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LEVOSIMENDAN IN PATIENTS WITH ISCHAEMIC HEART DISEASE

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ACADEMIC DISSERTATION

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To My Family

ABSTRACT

Levosimendan is a new drug for the treatment of heart failure. Its mechanism of action includes calcium sensitization of contractile proteins and the opening of ATP-sensitive potassium channels. The combination of positive inotropy with possible anti-ischaemic effects *via* potassium channel opening may offer benefits in comparison with currently available intravenous inotropes, which are contraindicated in patients with ongoing myocardial ischaemia. The active levosimendan metabolite OR-1896, with properties similar to those of the parent drug, significantly prolongs the duration of the haemodynamic effects of levosimendan.

The main aims of the present study were to investigate: 1) the clinical effects and safety of intravenous and oral levosimendan and 2) the pharmacodynamics and pharmacokinetics of intravenous and oral levosimendan and its metabolites in patients with ischaemic heart disease.

In the four studies included in this thesis levosimendan was administered intravenously or orally to 557 patients with ischaemic heart disease with or without concomitant heart failure. Study I included patients with acute myocardial infarction, complicated by left ventricular failure. Studies II to IV included patients with chronic ischaemic heart disease; in studies II and IV the ischaemic heart disease was complicated by severe chronic heart failure. Non-invasive haemodynamic measurements were used in all studies, and blood samples were drawn for pharmacokinetic evaluation in studies II to IV. Safety of the patients was followed by ECG recordings, adverse event inquiries and laboratory assessments.

Intravenous levosimendan, administered as a 6-hour infusion in doses of 0.1 or 0.2 μg/kg/min did not cause clinically significant hypotension or ischaemia in comparison with placebo and reduced worsening heart failure and short- and long-term mortality. Increase in incidence of hypotension and ischaemia was seen with a dose of 0.4 μg/kg/min. Both intravenous and oral levosimendan possessed a moderate positive inotropic effect. Vasodilatory effect was more pronounced with intravenous levosimendan. A chronotropic effect was seen in all studies; however, it was not accompanied by any increase in arrhythmic events. The formation of levosimendan metabolites after oral dosing increased linearly with the daily dose of the parent drug, leading to increased inotropic and chronotropic response, especially with the doses of 6 and 8 mg daily. Levosimendan was well tolerated in all studies.

In conclusion, levosimendan was safe and effective in the treatment of patients with acute and chronic ischaemia. The risk-benefit ratio of intravenous levosimendan is favourable up to the dose of 0.2 μg/kg/min. The daily dose of oral levosimendan in patients with ischaemic heart failure should not exceed 4 mg due to an increase in chronotropic response.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. Moiseyev VS, Põder P, Andrejevs N, Ruda MY, Golikov AP, Lazebnik LB, Kobalava ZD, Lehtonen LA, Laine T, Nieminen MS, Lie KI. Safety and efficacy of a novel calcium sensitizer, levosimendan, in patients with left ventricular failure due to an acute myocardial infarction. A randomized, placebo-controlled, double-blind study (RUSSLAN). Eur Heart J 2002;23(18):1422-32.

II. Põder P, Eha J, Sundberg S, Antila S, Heinpalu M, Loogna I, Planken Ü, Rantanen S, Lehtonen L. Pharmacokinetic-pharmacodynamic interrelationships of intravenous and oral levosimendan in patients with severe congestive heart failure. Int J Clin Pharmacol Ther 2003;41(8):365-73.

III. Põder P, Eha J, Antila S, Heinpalu M, Planken Ü, Loogna I, Mesikepp A, Akkila J, Lehtonen L. Pharmacodynamic interactions of levosimendan and felodipine in patients with coronary heart disease. Cardiovasc Drugs Ther 2003;17(5-6):451-8.

IV. Põder P, Eha J, Sundberg S, Antila S, Heinpalu M, Loogna I, Rantanen S, Lehtonen L. Pharmacodynamics and pharmacokinetics of oral levosimendan and its metabolites in patients with severe congestive heart failure: a dosing interval study. J Clin Pharmacol 2004;44(10):1143-50.

ABBREVIATIONS

ACC	American College of Cardiology
ACE	angiotensin-converting enzyme
ACS	acute coronary syndrome
ADP	adenosine diphosphate
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
AMI	acute myocardial infarction
ARB	angiotensin II receptor blocker
ASA	acetylsalicylic acid
ATP	adenosine triphosphate
AUC	area under concentration-time curve
AV	atrioventricular
BNP	b-type natriuretic peptide
BP	blood pressure
CABG	coronary artery by-pass grafting
CCS	Canadian Cardiovascular Society
CO	cardiac output
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CABG	coronary artery by-pass grafting
CHF	chronic heart failure
CI	confidence interval
CK	creatine kinase
CK-MB	MB-fraction of creatine kinase
Cl_{tot}	total clearance
C_{max}	maximum concentration
COX	cyclooxygenase
CVD	cardiovascular disease
dBP	diastolic blood pressure
dp/dt	rate of rise of intraventricular pressure
DTI	direct thrombin inhibitor
ECG	electrocardiogram
eNOS	endothelial NO synthase
ESC	European Society of Cardiology
Gp	glycoprotein
HDL	high density lipoprotein
HMG-CoA	hydroxylmethylglutaryl coenzyme A
HR	heart rate
IHD	ischaemic heart disease
K^+_{ATP} channels	ATP-sensitive potassium channels
LDL	low-density lipoprotein
LMWH	low molecular weight heparin
LS	levosimendan
LVEF	left ventricular ejection fraction
LVD	left ventricular dysfunction
LVF	left ventricular failure
MMP	matrix metalloproteinase
MRT	mean residence time

NO	nitric oxide
NSTE ACS	non ST-elevation acute coronary syndrome
NSTEMI	non ST-elevation myocardial infarction
NYHA	New York Heart Association
PCI	percutaneous coronary intervention
PCWP	pulmonary capillary wedge pressure
PDE	phosphodiesterase
PEP	primary endpoint
PET	positron emission tomography
PKA	protein kinase A
PKC-ε	protein kinase C-epsilon
PTCA	percutaneous transluminal coronary angioplasty
QS2	electromechanical systole
QS2i	heart rate corrected electromechanical systole
RAAS	renin-angiotensin-aldosterone system
Rt-PA	recombinant tissue plasminogen activator
sBP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
STEMI	ST-elevation myocardial infarction
t_{max}	time of maximum concentration
$t_{1/2}$	terminal elimination half-life
TNFα	tumour necrosis factor alpha
UFH	unfractionated heparin
UA	unstable angina
VEGF	vascular endothelial growth factor
V_{ss}	apparent volume of distribution at steady-state
VSMC	vascular smooth muscle cell
VT	ventricular tachycardia
V_z	apparent volume of distribution based on the terminal phase
WHO	World Health Organisation
λ_z	terminal elimination rate constant

1. Introduction

Ischaemic heart disease (IHD) belongs to the entity of cardiovascular disease (CVD), together with hypertension, stroke and valvular, muscular and congenital heart disease. About 15% of worldwide mortality is attributable to IHD, making it the leading cause of death globally (1). By 2020 it is expected that IHD will be the largest cause of disease burden worldwide (2). In USA more than 12 million people currently suffer from IHD and in 2000 the economic cost of IHD was estimated at about 120 billion USD (3).

Recent epidemiological data have shown that mortality due to IHD in industrialised countries is decreasing (4). The reasons for such a decrease include the introduction of new treatment measures and the reduction in the incidence of the major risk factors of IHD (hypercholesterolemia, hypertension, diabetes and smoking). For example in USA the mortality due to IHD has declined by 50% over the last 30 years (5). Similar trends have been observed also in other Western countries (4, 6).

Current therapy of IHD consists of pharmacological therapy and revascularisation procedures. The main goals of these treatment measures are establishing reperfusion in coronary arteries, enhancing coronary blood flow, reduction of myocardial oxygen consumption and the incidence of arrhythmic disorders and, in patients with acute myocardial infarction (AMI), also limitation of the infarct size. IHD is the most important and most common contributor to the development of heart failure, accounting for up to 50% of cases (7, 8). The prognosis of patients with heart failure due to IHD remains poor despite intensive pharmacological therapy and increasing utilization of surgical interventions.

Treatment with positive inotropic drugs is currently indicated for patients with severe chronic or acute heart failure to improve the pump function of the heart (7, 9, 10). Beta-adrenergic agonists and phosphodiesterase inhibitors improve cardiac contractility by increasing intracellular calcium concentration. This mechanism, however, leads to increase in myocardial oxygen consumption. Increase in oxygen demand further leads to the increase of arrhythmic and ischaemic complications, which can easily occur in ischaemic patients, whose haemodynamics is unstable. Therefore these drugs have not been widely used in patients with current or recent ischaemia. Furthermore, clinical trial evidence regarding positive inotropic drugs in these patients is very limited (10-13).

Levosimendan is a drug that possesses a novel mechanism of positive inotropy. It is a calcium sensitizer, meaning that the drug augments myocardial contractility by increasing myofilament sensitivity to calcium by binding to cardiac troponin C in a calcium-dependent manner (14-17). This mechanism allows the achievement of positive inotropic effect without increasing intracellular calcium concentrations. Levosimendan also opens ATP-sensitive potassium channels (K^+_{ATP}) in vascular and cardiac tissue, thereby producing vasodilatory and possibly also anti-ischaemic effects (18-20). Levosimendan inhibits also cardiac and smooth muscle phosphodiesterase, being a phosphodiesterase (PDE) III inhibitor (21).

Previous studies have shown that levosimendan does not significantly increase myocardial oxygen consumption in healthy volunteers or in patients with heart failure (22, 23). In theory levosimendan may therefore be safer than conventional inotropes in patients with acute or chronic ischaemia.

The aim of this thesis was to study the effects of levosimendan in patients with stable IHD or AMI, with and without concurrent heart failure.

