of contractile proteins, has shed new light on inotropic therapy, and, importantly, has reduced mortality in acute HF patients. The compounds which have been selected for consideration are limited only to positive inotropic compounds that produce Ca^{2+} sensitization of contractile proteins. The differences between various Ca^{2+} sensitizing mechanisms, mainly focuses on the relaxation of cardiac muscle [16]. Levosimendan is the first available Ca^{2+} sensitizers. It increases CC without increasing MVO. This drug has no proarrhythmic effects and has anti-ischemic properties. Its hemodynamic effects, similar or superior to those of catecholamines, persist during one week.

In a group of HF patients levosimendan was associated with a mortality reduction and in comparison with dobutamine. This inotropic agent appears very promising and it will be widely used [17]. Cardiogenic shock is a condition associated with high mortality. The data base for choice of treatment is insufficient, but Ca^{2+} sensitizers and new understanding have lead to some improvement in the prognosis. The treatments of cardiogenic shock, acute MI, cardiogenic shock are with emphasis on the role of inotropic drug therapy. A patient with cardiogenic shock complicating MI because of occlusion of the left-main coronary artery was treated with acute revascularization, intra-aortic balloon counterpulsation (IABP) and levosimendan. Early revascularization is a key factor in the treatment of cardiogenic shock; rapid transfer of patients to a revascularization centre is recommended. IABP should be considered after successful revascularization because of post-ischaemic dysfunction that persists despite restoration of epicardial blood flow. The Ca^{2+} overload of the cardiomyocytes and increased mortality. The Ca^{2+} sensitizer is promising, but more controlled clinical trials are needed [18]. The Ca^{2+} sensitizers are shares the in vitro properties of Ca^{2+} sensitization and PDE inhibition. Levosimendan, as it stabilizes the interaction between Ca^{2+} and tnC by binding to tnC in a Ca^{2+}-dependent manner, improving inotropy. It does not exhibit clinically relevant PDE inhibition at therapeutic concentrations. It also exerts vasodilatory effects, possibly through activation of several K^+ channels and other less well characterized mechanisms. The pharmacokinetics of levosimendan is similar in healthy human and patients with HF and remains relatively unaltered by age, sex, and organ dysfunction. In clinical studies, levosimendan exerted potent dose-dependent positive inotropic and vasodilatory activity. Unlike conventional inotropes, levosimendan is not associated with significant increases in MVO, proarrhythmia, or neurohormonal activation. The most common adverse effects are attributable to the vasodilation. The trials demonstrated favorable hemodynamic effects, improved tolerability, and a possible mortality benefit over dobutamine and placebo in patients who had acute symptoms of failure and required inotropic therapy. The long-term effect on patient outcomes is being confirmed in ongoing placebo- and inotrope-controlled trials. Levosimendan appears to be an effective inodilator devoid of the detrimental effects of conventional inotropes. It may provide a promising alternative to conventional inotropes for patients with acutely decompensated HF [19]. During HF, alterations occur in contractile protein expression and phosphorylation, which may influence the effects of Ca^{2+}-sensitizers. Two different Ca^{2+}-sensitizers, EMD 53998 (10 μM), which exerts its influence through the actin-myosin interaction, and OR-1896 (10 μM) (active metabolite of levosimendan), which affects the Ca^{2+}-sensory function of the thin filaments. The maximal force at saturating Ca^{2+} concentration and the resting force in the virtual absence of Ca^{2+} did not differ between the failing and non-failing myocytes, but the Ca^{2+} concentration required to induce the half-maximal force was significantly lower in the failing than in the non-failing myocytes. This difference in Ca^{2+}-sensitivity, however, was abolished during mimicked ischemia. EMD 53998 increased fource and resting force by approximately 15% of fource and greatly enhanced the Ca^{2+}-sensitivity of force production. OR-1896 did not affect fource and resting force, and provoked a small, but significant Ca^{2+}-sensitization. These effects were comparable in the donor and failing myocytes, but, in contrast with OR-1896, EMD 53998 considerably diminished the difference in the Ca^{2+}-sensitivities between the failing and non-failing myocytes. The action of Ca^{2+}-sensitizers under mimicked ischemic conditions was impaired to a similar degree in the donor and the failing myocytes. The Ca^{2+}-activation of the myofibrillar system is altered in end-stage human HF. This modulates effects of Ca^{2+}-sensitizers both under control and under mimicked ischemic conditions [20].