2. Review of the literature

2.1 Pathophysiological features of ischaemic heart disease

Atherosclerosis

Atherosclerosis is a chronic inflammatory condition, together with endothelial dysfunction, which advances to a clinical event by the formation of atherosclerotic plaques and the induction of plaque rupture, which leads to thrombosis (24-26). Endothelial dysfunction is considered an early marker for atherosclerosis, preceding angiographic or ultrasonic evidence of atherosclerotic plaque formation. Damage to the endothelium upsets the balance between vasoconstriction and vasodilation and initiates the processes that promote or exacerbate atherosclerosis; these include endothelial permeability, platelet aggregation and generation of cytokines (27, 28). The hallmark of endothelial dysfunction is impairment of the nitric oxide-mediated vasodilation.

Role of nitric oxide (NO) in atherosclerosis and ischaemia

NO is formed in endothelial cells from its precursor L-arginine *via* the enzymatic action of endothelial NO synthase (eNOS) (29). In the vascular wall, NO activates soluble guanylate cyclase in vascular smooth muscle cells (VSMCs), leading to elevation of cyclic guanosine monophosphate (cGMP), activation of cGMP-dependent protein kinase (PKG), and vasorelaxation (30). It has been proposed that oxidation of low-density lipoprotein (LDL) is a major mechanism of atherosclerosis (26). Since NO prevents oxidative modification of LDL, impaired production or activity of NO leads to events that promote atherosclerosis, such as vasoconstriction, platelet aggregation, smooth muscle cell proliferation and migration, leukocyte adhesion and oxidative stress (27). Oxidized LDL cholesterol increases the synthesis of caveolin-1, which by inactivating eNOS, inhibits production of NO (31). In patients with dysfunctional endothelium, the loss of flow-mediated and catecholamine-stimulated NO release permits the vasoconstriction by catecholamines. Thus the reduced production of NO contributes to impaired vasodilation and exaggerated coronary vasoconstriction and thereby also to myocardial ischaemia (32-34).

In the later stages of atherosclerosis ultrastructural changes will take place. Atherosclerotic plaque formation starts with the development of a fatty streak. Several phases can be identified in streak development, e.g. extracellular lipid formation, leukocyte accumulation and foam cell and lesion formation (26, 35). In the next phase of evolution of the atheroma - development of a fibrofatty lesion - smooth muscle cells divide and elaborate extracellular matrix, promoting extracellular matrix accumulation in the growing atherosclerotic plaque (36, 37). In addition, neovascularization occurs in atherosclerotic plaques (38). During the progress to advanced lesions, fatty streaks tend to form a fibrous cap that walls off the lesion to lumen. In the beginning the plaque grows outwards, leading to an increase in the diameter of the artery. At some point when the artery can no longer compensate by dilation, the lesion intrudes into the lumen and alters blood flow (39, 40).

The late stages are marked by calcification and rupture of the plaque. Calcification is promoted by smooth muscle cells, by enhanced secretion of bone morphogenetic proteins, which suggests that plaque calcification can be regulated similarly to bone formation (41, 42). Plaque rupture, which is the predominant cause of thrombosis, can be defined as a disruption of an area of the fibrous cap, whereby the overlying thrombus is in continuity with the lipid core (43). Plaques with active inflammation, thin cap with large lipid core, endothelial denudation with superficial platelet aggregation, and plaques with fissured caps and stenotic plaques, are considered to be “vulnerable” plaques (44, 45). Another process leading to thrombosis is called endothelial

erosion. In this case the plaque itself is intact. The erosion is caused by an extension of the process of endothelial denudation whereby large areas of the surface of the subendothelial connective tissue of the plaque are exposed (46, 47).

The rupture of plaques is considered to be the common pathophysiological substrate of *acute coronary syndromes (ACS),* involving *unstable angina (UA), and transmural (STEMI)* and *non-transmural myocardial infarction (NSTEMI).* When episodes of *stable angina* are associated with plaque rupture associated with intraplaque thrombus, then UA is associated with thrombi that project, but do not occlude the lumen of the coronary artery, thus preserving some antegrade flow in the artery. Several potential mechanisms of UA attacks, such as constriction of coronary artery, intermittent change in size of thrombus and platelet disposition, have been proposed. Acute myocardial infarction (AMI), on the other hand, occurs when total coronary artery occlusion develops. In case of transmural (STEMI) infarction, occlusion develops over a relatively short time frame of a few hours and persists for at least 6-8 hours. The infarcted tissue is a structurally homogenous entity, i.e. all the involved myocardium dies at around the same time. Non-transmural (NSTEMI) infarcts have a different structure, built up by the coalescence of many small areas of necrosis of very different ages. A factor in limiting the spread of necrosis and preserving the subepicardial zone is the existence of collateral flow in the affected artery. The development of AMI results in apoptosis and necrosis of myocardiocytes (46).

Apoptosis and necrosis

The common view on how cardiomyocytes die during or after myocardial ischaemia or infarction has changed in recent years. For a long time necrosis was regarded as the sole cause of cell death. Now recent studies indicate that also apoptosis plays an important role in the process of tissue damage. Although both apoptosis and necrosis result in the death of the cell, they differ regarding several morphological and cellular regulatory features (48).

Necrosis is characterised by the rapid loss of cellular homeostasis, rapid swelling as a result of the accumulation of water and electrolytes, early plasma membrane rupture and disruption of cellular organelles (48). Different patterns of necrosis have been described. Coagulation necrosis, resulting from severe, persistent ischaemia, is present usually in the central region of infarction. The coagulation necrosis results in the arrest of muscle cells in the relaxed state and is characterised by shrinkage and loss of nucleus (49). The other form, contraction band necrosis, results primarily from severe ischaemia followed by reflow (reperfusion). It is caused by calcium ion influx into dying cells, resulting in the arrest of cells in the contracted state and is characterised by contracted myofibrils in contraction bands and mitochondrial damage with calcification and vascular congestion (49).

Apoptosis was first introduced in a paper by a group of pathologists studying cell population regulation (50). Apoptosis is defined as a form of cell death that involves genetic and molecular programs, *de novo* protein expression and unique cellular phenotype (48, 51, 52). It is characterised by shrinkage of the cell and nucleus. Nuclear chromatin is condensed into sharply delineated masses and eventually breaks up. Then the cell detaches from the surrounding tissue. At this stage, extensions bud out from its membranes, which seal off to form membrane enclosed vesicles, called apoptotic bodies, containing condensed cellular organelles and nuclear fragments. These apoptotic bodies are either rapidly phagocytosed by neighbouring cells or undergo degradation, which resembles necrosis in a process called secondary necrosis. In contrast to necrosis, apoptosis is generally considered not to trigger an inflammatory response (48, 51-53).

In the cardiovascular system apoptosis has been found in addition to AMI also in association with dilative cardiomyopathy and conduction system disorders (54-56). Apoptosis is also a feature of atherosclerosis, evidenced by increased expression of molecular markers in atherosclerotic tissue (57, 58). Multiple studies have found apoptosis in atherosclerotic coronary, carotid and aortic arteries and in smooth muscle cells of the media underlying atherosclerotic lesions (58-60).

Several stimuli and pathways that elicit cardiomyocyte apoptosis have been identified. They include ischaemia (especially when followed by reperfusion) (61, 62), oxygen radicals (H_2O_2, O_2^-) (63), caspases (64), tumour necrosis factors (65, 66), and nuclear factor-kappaB (67).

Three consequences of cardiomyocyte apoptosis and necrosis can be envisioned:

1) compromise in cardiac contractility due to loss of myocytes;

2) conduction disturbances leading to arrhythmias;

3) cardiac remodelling due to disruption of the geometrical alignment of myocytes.

Cardiac remodelling

Cardiac remodelling is the central mechanism of heart failure progression in patients with IHD, occurring usually as a consequence of AMI. Postinfarction remodelling can be divided into an early phase (within 72 hours) and a late phase (beyond 72 hours) (68).

Early remodelling

Early remodelling involves expansion of the infarct zone and collagen degradation, which may result in early ventricular rupture or aneurysm formation (69). Infarct expansion results from the degradation of the intermyocyte collagen struts by serine proteases and from the activation of matrix metalloproteinases (MMPs) released from neutrophils (70). Infarct expansion occurs within hours of myocyte injury, results in wall thinning and ventricular dilatation, and causes the elevation of diastolic and systolic wall stresses (71). Substantial changes in circulatory haemodynamics trigger the sympathetic adrenergic system, which stimulates catecholamines, activates the renin-angiotensin-aldosterone system (RAAS), and stimulates the production of endothelins, and atrial and b-type natriuretic peptides (ANP and BNP). Positive inotropic, chronotropic and also vasodilatory effects from this sympathetic stimulation result in hyperkinesis of the noninfarcted myocardium and temporary circulatory compensation by reduction of systemic vascular resistance and left ventricular filling pressure (68). However, although the neurohormonal activation initially serves an adaptive role, in later stages the responses become pathological and contribute adversely to remodelling and ultimately to the progress of heart failure. In addition, neurohormonal activation may precipitate further ischaemia by increasing oxygen demand and predisposing to arrhythmias (72).

Late remodelling and scar formation

Late remodelling involves the left ventricle globally and is associated with time-dependent dilatation, distortion of ventricular shape and hypertrophy. Hypertrophy is an adaptive response during postinfarction remodelling that offsets increased load, attenuates progressive dilatation, and stabilizes contractile function (73). It is initiated by neurohormonal activation, myocardial stretch, activation of the RAAS, and by paracrine/autocrine factors. Especially enhanced norepinephrine release contributes to the hypertrophy.

Myocardial repair and scar formation is triggered by cytokines released from injured myocytes. Before collagen synthesis tissue repair is initiated by the formation of a fibrin-fibronectin matrix to which myofibroblasts become adherent (74). Deposition of collagen occurs predominantly in the infarct zone, but also in noninfarcted myocardium. Collagen is detectable microscopically by day 7 and its deposition then increases dramatically, such that by 28 days, the necrotic myocytes are entirely replaced by fibrous tissue. After the formation of a scar that equilibrates distending and restraining forces, collagen formation is down-regulated and most fibroblasts undergo apoptosis (70).