The effects of levosimendan and its stereoisomer dextrosimendan on the cardiac contractile were using skinned fibers obtained from guinea pig hearts. Levosimendan was found to be more effective than dextrosimendan. The concentrations of levosimendan and dextrosimendan at EC_{50} were 0.3 and 3 μM. The difference in efficacy as Ca^{2+} sensitizers, the binding of the two stereoisomers on cardiac troponin C was studied in the absence and presence of two peptides of cardiac troponin I. The two stereoisomers interacted with both domains of cardiac troponin C in the absence of cardiac troponin I. In the presence of cardiac tn-I (32-79) and cardiac tn-I (128-180), the binding of both levosimendan and dextrosimendan to the C-terminal domain of cardiac troponin C was blocked and only the binding to the N-terminal domain was observable. Differences in the overall binding behavior of the two isomers to cardiac troponin C were focused in order to discuss their structure to activity relation. The action of levosimendan as a Ca^{2+} sensitizer and positive inotrope relates to its stereoselective binding to Ca^{2+}-saturated cardiac troponin C [21]. The levosimendan improves CC without causing an increase in (Ca^{2+}) i and cAMP concentrations. It also has a vasodilator action due to an opening of the ATP-sensitive K^+ channels. In a clinical trial levosimendan was compared with dobutamine in patient with severe low-

output CHF. The pre-defined hemodynamic improvement was achieved in 28% of patients receiving levosimendan compared to only 15% with dobutamine. Levosimendan also reduced the mortality more than dobutamine. Levosimendan produced less MI and cardiac arrhythmias than dobutamine. Ca^{2+} sensitizers offer therapeutic possibility in patients with decompensated low-output HF [22]. The increases and decreases in Ca^{2+} levels in myocytes regulate the contraction and relaxation of the heart. Therapeutic agents can improve or interfere with this delicate balance. Ca^{2+} sensitizers enhance cardiac contraction by improving the use of Ca^{2+} that is available, the cell with excessive Ca^{2+}, as is the case with traditional inotropes. Levosimendan is used for the treatment of patients with acute HF. In clinical studies, levosimendan increased CO and stroke volume without significantly increasing oxygen demand. By its additional action as a vasodilator (via K^+ channel opening), levosimendan also corrects the hemodynamic decompensation, thus lowering the pulmonary capillary wedge pressure and SVR. Furthermore, levosimendan increases the coronary circulation thus leading to an improved function of the stunned myocardium and lessened ischemia. Levosimendan, Ca^{2+}-sensitizing action, along with its complementary vasodilator properties, make this drug a highly promising agent for the treatment of patients with acute HF [23].

4. Clinical Signifigance of Ca Sensitizer Alone and in Combination with Classic Inotropic Agent

For increasing CC in patients with HF, catecholamines, PDE-III inhibitors, and Ca^{2+} sensitizers are available. Improving myocardial performance with catecholamines and PDE inhibitors leads to increased (Ca^{2+}) i concentration as an unavoidable side effect. An increase in (Ca^{2+}) i can induce harmful arrhythmias and increases the energetic demands of the myocardium. The PDE inhibitors have raised concerns about the safety of positive inotropic treatment for HF. Ca^{2+} sensitizers, improve myocardial performance by directly acting on contractile proteins without increasing (Ca^{2+}) i load. Thus, they avoid the undesired effects of an increased (Ca^{2+}) i load. Ca^{2+} sensitizers may enhance myocardial performance without increasing MOV and without provoking fatal arrhythmias. Two Ca^{2+} sensitizers are available for the treatment of HF in humen. Pimobendan is a drug with positive inotropic effects that additionally inhibits the production of proinflammatory cytokines. However, it exerts a significant inhibition of PDE at clinically relevant doses. Levosimendan is with no major inhibition of PDE at clinically relevant doses. It opens ATP-dependent K^+ channels and thus has vasodilating and cardioprotective effects. The treatment of stable HF with pimobendan and on the short-term treatment of unstable HF with levosimendan is presented [24]. Regulation of CC by cardiotonic agents is achieved by an increase in (Ca^{2+}) i mobilization, an increase in Ca^{2+} binding affinity to troponin C, or facilitation of the process subsequent to Ca^{2+} binding to troponin C. The cAMP mediates the regulation induced by Ca^{2+} mobilizers such as β-adrenoceptor agonists and selective PDE-III inhibitors acting through the upstream mechanism. These agents act likewise on the central mechanism to decrease Ca^{2+} sensitivity of troponin C in association with the cAMP-mediated phosphorylation of troponin I. In addition to such a well-known action of cAMP, Ca^{2+} sensitizers, such as levosimendan, OR-1896, and UD-CG 212 Cl, require the cAMP-mediated signaling for induction of Ca^{2+} sensitizing effect. These agents shift the $[Ca^{2+}]$ -force relationship to the left, but their positive inotropic effect (PIE) is inhibited by carbachol, which suppresses selectively the cAMP-mediated PIE. These findings imply that cAMP may play a crucial role in increasing the myofilament Ca^{2+} sensitivity. No clinically available cardiotonic drugs act via Ca^{2+} sensitization, but the PIE of pimobendan and levosimendan is partly mediated by an increase in myofilament Ca^{2+} sensitivity. Cardiotonic agents with Ca^{2+} sensitizing action are more effective than agents that act purely via the upstream mechanism in clinical settings. The effectiveness of Ca^{2+} sensitizers in long-term therapy for CHF patients [25]. However, currently available therapies, which are based on three basic mechanisms of action (diuresis, exogenous vasodilators, and cAMP-dependent positive inotropes), have significant limitations that have encouraged the development of newer agents. The leading medications are representatives of three different therapeutic approaches, which include endogenous vasodilatory neurohormones (nesiritide), Ca^{2+} sensitizers (levosimendan), and neurohormonal antagonists (tezosentan). These three agents represent a new generation of therapeutics for this important medical problem and may provide to treat symptoms and also to improve longer-term clinical outcomes [26].