2.2 Consequences of myocardial ischaemia

Myocardial stunning and hibernation

After a brief episode of severe ischaemia, there is a period of prolonged myocardial dysfunction with a gradual return of contractile activity. This condition has been termed as *myocardial stunning* (75). Myocardial stunning has been observed in patients with IHD in a variety of clinical conditions, such as early thrombolytic reperfusion after AMI, percutaneous transluminal coronary angioplasty (PTCA), delayed recovery from angina pectoris and ischaemic cardioplegia in connection with coronary artery by-pass grafting (CABG) (76-78). Stunning is currently considered to occur *via* three synergic mechanisms:

a) generation of oxygen derived free radicals

b) increase of cytosolic calcium and reduction in sensitivity of myofilaments to calcium

c) loss of myofilaments (79).

In case of stunning, myocardial ischaemia, followed by reperfusion, results in increased production of superoxide and hydroxyl radicals, the targets of which are sarcolemmal Na^+ K^+ - and Ca^{2+}-stimulated ATPase and in sarcoplasmatic reticulum Ca^{2+}-stimulated ATPase. This causes increased influx of calcium through sarcolemma and decreased calcium reuptake by sarcoplasmatic reticulum, which results in cellular calcium overload and impaired excitation-contraction coupling. Ischaemia followed by reperfusion results in decreased calcium sensitivity of myofilaments. Recovery from stunning takes from hours to days, depending on the duration of the occlusion. Full mechanical recovery from stunning may take from days to weeks (76-78).

The term *myocardial hibernation* was introduced in the early 1980s; it refers to the presence of impaired left ventricular function due to reduced coronary blood flow that can be improved by revascularisation (80, 81). The molecular basis of hibernation has not been extensively investigated so far. It has been shown that hibernating myocardium exhibits a molecular phenotype that on a regional basis is similar to that found in end-stage ischaemic cardiomyopathy (82). There is evidence that hibernation is related to partial inhibition of cytochrome oxidase during hypoxia, which allows mitochondria to function as the oxygen sensors, limiting ATP utilization and oxygen consumption (83). A recent study has also demonstrated the upregulation of genes and corresponding proteins involved in anti-apoptosis (IAP, caspase inhibitor), growth (VEGF, vascular endothelial growth factor) and cytoprotection (hypoxia-inducible factor -1α, heat-shock protein HSP70) (84).

Hibernating myocardium is present in approximately one third of patients with IHD and impaired left ventricular function (85, 86). The time course of recovery of hibernating myocardium after revascularisation varies from days to months. In many cases, however,

recovery remains incomplete (87). Detection of hibernating myocardium is based on finding still viable akinetic or hypokinetic segments of left ventricle. Different methods are used for detection of myocardial viability and contractile reserve, e.g. dobutamine stress echocardiography, thallium-201 redistribution study, imaging with technetium-99m-sestamibi and positron-emission tomography (88).

Both stunning and hibernation play an important clinical role, since they contribute to the process and progress of heart failure in patients with IHD. A major unresolved issue that has important pathophysiological and clinical implications is whether repetitive episodes of myocardial stunning can account for at least some of the clinical manifestations of hibernation. Indeed, it has been demonstrated that recurrent stunning can cause prolonged, reversible dysfunction. The main difference between stunning and hibernation is that regional perfusion is normal or near normal in stunning, but low in hibernation. If regional perfusion is not measured simultaneously with regional contractile function (and it is rarely done in clinic), the reversible dysfunction associated with repetitive stunning may mimic hibernation. On the other hand, it is well known that many patients with IHD experience recurrent episodes of ischaemia in the same territory, so the myocardium may remain reversibly depressed for extended periods of time. It is therefore possible that in some clinical cases in which reversible left ventricular dysfunction is thought to be secondary to hibernation, the depressed contractility is in fact secondary to repetitive stunning (89).

The treatment of these two forms of myocardial dysfunction is different. Several trials have demonstrated that patients with hibernating myocardium with left ventricular dysfunction appear to have better outcome after revascularisation (90-92). Myocardial stunning, on the other hand, can be reversed by using positive inotropic agents. Positive inotropic drugs have been useful in the post-cardiopulmonary by-pass setting and in patients who experience severe heart failure after successful reperfusion (93-95). Since one of the mechanisms of stunning is desensitization of myofilaments to calcium, an inotrope that would increase the sensitivity of myofilaments, would in theory be a very useful pharmacological tool to overcome stunning.

Ischaemic preconditioning

Myocardial ischaemic preconditioning is a phenomenon by which the brief episodes of myocardial ischaemia increase the ability of the heart to tolerate a subsequent period of ischaemic injury. The stimulus for preconditioning is the reduction of coronary blood flow. The protection obtained has been characterized both in terms of time course and various end points in cellular injury (96). Preconditioning has been shown to reduce reperfusion arrhythmias (97), slow energy metabolism during early stages of ischaemia (98), improve post-ischaemic recovery of function (99), protect coronary endothelium (100), and increase the resistance of isolated myocytes to hypoxia (101) and ischaemia (102).

Preconditioning can be divided according to its time course to “early” and “late” preconditioning (96). Typically in *early* preconditioning, a 5-minute period of ischaemia followed by up to 60-minute reperfusion prior to the repeat ischaemic episode results in salvage (103). If the time between the initial and repeated ischaemia is prolonged to 24-96 h, a protective effect may also be seen and is called as *late* preconditioning (104). Unlike the early preconditioning the late preconditioning protects against not only myocardial infarction, but also against myocardial stunning (105).

Adenosine and bradykinin are the most well-studied triggers of preconditioning (106, 107). The protective effect of ischaemic preconditioning is thought to be due to intracellular mediators, three types of which have been described in literature: K^{+}_{ATP} channels, protein kinase C-epsilon

(PKC-ε) and protein kinase A (PKA) (108-110). Two K^+_{ATP} channels exist in different locations in cardiomyocytes: in the sarcolemma and the mitochondria. K^+_{ATP} channels open whenever ATP declines substantially, as during brief ischaemic periods (111). Blocking the K^+_{ATP} channel eliminates the preconditioning effect (112, 113). Sarcolemmal K^+_{ATP} channels can be blocked by sulfonylureas and 5-hydroxycanoate (5HD), whereas mitochondrial K^+_{ATP} channels can be opened by diazoxide and blocked with low concentrations of 5HD (96, 113). It is suggested that the lack of cardioprotective effect by sulfonylureas may be due to blunting of the K^+_{ATP} channel dependent component of the preconditioning response (114). The protein kinases play a role in preconditioning possibly through kappa-opioid receptors, Rho-kinase inhibition and actin cytoskeletal deactivation (110). For *late* preconditioning, also additional stimuli, including heat stress, rapid ventricular pacing, exercise, endotoxins, interleukin-1, TNFα, reactive oxygen species, NO donors and adenosine receptor agonists have been proposed (96).

Ischaemic preconditioning has also important clinical implications. It has been shown that preinfarction angina is associated with a subsequent reduction of the infarct size (115, 116). Ischaemic preconditioning is also responsible for the "warm up" or "walk through angina" phenomenon, and it has been shown to protect against ventricular tachyarrhythmias following balloon occlusion in PTCA, in variant angina and following CABG (117, 118). Administration of drugs that induce or enhance ischaemic preconditioning have therefore potential to decrease myocardial injury, cell death, preserve ventricular function and reduce mortality. Such agents could include adenosine and its agonists, PKC agonists, NO donors and K^+_{ATP} openers.

2.3 Clinical presentation of ischaemic heart disease

Ischaemic heart disease is caused by an imbalance between the supply and demand of oxygen to the heart. The condition is most often caused by the narrowing of coronary arteries and an associated reduction in the flow of oxygenated blood. The disease may be symptomatic or asymptomatic and it may have a stable or a progressive course. IHD is classified on the basis of symptomatology and severity (119-122).

Stable angina pectoris

Stable angina pectoris is the main symptom/form of IHD. The pathological substrate for angina is almost invariably atheromatous narrowing of the coronary arteries. It is usually considered that a coronary artery must be narrowed by at least 50-70% in luminal diameter before coronary blood flow is inadequate to meet the metabolic demands of the heart with exercise or stress (120-122). The importance of stenosis depends also on the length and number of stenoses.

Angina pectoris results from myocardial ischaemia, which is caused by an imbalance between myocardial oxygen requirements and oxygen supply. Increased oxygen demand may occur due to increase in heart rate, left ventricular wall stress or contractility. Oxygen supply, on the other hand, is determined by coronary blood flow and coronary arterial oxygen content. The precipitating factors causing angina due to increased myocardial oxygen consumption include exercise, mental stress, fever, cold, tachycardia from any cause, thyrotoxicosis, and hypoglycaemia (123). Typical angina pectoris is substernal, across mid-thorax, anteriorly, can locate also in arms, shoulders, neck, and interscapular region. It is characterized by a burning, heavy or squeezing feeling, is precipitated by exertion or emotion and is promptly relieved by rest or by nitroglycerin. The typical episode of angina pectoris usually begins gradually and reaches its maximum intensity over a period of minutes before dissipating (119-122, 124). If the symptoms remain the same for several weeks and constantly occur under the same physical or mental stress, the condition is described as "stable" angina pectoris (119-122).

Stable angina pectoris is classified by severity into 4 classes according to the Canadian Cardiovascular Society (125, 126) (Table 1).