In addition to sensitizing troponin to (Ca^{2+}) i, levosimendan has been shown to inhibit PDE-III, which may contribute to its positive inotropic effect, and open ATP-sensitive K^+ channels (K (ATP)), which may produce vasodilation. Unlike available intravenous inotropes, levosimendan does not increase myocardial oxygen utilization, has not been shown to be proarrhythmic, and has been used effectively in the presence of β-blocking medications. Levosimendan also has not been shown to impair ventricular relaxation. Clinical studies of levosimendan have demonstrated short-term hemodynamic benefits of levosimendan over both placebo and dobutamine. The mortality benefits of levosimendan over dobutamine up to 180 days after treatment. Levosimendan with other positive inotropes, namely milrinone, are lacking. Levosimendan treatment appears to be well-tolerated, with the primary adverse events being headache, hypotension and no clinically significant drug-drug interactions have been reported. The levosimendan will depend on the results of ongoing trials [27]. The Ca^{2+} sensitizers act on the central mechanism Ca^{2+} binding affinity of tnC) and/or downstream mechanisms of cardiac E-C coupling. Ca^{2+} sensitizers have mechanistic and energetic advantages over the agents that act through the upstream mechanism [(Ca^{2+}) i mobilization]. The Ca^{2+} sensitizers and the agents that act through cAMP-mediated signaling process have been postulated to belong to different classes, however, experimental findings revealed that certain Ca^{2+} sensitizers, such as levosimendan, OR 1896 and UD-CG 212 Cl,

require cAMP-mediated signaling for induction of the Ca^{2+} sensitizing effect. No clinically available agents act primarily via Ca^{2+} sensitization, but the positive inotropic effect of pimobendan and levosimendan is partly due to an increase in myofilament Ca^{2+} sensitivity.

5. Pharmacological Mechanism of Levosimendan

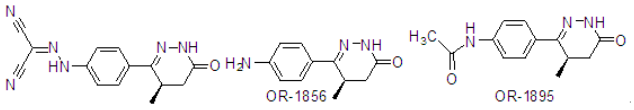
These agents are Ca^{2+} sensitizer and PDE3 inhibitor. The contribution of Ca^{2+} sensitizing effect of these agents to improve the hemodynamics in patients with HF is uncertain [28]. Compounds that sensitize cardiac muscle to Ca^{2+} by intervening at the level of regulatory thin filament proteins would have potential therapeutic benefit in the treatment of MI. Two Ca^{2+} sensitizers, EMD 57033 and levosimendan are bind to cTnC. In the absence of Ca^{2+}, neither drug interacted with cTnC. In the presence of Ca^{2+}, one molecule of EMD 57033 bound specifically to the C-terminal domain of free cTnC. The presence of levosimendan had no apparent effect on the Ca^{2+} binding affinity of cTnC. Changes in the N-terminal methionine methyl chemical shifts in cTnC upon association with cTnI suggest that cTnI associates with the A-B helical interface and the N terminus of the central helix in cTnC. However, levosimendan covalently bound to a small percentage of free cTnC after incubation with the protein. The levosimendan exerts its positive inotropic effect by mechanisms that do not involve binding to cTnC [29]. Levosimendan is a vasodilator, but its mechanism is not well understood. The cardiac target protein of levosimendan, troponin C, is a Ca^{2+}-binding EF-hand protein. This raises the possibility that levosimendan may also interact with smooth muscle EF-hand proteins, such as, calmodulin, the regulatory myosin light chains, or S100 proteins. The effects of levosimendan on $[Ca^{2+}]$ i, and force in porcine coronary arteries, with receptor-mediated (U46619) or KCl stimulation. At high levels of stimulation, levosimendan decreased force without changing or increasing $[Ca^{2+}]$ i, measured. With lower levels of U46619, levosimendan (1 µM) lowered force by 70% and reduced $[Ca^{2+}]$ i by 38%. The relationship between force and $[Ca^{2+}]$ i for KCl stimulation are significantly rightward shifted, indicating Ca^{2+} desensitization by levosimendan. In contrast, PDE-III inhibitor, milrinone, does not shift the force- Ca^{2+} relations but elicits relaxation via lowering $[Ca^{2+}]$ i. Levosimendan relaxes coronary arteries and lowers $[Ca^{2+}]$ i by mechanisms different than milrinone. Lowering of $[Ca^{2+}]$ i by levosimendan consistent with opening of K^+ channels and a relaxation that is independent of $[Ca^{2+}]$ i. Evidence points to a novel mechanism that might involve the direct effect of levosimendan on the smooth muscle contractile or regulatory proteins themselves [30].

The positive inotropic effects of cAMP-increasing drugs (catecholamines, PDE-inhibitors) are diminished in the failing myocardium. Hence, the usefulness and mechanism of the two Ca^{2+} sensitizers, levosimendan and CGP 48506 in preparations from end-stage failing human hearts since the exact mechanism of the positive inotropic effects is not yet clearly understood. Ca^{2+} sensitization was investigated in skinned fibers and PDE activity was measured in ventricular homogenate. The levosimendan (1 µmol/l) increased cAMP content. CGP 48506 is an inotropic agent with Ca^{2+}-sensitizing properties in the human heart that is devoid of inhibitory activity on human cardiac PDE isoenzymes. The positive inotropic therapy can be useful for the bridging treatment of HF before transplantation. The levosimendan is showing less-effective inotropic effects accompanied by increased cAMP levels [31]. The levosimendan is acting through TnC, accelerated the proportional association rate and decelerated the dissociation rate of crossbridges. The effect of levosimendan on crossbridge kinetics occurred before the peak twitch tension was achieved. Thus, the compound did not change the actual relaxation phase of twitch tension. Since the effect on the association was more pronounced than on the dissociation of crossbridges, levosimendan shifted the entire twitch tension curve to the left. Based on the dissociation rate analysis levosimendan seems to act preferentially as a Ca^{2+} m sensitizer at low concentrations. At high concentrations the PDE-3 inhibitory properties of levosimendan modulated its effect on the early relaxation processes. In contrast, PDE3 inhibition is probably the primary mechanism of action for MCI-154. Pimobendan, and EMD 53998 at low concentrations, whereas their direct effects on crossbridge kinetics contributed to the positive inotropic action at high concentrations. The Ca^{2+} sensitizing mechanisms of these compounds seemed to be based almost exclusively on the decelerating effect on dissociation of crossbridges [32]. The role of cardiac troponin C (cTnC) as a target protein for the Ca^{2+} sensitization by levosimendan, pimobendan, MCI-154 and EMD 53998 was evaluated using purified recombinant human cTnC. For determination of Ca^{2+}- and magnesium (Mg^{2+})-dependent binding of the compounds to cTnC a new type of cTnC-HPLAC column was used. Furthermore, dansylated cTnC was utilized to study the effect of the Ca^{2+} sensitizing compounds on Ca^{2+}-induced conformation of cTnC. Levosimendan showed Ca^{2+}-dependent and to a lesser extent Mg^{2+}-dependent retention in the cTnC column. The findings indicate that levosimendan binds both to the N-terminal and C-terminal domains of cTnC. In agreement with this, only levosimendan shifted the Ca^{2+}induced fluorescence curve of dansylated cTnC to the left. The mechanism of Ca^{2+}sensitizing effect of levosimendan, unlike that of the other Ca^{2+} sensitizers is based on Ca^{2+}-dependent binding to the N-terminal domain of cTnC. This is proposed to amplify the trigger of contraction induced by cTnC in the cardiac muscle [33]. Levosimendan is a novel positive inotropic drug targeted to increase contraction force of the heart through its Ca^{2+}-dependent binding to troponin C (cTnC). The Ca^{2+}-sensitizing effect of levosimendan on contractile proteins as well as its positive inotropic and lusitropic effects in paced guinea pig papillary muscle. The Ca^{2+} sensitization induced by levosimendan in fibers skinned with saponin was dependent on the perforation velocity of cell membranes. Levosimendan was almost ineffective in slowly perforated fibers, but was the most potent Ca^{2+} sensitizer in fibers with rapidly perforated cells. The perforation-dependent Ca^{2+} sensitization was probably due to changes in phosphorylation state of contractile proteins during the slow dissection of fibers. It is noteworthy that the Ca^{2+}-sensitizing effect of levosimendan was not affected by acidic pH. Levosimendan at therapeutically relevant (0.3-10 µM)