Table 1. Classification of stable angina pectoris

Class I	Angina occurs with strenuous or rapid or prolonged exertion at work or recreation. Ordinary physical activity, such as walking and climbing stairs, does not cause angina.
Class II	Slight limitation of ordinary activity. Angina occurs on walking or climbing stairs rapidly, walking uphill, walking or stair climbing after meals, in cold, in wind or when under emotional stress, or only during the few hours after awakening. Walking more than two blocks on the level and climbing more than one flight of ordinary stairs at a normal pace and in normal conditions.
Class III	Marked limitation of ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight in normal conditions.
Class IV	Inability to carry on any physical activity without discomfort – anginal syndrome may be presented at rest.

Unstable angina pectoris and Prinzmetal (variant) angina

The currently used definition of unstable angina pectoris depends on the presence of one or more of the following features:

1) Crescendo angina (more severe, prolonged or frequent) superimposed on an existing pattern of relatively stable, exertion-related angina pectoris

2) Angina pectoris of new onset (usually within 1 month), which is brought on by minimal exertion

3) Angina pectoris at rest as well as with minimal exertion (119).

A classification of UA is presented in Table 2 (127).

Table 2. Classification of unstable angina pectoris

Class I	New-onset, severe or accelerated angina. Patients with angina of less than 2 months' duration, severe angina or angina occurring three or more times per day or angina that is distinctly more frequent and precipitated by distinctly less exertion. No rest pain in the last 2 months.
Class II	Angina at rest. Subacute. Patients with one or more episodes at rest during the preceding month, but not within the preceding 48 hours.
Class III	Angina at rest. Acute. Patients with one or more episodes at rest within the preceding 48 hours.

Prinzmetal's (variant) angina is an unusual and uncommon form of angina secondary to myocardial ischaemia that occurs almost exclusively at rest, is usually not precipitated by physical exertion or emotional stress and is associated with ST-segment elevations in electrocardiogram (128-130). Variant angina is demonstrated to be due to coronary artery spasm, which narrows the coronary artery resulting in myocardial ischaemia (129, 130). Endothelial dysfunction, an increased platelet aggregation together with changes in autonomic tone can trigger the vasospasms (131, 132). Also dysfunction of K^{+}_{ATP} channels may have role in variant angina (133).

Silent ischaemia

Silent myocardial ischaemia is defined as objective documentation of myocardial ischaemia in the absence of angina or anginal equivalents. It has been explained by the ability of patients to produce endogenous opioids that raise the pain threshold, by autonomic neuropathy and also by a defect in the cerebral cortex. Silent ischaemia and infarction are more frequent in the elderly, women and diabetics (134). Patients with silent ischaemia can be divided into three types: Type I patients have no symptoms at any time in spite of obstructive IHD, type II patients have silent ischaemia after experiencing AMI, and patients of type III, the most common group, have either concurrent chronic stable angina, UA or Prinzmetal angina (134). Patients experiencing episodes of silent myocardial ischaemia have been found to have a worse prognosis compared with those without silent ischaemia (135-138).

Acute myocardial infarction

Acute myocardial infarction (AMI) represents the most critical and serious form of IHD. Although the death rate from AMI has continuously declined over the past decades, its development together with all complications is fatal for about one third of the patients (139, 140).

Almost all AMIs result from coronary atherosclerosis, generally with superimposed coronary thrombosis. During the natural evolution of atherosclerotic plaques an abrupt transition may occur, characterized by plaque rupture. After plaque rupture there is an exposure of substances that promote platelet activation and aggregation, thrombin generation and ultimately thrombus formation. The thrombus interrupts the blood flow and leads to an imbalance between oxygen supply and demand and, if this imbalance is severe and persistent, to myocardial necrosis. After onset of infarction, the first ultrastructural changes are noted already within 20 minutes. The first irreversible changes are seen after about 1-2 hours from onset of AMI. After about 6 hours of continuous occlusion the entire jeopardised area becomes necrotic. The infarction process results in the formation of a fibrous scar with interspersed intact muscle fibres after about 6 weeks from onset of the process (140).

The WHO criteria for diagnosis of AMI required that at least two of three elements be present for diagnosis; these criteria have been used for several decades for diagnosis of AMI (141). Recently, however, mainly for purposes of risk stratification and subsequent treatment, a revised definition of AMI has been proposed by European and American cardiology societies (Table 3) (142).

Table 3. Diagnostic criteria of acute myocardial infarction

WHO	1) Definite ECG **or** 2) Typical or atypical symptoms or inadequately described symptoms, together with probable ECG or abnormal enzymes **or** 3) Typical symptoms with abnormal enzymes with ischaemic or non-codable ECG or ECG not available **or** 4) Fatal case, whether sudden or not, appearance of fresh myocardial infarction and/or recent coronary occlusion found at necropsy
ESC and ACC	1) Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following: a) ischaemic symptoms b) development of pathological Q-waves on ECG c) ECG changes indicative of myocardial ischaemia (ST-segment elevation or depression) d) coronary artery intervention (e.g. coronary angioplasty) 2) Pathological findings of an AMI

Heart failure and cardiogenic shock

Heart failure is a syndrome in which patients should have the following features: symptoms of heart failure (typically breathlessness or fatigue, either at rest or during exertion, or ankle swelling), and objective evidence of cardiac dysfunction (7). The clinical symptoms and signs of heart failure also include hepatojugular reflux, jugular venous distension, gallop rhythm, proteinuria, pulmonary rales, cyanosis and ascites. In addition, B-type natriuretic peptide, a protein released from the left ventricle in response to volume expansion and pressure overload, has been recently introduced as the first blood marker for identification of patients with heart failure (143, 144). Most heart failure is associated with left ventricular systolic dysfunction. Diastolic heart failure is diagnosed when symptoms and signs of heart failure occur in the presence of normal systolic function (7, 9).

IHD is considered to be the commonest cause of systolic dysfunction and heart failure in the current era (7, 9). Despite different new treatment initiatives, heart failure in its different clinical forms has remained the most common cause of death in patients with IHD. Three main underlying mechanisms of heart failure can be identified in patients with IHD:

1) Permanent myocyte loss due to infarction with scar formation

2) Chronic dysfunction in viable myocardium subtended by stenosed coronary arteries which recovers after revascularisation (hibernating myocardium)

3) Changes in the remote myocardium (adverse remodelling) (145).

Table 4 shows the New York Heart Association (NYHA) classification of chronic heart failure, which is based on the relation between symptoms and the effort to provoke them (146).

Table 4. NYHA classification of chronic heart failure

Class I	Ordinary physical activity does not cause symptoms.
Class II	Slight limitation of physical activity. Ordinary physical activity results in symptoms.
Class III	Marked limitation of physical activity. Less than ordinary physical activity leads to symptoms.
Class IV	Inability to carry on any physical activity without discomfort. Symptoms of heart failure are present at rest. With any physical activity, increased discomfort is experienced.

In patients with chronic angina, heart failure often develops over time, i.e. the symptoms and worsening of NYHA heart failure class progress together with the progression of symptoms of IHD. However, the acute worsening of current IHD status may provoke rather rapid onset of symptoms and signs, a status defined as acute heart failure. There can be two clinical presentations – acute decompensated heart failure ("acute-on chronic") or *de novo* acute heart failure. Both are characterised by rapid development of severe left ventricular failure and pulmonary oedema. However, whereas acute decompensated heart failure is characterised also by increased fluid retention (increased peripheral oedema), *de novo* acute heart failure usually occurs without clinical signs of peripheral oedema. Very often *de novo* acute heart failure occurs in patients with AMI, the severity being highly dependent on the extent of damage to the myocardium (10). A classification of heart failure after AMI, based on physical signs, was first published in 1967 by Killip and colleagues. It has proved useful for clinical characterisation and prognosis of these patients (Table 5) (147-149).

Table 5. Killip classification of heart failure after AMI

Class I	No signs of congestive heart failure
Class II	S3 gallop and bibasilar rales
Class III	Acute pulmonary oedema
Class IV	Cardiogenic shock

The most severe expression of acute heart failure is cardiogenic shock, which is associated with extensive damage to the left ventricular myocardium. Cardiogenic shock occurs when more than 40% of the myocardium is destroyed; it is observed in about 5-10% of patients with AMI and it is more common in patients with ST-elevation. Cardiogenic shock is characterized by marked and persistent hypotension with systolic blood pressure (sBP) less than 90 mmHg (or in chronically hypertensive patients a drop in sBP of 30 mmHg or more), reduced cardiac index, elevated pulmonary capillary wedge pressure (PCWP) and evidence of vital organ hypoperfusion (oliguria, cool extremities, acidosis). The timing of shock varies, but it occurs most commonly within 48 hours of the onset of AMI (150).

A recent observational study in USA, carried out in 775 hospitals has revealed some decline in overall in-hospital cardiogenic shock mortality, which is probably related to more aggressive use of revascularization procedures. Mortality, however, remains high, being about 50% (151).

2.4 Therapy of IHD

2.4.1 Therapy to improve prognosis

Beta-adrenergic receptor blockers

Beta-adrenergic receptor blockers constitute a cornerstone of IHD therapy. The action of beta-blockers depends on their ability to cause competitive inhibition of the effects of catecholamines on beta-receptors, which reduces myocardial oxygen requirements by slowing the heart rate, increasing the time for coronary perfusion and by reducing blood pressure. Thus, in case of impaired myocardial perfusion beta-blockers favorably alter the imbalance between oxygen supply and demand, thereby eliminating ischaemia (120-122, 152). Beta-blockers can be divided into three classes: 1) nonselective $beta_1$ and $beta_2$ receptor blockers (such as propranolol), 2) selective $beta_1$ receptor blockers (such as metoprolol and atenolol), and 3) beta-blocker-vasodilators (such as carvedilol and bucindolol) (120-122).

The efficacy of beta-blockers has been shown in numerous clinical trials in patients with stable angina, given either alone or together with other antianginal agents (153-158). Efficacy has been similar to that seen with calcium antagonists and nitrates, however, beta-blockers have had better tolerability (159, 160). The effect of beta-blockers on the prognosis of stable angina has not been established, although in UA several trials have shown benefit of beta-blockers in reducing the incidence of subsequent AMI or recurrent ischaemia (161-164).