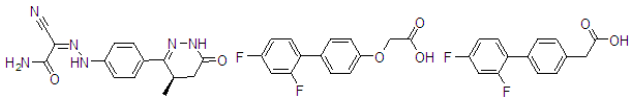
concentrations markedly increased Ca^{2+} sensitivity more potent than EMD 53998, pimobendan, and MCI-154. The lack of effect of levosimendan on maximum tension supports the hypothesis that levosimendan increases Ca^{2+} sensitivity through its action on cTnC. Unlike EMD 53998, levosimendan did not increase myosin ATPase activity, indicating that it did not increase the cycling rate of myosinactin crossbridges. In paced papillary muscles, levosimendan induced positive inotropic effect without changing relaxation time. Thus, levosimendan was devoid of the main negative factors described for Ca^{2+} sensitizers [34]. The mechanisms of actions of the Ca^{2+}-sensitizing agents such as levosimendan, pimobendan, MCI-154, and EMD 53998 by using purified human recombinant cTnC, the role of cTnC as a target protein for these compounds. The Ca^{2+}-dependent binding to cTnC in a cTnC and the stabilizing effects of the compounds on the Ca^{2+}-induced conformation of dansylated cTnC were studied. Only levosimendan showed Ca^{2+}-dependent action on cTnC. The levosimendan was the most potent Ca^{2+} sensitizer in skinned fiber experiments. EMD 53998 and MCI-154 but not levosimendan and pimobendan, increased myosin ATPase activity, indicating that they may enhance the cycling rate of myosin-actin crossbridges. By analyzing the velocity (dT/dt) of isometric tension development in paced papillary muscles, it was shown that levosimendan probably enhances the association rate but decreases the dissociation rate of myosin-actin crossbridges. Levosimendan does not seem to affect the actual relaxation phase. The other Ca^{2+} sensitizers, however, appear to act mainly by decreasing the dissociation rate of crossbridges. The weak Ca^{2+}-sensitizing effect of pimobendan may be based on indirectly mediated increase in affinity of cTnC for Ca^{2+}. MCI-154 might act in a similar way but, like EMD 53998, MCI-154 also acts on myosin-actin crossbridges. We suggest that levosimendan binds in a Ca^{2+}-dependent manner to the N-terminal domain of cTnC, which magnifies the extent of the contraction produced by cTnC when it is Ca^{2+}-activated [35].