The benefit of using beta-blockers after AMI has been confirmed in well-controlled randomized clinical trials (Table 6). Use of beta-blockers undoubtedly improves both short- and long-term outcome, reduces the infarct size and is effective in the long-term secondary prevention of AMI (165-170).

Beta-blockers were contraindicated for several years in patients with heart failure. However, well-controlled large randomized trials, such as the CIBIS II, MERIT-HF and COPERNICUS trials have now established their beneficial effect on mortality, which has been reduced by approximately one third in this population (171-173) (Table 6). The most extensively studied beta-blocker in this respect has been carvedilol, which has shown benefit over metoprolol (174). CAPRICORN and CHRISTMAS trials showed beneficial effects of carvedilol also in patients with IHD complicated by left ventricular dysfunction (LVD) (86, 175).

Table 6. Major clinical trials with beta-blockers

Study (ref.)	N	Patients	Drug	Comparator	Primary endpoint (PEP)	Result of PEP
ISIS-1 (166)	16027	AMI	atenolol	placebo	vascular mortality	↓ by 15%
MERIT-HF (172)	3991	NYHA II-IV	metoprolol	placebo	all-cause mortality	↓ by 34%
COMET (174)	3029	NYHA II-IV	carvedilol	metoprolol	1) all-cause mortality 2) all-cause mortality or hospitalisation for any reason	1) ↓ by 17% 2) ↓ by 6%
BEST (176)	2708	NYHA III-IV	bucindolol	placebo	all-cause mortality	↓ by 10%
CIBIS II (171)	2647	NYHA III-IV	bisoprolol	placebo	all-cause mortality	↓ by 34%
COPERNICUS (173)	2289	Severe HF	carvedilol	placebo	all-cause mortality	↓ by 35%
CAPRICORN (175)	1959	LVD after AMI	carvedilol	placebo	all-cause mortality or CV hospitalisation	↓ by 8%
CHRISTMAS (86)	387	CHF due to ischaemic LVD	carvedilol	placebo	LVEF	↑ by 3%

In summary, based on their effects on morbidity and mortality, beta-blockers should strongly be considered as initial therapy of all forms of IHD, including its complications, such as heart failure and arrhythmias.

Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers

Angiotensin-converting enzyme (ACE) inhibitors have been in clinical use from the 1970s. They inhibit the enzyme that converts the inactive angiotensin I to active angiotensin II and they also inhibit bradykinin degradation. The main effect resulting from this mechanism is the prevention of cardiac remodelling as shown in different animal models of AMI and heart failure (177, 178).

ACE inhibitors have been widely used for the treatment of hypertension and chronic heart failure (7). Recently their effects have been investigated in patients with chronic IHD (Table 7).

In the HOPE study ramipril reduced the risk of death, myocardial infarction and stroke (179). In the largest clinical trial investigating the effects of ACE inhibitors, the EUROPA trial, perindopril reduced the incidence of cardiovascular (CV) death, AMI and cardiac arrest (180). This effect was, however, not confirmed in the PEACE trial with trandolapril (181). No outcome trials have been performed in patients with UA, however, there is evidence that ACE inhibitors can reduce ischaemic injury also in this setting (182, 183).

Most of the evidence regarding effects of ACE inhibitors in patients with IHD has been obtained in studies in patients with AMI, especially in patients with LVD. There is now unequivocal evidence that ACE inhibitors reduce mortality in this patient population (184-186) (Table 7).

Randomized, placebo-controlled clinical trials with ACE inhibitors in heart failure patients with various degrees of HF severity have shown consistently a positive effect on outcome. The most striking effect was seen in the CONSENSUS I trial with enalapril in NYHA class IV patients, where mortality was reduced by 40% at 6 months (187). In the SOLVD trial, with moderate heart failure patients, the reduction of mortality among patients receiving enalapril was less marked, being 16% (188). A meta-analysis, including enalapril, captopril, ramipril, quinapril and lisinopril trials has confirmed the effect of ACE inhibitors in the reduction of morbidity and mortality in patients with heart failure (189).

Table 7. Major clinical trials with ACE inhibitors

Study (ref.)	N	Patients	Drug	Comparator	Primary results
SAVE (184)	2231	LVD after AMI	captopril	placebo	all-cause mortality↓ by 19%, CV mortality ↓ by 37%
AIRE (185)	2006	LVD after AMI	ramipril	placebo	all-cause mortality ↓ by 27%
TRACE (186)	1749	LVD after AMI	trandolapril	placebo	all-cause mortality↓ by 22%, CV mortality ↓ by 25%
EUROPA (180)	12218	Stable IHD	perindopril	placebo	CV mortality, AMI, or cardiac arrest ↓ by 20%
HOPE (179)	9297	Patients at high risk for CV events	ramipril	placebo	AMI, stroke, or CV mortality ↓ by 22%
PEACE (181)	8290	Stable IHD	trandolapril	placebo	CV mortality, AMI, or coronary revascularization ↓ by 4%
CONSENSUS I (187)	253	NYHA IV	enalapril	placebo	all-cause mortality↓ by 40%
SOLVD (188)	2589	NYHA II-III	enalapril	placebo	all-cause mortality↓ by 16%

About 80% of angiotensin II is generated via the ACE pathway. Since ACE inhibitors do not fully suppress angiotensin II production because there are other pathways through which angiotensin II can be produced, angiotensin II receptor blockers (ARBs) were theoretically thought to have great potential because of their ability to directly block angiotensin II produced through any pathway (190). A large body of literature has accumulated examining the effects of ARBs (Table 8). In placebo-controlled trials in patients with chronic heart failure they were superior to placebo in reducing hospitalisations and worsening heart failure, but not superior to placebo in all-cause mortality (191, 192). Trials comparing ARBs with ACE inhibitors have not revealed additional benefits of ARBs. The ELITE II study, which compared the effects of captopril and losartan in NYHA II-IV patients, demonstrated no statistically significant difference in mortality between the drugs (193). Similar results were seen in the OPTIMAAL and VALIANT studies with patients experiencing LVD after AMI (194, 195). Consequently,

ACE inhibitors have maintained their role as first choice treatment in patients with both chronic heart failure and AMI. ARBs have a role in patients unable to tolerate ACE inhibitors.

Table 8. Major clinical trials with ARBs

Study (ref.)	N	Patients	Drug	Comparator	Primary results
Val-HeFT (191)	5010	NYHA II-IV	valsartan	placebo	mortality/morbidity ↓ by 13%
CHARM (192)	7601	NYHA II-IV	candesartan	placebo	all-cause mortality ↓ by 9%
ELITE II (193)	3152	NYHA II-IV	losartan	captopril	all-cause mortality ↑ by 13%
OPTIMAAL (194)	5477	LVD after AMI	losartan	captopril	all-cause mortality ↑ by 13%
VALIANT (195)	14703	LVD after AMI	valsartan, valsartan + captopril	captopril	no difference in all-cause mortality

Lipid-lowering agents

Elevated LDL-C and triglyceride levels together with reduced HDL-C in IHD patients are well recognised risk factors of IHD with evidence supporting benefits of intensive LDL-C reduction on IHD risk (196). Currently there are five classes of drugs available for the treatment of dyslipidaemia:

a) hydroxylmethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, i.e. statins

b) fibric acid derivatives, i.e. fibrates

c) nicotinic acid

d) bile acid binding agents, i.e. resins, and

e) cholesterol absorption inhibitors

Of the above classes, statins and fibrates are widely used in clinical practice.

Statins are the most potent LDL-C lowering agents and also the most extensively tested of the five classes of drugs in different clinical settings, including primary prevention. Results with statins show that the benefits of cholesterol lowering therapy extend into all forms of atherosclerotic vascular disease (197, 198). Large-scale placebo-controlled trials have been performed with statins in patients with history of stable angina, UA or AMI, with results showing that treatment with statins reduce both cardiovascular and all-cause mortality and incidence of major cardiovascular events, such as AMI and stroke (199-201).

Several recent clinical trials have examined the use of statin medications early in the clinical course of ACS, with results showing that when the statins are used during hospital admissions for ACS, patients experience decreased recurrent AMI, lower death rates, and fewer repeat hospitalizations for ischaemia (202-204). This information suggests that all patients admitted for ACS should be treated with statins as drug of choice for dyslipidemias, regardless of cholesterol levels (Table 9).

Table 9. Clinical trials with statins in patients with IHD

Study (ref.)	N	Patients	Drug	Comparator	Primary results
4S (199)	4444	angina pectoris or AMI	simvastatin	placebo	all-cause mortality↓ by 30%
CARE (200)	4159	AMI	pravastatin	placebo	fatal coronary event or nonfatal AMI ↓ by 24%
LIPID (201)	9014	angina pectoris or AMI	pravastatin	placebo	mortality due to IHD↓ by 22%
RIKS-HIA (202)	19599	AMI	different statins	-	all-cause mortality↓ by 25%
MIRACL (203)	3086	unstable angina or AMI	atorvastatin	placebo	death, AMI, cardiac arrest or recurrent ischaemia ↓ by 16%
A to Z (204)	4497	ACS	early simvastatin	late simvastatin	CV death, AMI, readmission for ACS or stroke ↓ by 11%

Fibrates are the second-best-studied class of lipid-lowering agents; they have reduced clinical events as a monotherapy. Fibrates are used in patients with low HDL-C levels, hypertriglyceridemia and combined hyperlipoproteinemia. By inducing the elevation of HDL-C levels, reduction of triglyceridemia and inflammatory state, fibrates attenuate the atherosclerotic burden (205). Such actions have translated into clinical benefit as demonstrated by the reduction in morbidity and mortality in both primary and secondary intervention trials (206-208).

Recently a new lipid-lowering agent, the cholesterol absorption inhibitor ezetimibe has been introduced (209). It has been shown to be effective in both monotherapy and especially in combination with statins (210, 211). In addition to potentiating the LDL-C lowering effects of statins and diminishing the clinical variability in response to statin therapy, the combination of statins and ezetimibe may produce significant additional reductions in IHD risk.

Antiplatelet- and antithrombotic treatment

Since coronary thrombosis is involved in both the development of atheroma and its lethal complications, pharmacological manipulation of the haemostatic system, with the aim of preventing or reducing the incidence of coronary thrombosis, is therefore of central importance in the treatment of patients with IHD. Several different antiplatelet and antithrombotic drugs are used in the IHD setting (212).

Acetylsalicylic acid

Acetylsalicylic acid (ASA) was introduced in the late 1890s, but only in the 1950s were its antithrombotic effects noted. ASA exerts its effects primarily by interfering with the biosynthesis of cyclic prostanoids (thromboxane A2, prostacyclin, and other prostaglandins). ASA irreversibly acetylates the fatty acid cyclooxygenase (COX) and inhibits the thromboxane A2 pathway, the latter effect mediating its antithrombotic effects. In addition, some of the beneficial actions of ASA in patients with coronary artery disease may be independent of its antithrombotic effects - these include anti-inflammatory and antioxidant properties (213).

The best summary of the numerous trials of ASA in vascular disease have been the meta-analyses published by the Antithrombotic Trialists' Collaboration. One meta-analysis dealt with all types of antiplatelet treatments in a variety of secondary prevention trials in a wide ranging selection of patients at high risk for vascular events, including but not limited to unstable and stable angina, AMI, CABG, PTCA and heart failure. In all 195 trials, involving 135640 patients, were identified that compared antiplatelet treatment to controls. The predominant

antiplatelet agent used in these trials was ASA. Overall, the allocation to antiplatelet treatment reduced the combined outcome of any serious vascular event by a quarter, non-fatal myocardial infarction by a third, non-fatal stroke by a quarter, and vascular death by a sixth, with no adverse effect on other deaths. The effect was similar among all forms of IHD (214).

Heparin and warfarin

Heparin and its derivative, low-molecular-weight heparin (LMWH, such as enoxaparin, fraxiparin, and dalteparin), are the anticoagulants of choice when a rapid anticoagulant effect is required. Heparin binds to antithrombin and this complex inactivates a number of coagulation enzymes, including thrombin factor (IIa), factors Xa, IXa, XIa, and XIIa (215).

Unfractionated heparin (UFH) is not used as the sole antithrombotic drug in patients with ACS, rather it is combined with ASA and with thrombolytic therapy in patients with evolving AMI, and with GPIIb/IIIa antagonists in high-risk patients with UA or in patients undergoing high-risk PTCA (216-220). UFH has been evaluated in a number of randomized, double-blind, placebo-controlled clinical trials for the short-term treatment of patients with UA or NSTEMI (221-224). Meta-analysis of short-term results suggests that the combination of UFH and ASA reduces cardiovascular death and AMI in patients with UA by about 30% over that achieved by ASA alone (222). Another meta-analysis found a risk reduction of 33% in cardiovascular death and AMI with the combination of UFH and ASA compared to placebo (218).

LMWHs have been evaluated in numerous randomized trials in patients with UA or NSTEMI (225-230). A recent overview of the 6 largest trials comparing the effects of enoxaparin and UFH, covering 22000 patients, revealed an approximately 10-% relative reduction in risk of nonfatal AMI or death at 30 days of treatment with enoxaparin (231). In patients with Q-wave AMI, experience with LMWH is limited to studies in which most patients received thrombolytic therapy (232-234). The largest study enrolled 776 patients with AMI in a randomized, double-blind comparison of dalteparin with placebo, showing less thrombotic events in the dalteparin group (232).

Oral anticoagulation with warfarin has been examined in several trials with the rationale that prolonged treatment might extend the benefit of early anticoagulation with either UFH or LMWH. Indeed, in some trials warfarin showed benefit over ASA in reduction of reinfarction and death (235, 236). This effect, however, has not been consistent in all trials (237). Therefore, currently warfarin can be considered only as an alternative to ASA in long-term antithrombotic treatment of patients with IHD.

Clopidogrel

Clopidogrel, a new antiplatelet drug, inhibits adenosine diphosphate (ADP) from binding to one of its three known receptors on platelets, preventing ADP mediated upregulation of the glycoprotein (Gp) IIb/IIIa receptor as part of the amplification phase of platelet activation. Several trials have evaluated the efficacy of clopidogrel in patients with IHD. The CAPRIE trial with 19185 patients showed that clopidogrel was slightly more effective than ASA in reducing ischaemic complications in patients with atherosclerotic vascular disease (238). The CURE trial with 12 562 patients presenting with non-ST elevation ACS showed that combining clopidogrel with ASA leads to a further reduction in cardiovascular death, non-fatal AMI, and stroke (239). The PCI-CURE, a prospective observational study in 2658 patients enrolled in the CURE study who underwent a percutaneous coronary intervention (PCI) in response to refractory symptoms or adverse events, showed that clopidogrel was effective also as a pre-treatment before PCI, reducing the incidence of AMI and ischaemia (240).

Glycoprotein IIb/IIIa inhibitors

Activation of the glycoprotein (Gp) IIb/IIIa receptor on platelets is the final common pathway leading to platelet aggregation, intracoronary thrombus formation, and myocardial ischaemia. Several randomized controlled trials have examined the effect of four different intravenous Gp IIb/IIIa antagonists (abciximab, tirofiban, eptifibatide and lamifiban) in patients presenting with non-ST elevation ACS. There was, however, pronounced heterogeneity in the outcomes of these trials. The CAPTURE, PRISM PLUS and PURSUIT trials with 1265, 1915 and 10948 patients, respectively, showed a significant reduction in their primary endpoints at 30 days (216, 217, 241). In contrast, the PRISM, PARAGON and GUSTO IV trials with 3232, 2282 and 7800 patients, respectively, failed to show a significant difference in their primary endpoints at 30 days (242-244). The treatment effect of these agents appears to be greatest among those patients with elevation of troponin and those undergoing early PCI. The apparent divergence in efficacy among the various Gp IIb/IIIa inhibitors in the cardiac catheterisation laboratory and in ACS may be explained by differences in the level and duration of platelet inhibition achieved by the various regimens used in these different settings.

No reduction in ischaemic events with oral Gp IIb/IIIa antagonists has been demonstrated, despite clinical trial experience with over 40 000 patients and the study of four different oral Gp IIb/IIIa antagonists (orbofiban, sibrafiban, xemilofiban and lotrafiban) (245-248). Of greater concern is the emerging evidence suggesting an increase in mortality with these agents (249). The reason for this finding remains to be clarified, although there are data suggesting that Gp IIb/IIIa inhibitors may have a prothrombotic or proinflammatory effect (250, 251). Furthermore, there may be genetic differences between patients in their response to these agents (252).

Hirudin and bivalirudin

Hirudin and bivalirudin, known as direct thrombin inhibitors (DTIs), interact directly with thrombin and inactivate fluid-phase and bound forms of the enzyme. Since DTIs do not bind to plasma or tissue proteins, their anticoagulant effect is considered more predictable (253). Given the promising pharmacologic characteristics of DTIs, there have been a number of clinical trials that have investigated the role of these agents. In one meta-analysis of 11 randomized trials of DTIs versus UFH, in 35970 patients with STEMI, non ST-elevation acute coronary syndrome (NSTE ACS), or undergoing PTCA, DTIs were associated with a significantly lower risk of death or AMI at 30 days (254).

Multiple clinical trials have compared DTIs with UFH in patients undergoing PTCA. In the HELVETICA trial hirudin was associated with a significant reduction in early death, AMI, and urgent revascularization within 96 hours of PTCA, but a higher incidence of bleeding was seen with hirudin (255). The Bivalirudin Angioplasty Trial randomized 4098 patients undergoing PTCA for NSTE ACS or postinfarction angina to UFH or bivalirudin immediately before angioplasty. Bivalirudin was not significantly different from UFH in reduction of the incidence of in-hospital death, AMI, abrupt vessel closure, or cardiac deterioration necessitating PTCA (256). In the largest of the trials comparing DTIs with UFH, the HERO-2 trial in 17073 patients with STEMI, bivalirudin did not reduce mortality compared with UFH, but did reduce the rate of reinfarction within 96 h by 30% (257).

Fibrinolytic therapy and revascularisation procedures

Pharmacological reperfusion therapy for AMI was incorporated into the armamentarium of physicians in the 1980s and has had an extraordinarily beneficial impact on the outcome of

STEMI patients. The treatment is more efficient when administered between 0 and 6 h (30 deaths prevented per 1000 treated) than between 7 and 12 h (20 deaths prevented per 1000 treated). This underscores that the time window for effective treatment is limited as thrombi will grow and consolidate with time and increase the extent of irrevocable muscle necrosis (258).

Numerous large trials have verified the efficacy of different fibrinolytics in reduction of mortality and morbidity in STEMI patients (Table 10). In the first major study on fibrinolytics, the GISSI-1 study, streptokinase reduced total mortality by 19% compared with standard therapy (259). In the ISIS-2 study additional benefit was obtained by concomitant administration of ASA (260). The GISSI-2 and ISIS-3 studies evaluated the efficacy of alteplase, a recombinant tissue plasminogen activator (rt-PA), a drug capable of degrading fibrin of the thrombus more selectively than streptokinase. No specific differences regarding clinical endpoints were seen in either study in comparison with streptokinase (261, 262). In the GUSTO I trial alteplase combined with UFH provided a 14-% benefit over streptokinase combined with UFH, but an increased frequency of haemorrhagic strokes was seen with alteplase (263). The GUSTO III and ASSENT-2 trials with reteplase and tenecteplase, respectively, did not provide any additional survival benefit over alteplase (264, 265).