The problems with inotropic agents and describes the new concept of increasing cardiac myofilament sensitivity to Ca^{2+}. Presently used inotropic agents act by increasing the (Ca^{2+}) i concentration of Ca^{2+} in cardiac myocytes by either cAMP-dependent or cAMP-independent mechanisms. There is concern that elevation of cAMP and/or cytosolic Ca^{2+} might be proarrhythmic and increase mortality in patients with CHF. Ca^{2+} sensitization represents a approach to the treatment of CHF. Drugs that sensitize the contractile proteins to Ca^{2+} enhance myocardial contractility without changes in the cytosolic Ca^{2+} concentration. Ca^{2+} sensitization can be achieved by an increased affinity of tnC for Ca^{2+} (pimobendan), by stabilization of the Ca^{2+}-induced conformational change of troponin-C (levosimendan) or by direct interference with the myosin-actin interaction (MCI-154, EMD 53998, and EMD 57033). Ca^{2+} sensitization reduces the risk for Ca^{2+} overload and has a favorable effect on MVO. Inhibition of cardiac relaxation is a possible adverse effect of Ca^{2+} sensitizers owing to an expected higher level of contractile tension during diastole. The Ca^{2+} sensitizers have additional PDE-III inhibitory activity, which is associated with a positive lusitropic effect, but from the standpoint of mortality PDE inhibition might not be beneficial in the long run. Most Ca^{2+} sensitizers have a hemodynamic profile characteristic of inodilators. Clinical data on Ca^{2+} sensitizers are yet very sparse but ongoing clinical trials are awaited [36]. Levosimendan belongs to a new group of HF drugs, the Ca^{2+} sensitizers. Because these compounds are not yet available for clinical use, the adverse drug events (ADEs) during levosimendan treatment cannot be predicted in detail. The most common ADE seen in healthy volunteers is headache. The incidence of headache does not correlate well with the total daily dose of the drug. However, the controlled release formulations tested appear to cause vasodilatory symptoms more frequently than i.v. or rapid release oral formulations. The other typical vasodilatory ADEs seen in healthy volunteers are nausea, palpitation, and dizziness. Symptomatic hypotension is rarely encountered. It appears that HF patients tolerate the vasodilatory actions of the drug better than healthy volunteers. Only individual cases of headache, vertigo, and flushing have been reported, and injection site irritation has been the most commonly reported ADE. Patients who have received levosimendan have been monitored with an ambulatory ECG. Even though some increase in heart rate is seen with high doses of the drug, there are thus far no signs of an increased incidence of ventricular tachyarrhythmias, nor have there been any noteworthy changes in the clinical laboratory safety tests. The experience with levosimendan is limited thus far and long-term data are lacking. It can be concluded, however, that at least in i.v. dosing the drug is devoid of ADEs with significant medical seriousness [37]. Ca^{2+} sensitizers may influence myocardial energetics by their action on Ca^{2+} turnover and on crossbridge behavior. The effects of the Ca^{2+} sensitizer EMD-53998 on Ca^{2+} cycling, crossbridge behavior, and myocardial energy turnover were compared with the effects of an increase in extracellular Ca^{2+} and with the effects of the catecholamine isoproterenol. Relaxation time was decreased with isoproterenol, unchanged with high Ca^{2+}, and increased with EMD 53998. Ca^{2+} cycling-related energy consumption, as measured by tension-independent heat, increased by 234% with high Ca2+, by 439% with isoproterenol, and by 77% with EMD 53998. In contrast to high Ca^{2+} and isoproterenol, EMD 53998 increased economy of crossbridge cycling by increasing the force-time integral of the individual crossbridge cycle. The data indicate that EMD 53998 acts by PDE inhibition and myofilament Ca^{2+} sensitization. The latter effect is in part mediated by alteration of crossbridge behavior. Because of its effects on Ca^{2+} cycling and crossbridge function myocardial energy turnover was reduced significantly with EMD 53998, whereas energy turnover was unchanged with high Ca^{2+} and was increased with isoproterenol. The levosimendan was investigated in isolated failing human myocardium. Levosimendan dose-dependently increased isometric tension. The inotropic effect was associated with increased rate of relaxation and reduced relaxation time. Measurements of (Ca^{2+}) i using the photoprotein aequorin suggest that levosimendan acts by increasing myofilament Ca^{2+} sensitivity and by increasing cAMP due to PDE inhibition. However, the contribution of the cAMP system to the action of levosimendan appears to be rather small. The positive lusitropic effect of levosimendan may be consistent with the notion that levosimendan binds to tnC and increases Ca^{2+} sensitivity only at high (systolic) (Ca^{2+})

i concentrations [38]. Class I actions designate positive inotropic mechanisms that enhance the transmembrane Ca^{2+} current by various means, such as β-receptor stimulation (dobutamine), PDE inhibition (milrinone), direct stimulation of adenylate cyclase (forskolin), or direct modulation of Ca^{2+} channel gating (BAY K 8644). The mechanisms that lead to elevation of (Na^{+}) i activity either by inhibiting the Na, K pump (digitalis) or by increasing transmembrane sodium influx (DPI 201-106). Class III action involves a mechanism by which sensitivity of the myofilaments to Ca^{2+} increases (EMD 53998, levosimendan). This mechanism is not associated with apparent electrophysiologic manifestations. Positive inotropism is due to lengthening of the cardiac repolarization. The clinical implications of the various positive inotropic mechanisms reported [39].



Levosimendan



6. Discussion

Three classes of inotropic drugs are currently used, including beta-adrenergic agonists (mainly dobutamine), PDE inhibitors (such as milrinone) and Ca^{2+} sensitizers (levosimendan). Ca^{2+} sensitizers stimulate cardiac contractility without causing (Ca^{2+}) i overload or increasing myocardial oxygen demand [40]. The treating a patient with CHF is rapid and effective stabilization. This target often is attaining by the use of inotropic drugs. Classic inotropic agents (β-adrenergic agonists and PDE-3 inhibitors) provide short-term hemodynamic profit, but their long-term use has correlated with poor endurance rates. The Ca^{2+} sensitizers offers hemodynamic and symptomatic improvements without increasing cAMP and (Ca^{2+}) i concentrations. These drugs enhance CC without a concurrent increase in the risk of cardiac events and characterize a significant advance over classic positive inotropic drugs. The levosimendan is a promising drug for the treatment of CHF. The positive inotropic drugs affect multiple pathways with key roles in the pathophysiology of HF. The effect of levosimendan on mortality in patients with HF will expectantly decide the controversy as to whether levosimendan is superior to classic inotropic drug for the treatment of severe CHF.