Table 10. Major clinical trials with fibrinolytics in STEMI patients

Study (ref.)	N	Drug	Comparator	Primary Result
GISSI-1 (259)	11712	streptokinase	-	in-hospital mortality ↓ by 19%
GISSI-2 (261)	12490	alteplase	streptokinase	no difference between treatments regarding death and severe LV damage
ISIS-2 (260)	17817	streptokinase	ASA, placebo	5-week vascular mortality ↓ by 25% after streptokinase, ↓ by 42% after streptokinase+ASA
ISIS-3 (262)	41299	streptokinase	duteplase	no difference between treatments in 5-week mortality
GUSTO-1 (263)	41021	alteplase	streptokinase	30-day mortality ↓ by 14%
GUSTO-3 (264)	15059	reteplase	alteplase	no difference between treatments in 30-day mortality
ASSENT-2 (265)	16949	tenecteplase	alteplase	no difference between treatments in 30-day mortality

Revascularisation procedures, i.e. PCI (PTCA and stenting) and CABG, are now widely utilized in the treatment of IHD. A trial in stable angina, RITA-2 in 1018 patients, comparing efficacy of PTCA and medical therapy, showed that early intervention with PTCA was associated with greater symptomatic improvement, especially in patients with more severe angina (266). In long-term follow-up it was seen that an initial strategy of PTCA did not influence the risk of death or MI, but improved angina and exercise tolerance (267). In UA, the use of PTCA has been associated with an increased risk of AMI, need for emergency surgery and restenosis, compared with CABG (268). The introduction of intracoronary stents, both bare and drug-eluted stents together with new antiplatelet agents such as clopidogrel and glycoprotein IIb/IIIa inhibitors, have improved both short- and long-term outcomes in this patient population (269-273).

The success of PCI in the treatment of STEMI is highly dependent on timing. PCI is the preferred therapeutic option when it can be performed within 90 min of the first medical contact (so-called primary PCI). For several years primary PCI has been applied as an alternative to

fibrinolytic therapy. Several trials have been performed in order to compare the efficacy of primary PCI with that of fibrinolysis. Results have shown effective restoration of patency, less reocclusion, improved ventricular function and clinical outcome after PCI (274-279). The superiority of PTCA over fibrinolysis has also been seen in meta-analyses (280, 281). However, as time to reperfusion is critical, thrombolysis remains a valuable option for many patients with STEMI. The value of pre-hospital thrombolysis was evaluated in the CAPTIM study with 840 patients, where the outcomes of fibrinolysis were not different from those seen with PTCA (282). Due to the potential thrombotic reaction connected to primary PCI, pretreatment with thrombolytics, Gp IIb/IIIa inhibitors or both has been introduced in order to favourably influence the incidence of reinfarction and death (283-286). Primary PCI improved prognosis also in patients with cardiogenic shock (287, 288).

In summary, revascularisation procedures – CABG or PCI – have proved to be efficacious and shown to improve prognosis in many different patient populations. The decision regarding the specific procedure to be used is based on the number of involved vessels, comorbidities, degree of left ventricular dysfunction and also on how experienced the medical personnel is.

2.4.2 Symptomatic therapy

Nitrates

The clinical effects of nitrates were first described in the 19th century. Organic nitrates are prodrugs and must be biodegraded to have therapeutic effects. This biotransformation involves denitration of the nitrate, with the subsequent liberation of nitric oxide. Nitric oxide stimulates guanylyl cyclase, which reduces cytoplasmic calcium by inhibiting calcium inflow and by stimulating mitochondrial uptake of calcium. This leads to the conversion of guanosine triphosphate to cyclic guanosine monophosphate, which in turn causes vasodilation. The effects are seen in both coronary arteries and veins, resulting in reduction of preload and afterload. Therefore, nitrates are considered useful in the treatment of IHD and also heart failure (289, 290).

The most conventional and often used nitrate formulation is sublingual nitroglycerin, which is the drug of choice for the treatment of angina episodes in both stable and unstable form of angina and for the prevention of angina (289-291). Other preparations, such as oral isosorbide dinitrate, isosorbide 5-mononitrate as well as intravenous nitroglycerin, nitroglycerin ointment and transdermal patches are used in order to prolong the action of the drug and obtain more stable and longer-lasting clinical effects than seen with those after administration of sublingual nitroglycerin.

The major problem with the use of nitrates is the development of tolerance, which has been demonstrated to occur with all forms of nitrates (289, 290). The most widely accepted explanation to this is depletion of sulfhydryl groups, which are the crucial component of metabolic conversion of nitroglycerin to nitric oxide. The only practical strategy in tolerance management is to provide a nitrate-free interval. Also rebound effect after withdrawal of therapy has been observed, which requires that the withdrawal of nitrate therapy be done carefully (292).

Despite nitrates having been in clinical use for a long time, there is no long-term clinical trial evidence about their effects in patients with angina. Nitrates did not influence morbidity or mortality in patients with AMI in two large-scale clinical trials, GISSI-3 and ISIS-4 (293, 294). In patients with heart failure, in contrast, the combination of isosorbide dinitrate and the vasodilator-drug hydralazine did improve haemodynamics and decrease mortality (295, 296). In

patients with acute heart failure intravenous nitroglycerin and nitroprusside are widely accepted as first-line therapy, although prospective studies regarding their effect on prognosis have not been performed (297, 298). Thus, the use of nitrates in the treatment of IHD is largely based on pathophysiological considerations and clinical experience rather than evidence from randomized clinical trials.

Calcium antagonists

Calcium antagonists, introduced in clinical medicine in the 1960s, are a heterogenous group of compounds inhibiting calcium ion movement through channels in cardiac and smooth muscle membranes by blockade of voltage-sensitive L-type calcium channels. The efficacy of calcium antagonists in treatment of IHD is related to their ability to reduce myocardial oxygen demand and increase oxygen supply. They cause relaxation of vascular smooth muscle in both systemic arterial and coronary arterial beds. The blockade of the entry of calcium into myocytes results also in a negative inotropic effect. There are three major classes of calcium antagonists currently in clinical use: dihydropyridine-type (such as nifedipine, felodipine, amlodipine, lacidipine), phenylalkylamines (such as verapamil, gallopamil) and benzothiazepines (such as diltiazem) (299, 300).

Dihydropyridine-type calcium antagonists

Dihydropyridine-type calcium antagonists are particularly effective in dilation of vascular smooth muscle. Negative inotropic, chronotropic, and dromotropic effects are rather small. In rare instances they can lower blood pressure excessively with subsequent reflex tachycardia. Second-generation dihydropyridine-type calcium antagonists, such as amlodipine, felodipine and lacidipine are more vascular selective than calcium-antagonists of the first generation (nifedipine) (299, 300). Dihydropyridine-type calcium antagonists and their combination with beta-blockers have been shown to be efficacious in the treatment of stable angina pectoris and also in Prinzmetal (variant) angina (301-305).

The short-acting formulation of nifedipine has been studied mostly in patients with AMI and UA. Several early trials have shown that nifedipine increases mortality in this patient population (162, 306-308). A meta-analysis of 16 trials of short-acting nifedipine in patients both during and after myocardial infarction or with UA confirmed the existence of such an effect (309). The question remains whether this finding is relevant only to short-acting nifedipine, since there are very limited data on either long-acting nifedipine or the other dihydropyridines (such as felodipine or amlodipine) in this indication.

Second-generation dihydropyridine-type calcium antagonists have been evaluated in several clinical trials in patients with heart failure, however, without substantial efficacy. In the V-HeFT III trial, where felodipine was tested as a supplementary drug to enalapril, felodipine did not improve exercise tolerance, quality of life, or reduce hospitalizations or mortality (310). The finding was similar in the PRAISE trial with amlodipine (311). However, no harm has been observed with long-term treatment, which indicates that these drugs may be safely used in patients experiencing angina together with left ventricular dysfunction (312).

Recently some new evidence about the antioxidant properties of calcium antagonists has been presented indicating that these drugs can reduce the rate of progression of atherosclerosis (313-315). In the REGRESS study, co-administration of amlodipine or nifedipine with pravastatin caused a significant reduction in the appearance of new angiographic lesions (316). Recent results of the ELSA study show that lacidipine reduced the progression rate of intima-media thickening as compared to atenolol (317).

Phenylalkylamine-type calcium antagonists

Verapamil, the most often used phenylalkylamine-type calcium antagonist, dilates peripheral and coronary vessels, slows heart rate and has a marked negative inotropic effect. This results in a reduction in myocardial oxygen requirement, which makes verapamil a suitable treatment for different forms of IHD. Verapamil has been shown to be useful in the treatment of chronic stable angina, its efficacy being similar to that seen with beta-blockers (318-320). The INVEST study showed that a verapamil-trandolapril-based strategy was clinically as effective as an atenolol-hydrochlorothiazide-based strategy in hypertensive IHD patients (321). In the DAVIT II trial verapamil reduced the incidence of recurrent myocardial infarction and death (322) and a similar result has been seen in one meta-analysis (323). Verapamil is also useful in the treatment of variant angina (324).

Benzothiazepine-type calcium antagonists

Diltiazem, the most commonly used phenylalkylamine-type calcium antagonist, possesses vasodilator effects that are intermediate between those of dihydropyridine-type calcium antagonists and verapamil. Its vasodilator effects are less profound than those of dihydropyridines and its negative ino-and dromotropic effects smaller than those of verapamil. Diltiazem has been shown to be effective in the treatment of stable angina and its efficacy was better compared with other calcium antagonists (302, 325, 326). In patients with UA diltiazem has been more effective than nitrates in both short and long term and similar in efficacy to beta-blockers (327-330). Diltiazem has been found useful in the treatment of variant angina (324). In patients with AMI, diltiazem has improved left ventricular systolic function (331) and reduced the need of myocardial revascularization after thrombolysis (332). However, in the large-scale MDPIT study diltiazem had no effect on long-term prognosis (333).

In summary, calcium antagonists are effective in the treatment of stable angina and can be used in patients with UA or AMI in case of recurrent ischaemia despite beta-blockade (or in whom beta-blockers are contraindicated). Calcium antagonists are not recommended in patients with heart failure.