References

[1] Sakata Y. Clinical significance of calcium sensitizer. Clin Calcium. 2013; 23 (4): 575-82.

[2] Arai M. Calcium mobilizers and calcium sensitizers. Nihon Rinsho. 2011; 69 Suppl 9: 417-22.

[3] Giglioli C, Cecchi E, Landi D, Chiostri M, Spini V, Valente S, Gensini GF, Romano SM. Levosimendan produces an additional clinical and hemodynamic benefit in patients with decompensated heart failure successfully submitted to a fluid removal treatment. Congest Heart Fail. 2012; 18 (1): 47-53.

[4] van Hees HW, Andrade Acuña G, Linkels M, Dekhuijzen PN, Heunks LM. Levosimendan improves calcium sensitivity of diaphragm muscle fibres from a rat model of heart failure. Br J Pharmacol. 2011; 162 (3): 566-73.

[5] Robertson IM, Sun YB, Li MX, Sykes BD. A structural and functional perspective into the mechanism of Ca^{2+}-sensitizers that target the cardiac troponin complex. J Mol Cell Cardiol. 2010; 49 (6): 1031-41.

[6] Follath F. Newer treatments for decompensated heart failure: focus on levosimendan. Drug Des Devel Ther. 2009; 3:73-8.

[7] Meyer K, Klocke RC, Schipke JD, Gams E, Korbmacher B. Ca^{2+} sensitizer superior to catecholamine during myocardial stunning? Eur J Cardiothorac Surg. 2008; 34 (2): 326-31.

[8] Parissis JT, Andreadou I, Bistola V, Paraskevaidis I, Filippatos G, Kremastinos DT. Novel biologic mechanisms of levosimendan and its effect on the failing heart. Expert Opin Investig Drugs. 2008; 17 (8): 1143-50.

[9] Endoh M. Could Ca^{2+} sensitizers rescue patients from chronic congestive heart failure? Br J Pharmacol. 2007; 150 (7): 826-8. Epub 2007 Feb 26.

[10] Endoh M. Cardiac Ca^{2+} signaling and Ca^{2+} sensitizers. Circ J. 2008; 72 (12): 1915-25.

[11] Antoniades C, Tousoulis D, Koumallos N, Marinou K, Stefanadis C. Levosimendan: beyond its simple inotropic effect in heart failure. Pharmacol Ther. 2007; 114 (2): 184-97.

[12] Kasikcioglu HA, Uyarel H, Tartan Z, Kasikcioglu E, Ozturk R, Cam N. Do calcium sensitizers affect right ventricular functions in patients with chronic heart failure? Int J Cardiol. 2007; 118 (2): 246-8. Epub 2006 Sep 28.

[13] Papp JG, Pollesello P, Varró AF, Végh AS. Effect of levosimendan and milrinone on regional myocardial ischemia/reperfusion-induced arrhythmias in dogs. J Cardiovasc Pharmacol Ther. 2006; 11 (2): 129-35.

[14] González-Chon O, García López SM, Chacón Mercado MA, Arias Sánchez EA, Vega Zapata RE. Levosimendan: a new strategy in the treatment of heart failure. Arch Cardiol Mex. 2005; 75 Suppl 3: S3-130-9.

[15] Perrone SV, Kaplinsky EJ. Calcium sensitizer agents: a new class of inotropic agents in the treatment of decompensated heart failure. Int J Cardiol. 2005; 103 (3): 248-55.

[16] Haikala H, Pollesello P. Calcium sensitivity enhancers. IDrugs. 2000; 3 (10): 1199-205.

[17] Bonnefoy E, Trindade PT. Levosimendan, a revolution in the world of inotropic agents. Rev Med Suisse. 2005; 1 (21): 1425-6, 1428-9.

[18] Andersen GO, Eritsland J, Bjørnerheim R, Kløw NE, Jonassen A, Mangschau A. Cardiogenic shock-new therapeutic strategies. Tidsskr nor Laegeforen. 2005; 125 (10): 1318-21.

[19] Ng TM.Levosimendan, a new calcium-sensitizing inotrope for heart failure. Pharmacotherapy. 2004; 24 (10): 1366-84.

[20] Papp Z, Van Der Velden J, Borbély A, Edes I, Stienen GJ. Effects of Ca^{2+}-sensitizers in permeabilized cardiac myocytes from donor and end-stage failing human hearts. J Muscle Res Cell Motil. 2004; 25 (3): 219-24.

[21] Sorsa T, Pollesello P, Rosevear PR, Drakenberg T, Kilpeläinen I. Stereoselective binding of levosimendan to cardiac troponin C causes Ca^{2+}-sensitization. Eur J Pharmacol. 2004; 486 (1): 1-8.

[22] Follath F. Levosimendan in patients with low-output heart failure: lessons from the LIDO trial. Ital Heart J. 2003; 4 Suppl 2: 34S-38S.

[23] Erhardt LR. Is calcium sensitization the best strategy to improve myocardial contractility in acute heart failure? Ital Heart J. 2003; 4 Suppl 2: 27S-33S.

[24] Lehmann A, Boldt J, Kirchner J. The role of Ca^{2+}-sensitizers for the treatment of heart failure. Curr Opin Crit Care. 2003; 9 (5): 337-44.

[25] Endoh M. Mechanisms of action of novel cardiotonic agents. J Cardiovasc Pharmacol. 2002; 40 (3): 323-38.

[26] Teerlink JR. The development of new medical treatments for acute decompensated heart failure. Heart Fail Monit. 2002; 2 (4): 129-37.

[27] Nawarskas JJ, Anderson JR. Levosimendan: a unique approach to the treatment of heart failure. Heart Dis. 2002; 4(4):265-71.

[28] Endoh M. Mechanism of action of Ca^{2+} sensitizers--update 2001. Cardiovasc Drugs Ther. 2001; 15 (5): 397-403.

[29] Kleerekoper Q, Putkey JA. Drug binding to cardiac troponin C. J Biol Chem. 1999 Aug 20; 274 (34): 23932-9.

[30] Bowman P, Haikala H, Paul RJ. Levosimendan, a calcium sensitizer in cardiac muscle, induces relaxation in coronary smooth muscle through calcium desensitization. J Pharmacol Exp Ther. 1999; 288 (1): 316-25.

[31] Zimmermann N, Boknik P, Gams E, Herzig JW, Neumann J, Scholz H. Calcium sensitization as new principle of inotropic therapy in end-stage heart failure? Eur J Cardiothorac Surg. 1998; 14 (1): 70-5.

[32] Haikala H, Kaivola J, Nissinen E, Wall P, Levijoki J, Lindén IB. Cardiac troponin C as a target protein for a novel calcium sensitizing drug, levosimendan. J Mol Cell Cardiol. 1995; 27 (9): 1859-66.

[33] Haikala H, Levijoki J, Lindén IB. Troponin C-mediated calcium sensitization by levosimendan accelerates the proportional development of isometric tension. J Mol Cell Cardiol. 1995; 27 (10): 2155-65.

[34] Haikala H, Linden IB. Mechanisms of action of calcium-sensitizing drugs. J Cardiovasc Pharmacol. 1995; 26 Suppl 1: S10-9.

[35] Haikala H, Nissinen E, Etemadzadeh E, Levijoki J, Lindén IB. Troponin C-mediated calcium sensitization induced by levosimendan does not impair relaxation. J Cardiovasc Pharmacol. 1995; 25 (5): 794-801.

[36] Nielsen-Kudsk JE, Aldershvile J. Will calcium sensitizers play a role in the treatment of heart failure? J Cardiovasc Pharmacol. 1995; 26 Suppl 1: S77-84.

[37] Lehtonen L, Mills-Owens P, Akkila J. Safety of levosimendan and other calcium sensitizers. J Cardiovasc Pharmacol. 1995; 26 Suppl 1: S70-6.

[38] Hasenfuss G, Pieske B, Kretschmann B, Holubarsch C, Alpert NR, Just H. Effects of calcium sensitizers on intracellular calcium handling and myocardial energetics. J Cardiovasc Pharmacol. 1995; 26 Suppl 1: S45-51.

[39] Varro A, Papp JG. Classification of positive inotropic actions based on electrophysiologic characteristics: where should calcium sensitizers is placed? J Cardiovasc Pharmacol. 1995; 26 Suppl 1: S32-44.

[40] Rusca M, Liaudet L. Inotropic agents for treatment of acute heart failure syndromes in intensive care. Rev Med Suisse. 2009; 5 (229): 2512-5.