Other vasodilators

Several vasodilating drugs have been evaluated in treatment of IHD. One of the most extensively studied vasodilators is nicorandil, a drug with both nitrate-like and K^+_{ATP} channel opening properties (334). By virtue of this dual mechanism of action, the drug acts as a balanced coronary and peripheral vasodilator and reduces both preload and afterload (334). Nicorandil has been shown to induce ischaemic preconditioning in several animal and clinical studies (103, 335, 336). Studies in animal models of ischaemia-reperfusion-induced myocardial stunning or infarction indicate that nicorandil has cardioprotective effects (337-341). Various *in vitro* and animal studies have shown that the cardioprotective mechanism of nicorandil includes inhibition of oxidative stress-induced apoptosis of cardiac myocytes during reperfusion (342-344) and preservation of mitochondrial respiration and ATP production during reoxygenation (345, 346).

The efficacy of nicorandil has been evaluated in several clinical trials. The largest of these, the IONA study, was performed in 5126 patients with stable angina and showed reduction in major coronary events among patients receiving nicorandil (347). In the placebo-controlled CESAR 2 trial in patients with UA nicorandil reduced the incidence of myocardial ischaemia and had some antiarrhythmic effect (348). Nicorandil has also improved exercise capacity in patients with stable angina and the effect was comparable with those of nitrates, beta-blockers and

calcium antagonists (349-354). Studies in patients undergoing PTCA have shown that the administration of nicorandil enhances myocardial tolerance to ischaemia both in patients with angina and in patients with AMI (335, 355, 356). In patients with heart failure the effects of nicorandil were similar to those of nitroglycerin, however, less tachyphylaxis was seen in patients receiving nicorandil (357).

Molsidomine, a sydnonimine, resembles nitroglycerin in its mode of action. It has been studied mostly in patients with stable angina, in whom it was rather effective (358, 359). In patients with AMI, however, it has no favorable effects on either short- or long-term prognosis (360). Molsidomine has been studied also in patients with heart failure, but the data is very limited (361).

2.5 Positive inotropic drugs in the treatment of ischaemic heart failure

Positive inotropic drugs represent one of the oldest classes of drugs, used for treatment of both chronic and acute heart failure. During past decades new inotropic drugs have been synthesized and relatively thoroughly examined in different clinical settings.

Digitalis

Digitalis, the extract of the common foxglove plant *Digitalis purpurea*, has been used for treatment of edematous states, irregular heartbeats, and CHF for centuries. Digoxin's primary mechanism of action is the ability to inhibit membrane-bound alpha subunits of sodium-potassium ATPase (sodium pump), mainly located in the human myocardium. This inhibition promotes sodium-calcium exchange, which increases the concentration of intracellular calcium that is available to contractile proteins, resulting in an increase in the force of myocardial contraction (362-364). The beneficial effects of digoxin on HF may be related in part to its modulating effects on neurohormonal abnormalities, such as attenuation of carotid sinus baroreceptor discharge sensitivity, vagomimetic and sympathoinhibitory effects (365-368).

Several small and medium-size clinical trials have been performed with digoxin in heart failure. In the PROVED and RADIANCE studies, digoxin withdrawal in patients with systolic dysfunction was associated with a decrease in LVEF, renal function, exercise tolerance and quality of life and an increase in the incidence of worsening heart failure (369, 370). The largest trial with digoxin, the DIG trial, assessed all-cause mortality in 6800 patients with NYHA I-IV HF and decreased systolic function, with the patients receiving diuretics and ACE inhibitors. In this trial, digoxin had neutral effects on mortality, but hospitalizations related to worsening HF were significantly reduced. The effect was more pronounced in severe heart failure patients (371).

Phosphodiesterase inhibitors

Phosphodiesterase (PDE) III inhibiting drugs (such as amrinone, milrinone and enoximone) increase contractility by reducing the degradation of cyclic adenosine monophosphate (cAMP). In addition, they reduce both preload and afterload via vasodilation. The haemodynamic consequences of this action are reduced left ventricular afterload, increased cardiac output (CO) and reduced total peripheral resistance. Unlike sympathomimetic amines, PDE III inhibitors produce no tolerance and possess the distinct advantage of directly decreasing pulmonary vascular resistance (372-375).

Milrinone is the most widely used PDE III inhibitor. Two large prospective randomized studies have been conducted with milrinone, one with an oral and the other with an intravenous

formulation. The PROMISE study evaluated the efficacy of oral milrinone in 1088 patients with NYHA class III or IV congestive heart failure. As compared with placebo, milrinone was associated with a 28-% increase of all cause mortality (376). In the OPTIME-CHF trial 951 patients with decompensated heart failure were randomized to either intravenous milrinone or placebo. The study failed to show any benefits of milrinone over placebo in morbidity and mortality and milrinone was associated with a higher incidence of adverse events (377).

While amrinone has been largely abandoned as treatment of heart failure, mainly due to its ability to cause thrombocytopenia, low-dose oral enoximone has recently been evaluated in several small clinical trials, especially its effects during co-administration with beta-blockers (378-380).

Another drug, vesnarinone, a mixed PDE inhibitor and ion-channel modifier that has modest, dose-dependent, positive inotropic activity, but minimal negative chronotropic activity, has improved haemodynamics and quality of life in small trials (381-383). However, in the largest outcome study, the VEST trial with 3833 patients with severe heart failure, a dose-dependent increase in mortality was identified (384).

Dobutamine and dopamine

Dobutamine, a beta-adrenoreceptor agonist improves haemodynamics in patients with heart failure after cardiac surgery and AMI, both as long-term infusion and also in the outpatient setting (11-13, 93, 385-390). In several small trials the administration of dobutamine was associated with an increased risk of mortality, which may be due to dobutamine's chronotropic, proischaemic and proarrhythmic effects (391-393). Also, in the yet unpublished CASINO trial an adverse effect of dobutamine on survival was seen; however, the sample size of the trial – 299 patients – was again too small to provide conclusive evidence (394).

Dopamine is an endogenous catecholamine that is precursor to norepinephrine in the catecholamine synthetic pathway. At lower doses dopamine causes vasodilation of splanchic and renal arterial beds, whereas at higher doses peripheral vasoconstriction occurs as a result of alpha-adrenergic receptor stimulation (375, 395). Therefore, dopamine has been used in low doses to support renal perfusion (396-398). Nevertheless, sufficient evidence is still lacking on reduction in endpoints such as mortality or renal replacement therapy. In addition, several studies have failed to find expected specific renal effects of dopamine (399, 400). In conclusion, well-controlled clinical trials remain mandatory to assess the overall clinical effects of dopamine in treatment of both renal and heart failure.

Calcium sensitizers

These positive inotropic agents act by increasing the sensitivity of troponin C or some other part of the myofibrillar Ca^{2+} – binding apparatus to ionized calcium. There are several drugs with reported calcium sensitizing properties (sulmazole, isomazole, adibendan, meribendan, EMD 53998, DPI 201-106, Org 30029, APP 201-533, and MCI-154), but most of the clinical data come from two compounds: pimobendan and levosimendan.

Pimobendan is a calcium sensitizer with PDE III inhibitor properties, currently available for treatment of heart failure only in Japan. Both intravenous and oral formulations have been studied in patients with heart failure. Intravenous bolus doses had clear inotropic, vasodilatory and chronotropic effects in patients with heart failure, increasing CO and reducing systemic vascular resistance and left ventricular end-diastolic pressure (401, 402). In comparison with captopril, pimobendan appeared to be a stronger arterio-venodilator (403).

The effects of oral pimobendan were similar to those seen after intravenous administration (404-407). Several trials have evaluated long-term efficacy of pimobendan also during chronic oral administration. In those trials pimobendan increased exercise duration, peak oxygen uptake and improved quality of life (405, 408-411). The efficacy and safety were similar to those seen with enalapril, although a somewhat higher incidence of arrhythmias was seen in Holter-recordings (412, 413).

However, in the PICO trial with 317 patients with stable symptomatic heart failure and LVEF less than 45%, although pimobendan improved exercise duration, a trend towards increased mortality was seen in the pimobendan group and this effect was more pronounced among patients receiving concomitant digoxin (414). In contrast, the most recent placebo-controlled, double-blind study with pimobendan, the EPOCH study in 306 patients found that pimobendan significantly lowered morbidity and improved the physical activity of patients with stable NYHA II-III heart failure without increase in adverse cardiac events (415). Therefore, the role of pimobendan in the treatment of heart failure remains to be clarified and adequately powered prospective long-term mortality/morbidity trials are warranted in that respect.

2.6 Levosimendan

2.6.1 Chemistry

Levosimendan *[(R)-[[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl] hydrazono]propanedinitrile]* is the active enantiomer of simendan, a pyridazinone-dinitrile derivative. The structural formula of levosimendan is presented in Figure 1. Levosimendan is a moderately lipophilic drug with a molecular weight of 280.3 daltons. It is a weak acid with pK_a 6.2. Levosimendan is being developed in intravenous (Simdax®2.5 mg/ml infusion concentrate) and oral (0.5, 1, 2 and 4 mg hard gelatine capsules) formulations.

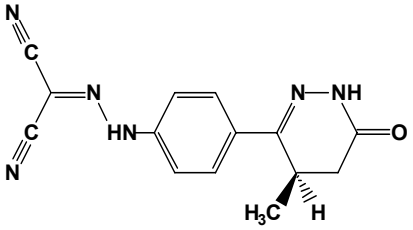


Figure 1. Structural formula of levosimendan.

2.6.2 Mechanism of action

Levosimendan exerts its effects mainly through two different mechanisms: positive inotropic effect, obtained by calcium sensitisation of the contractile proteins in cardiac muscle, and vasodilation, caused by opening the ATP-sensitive potassium channels in both vascular and cardiac tissue.

Positive inotropic effect

Levosimendan induces inotropy through the calcium-dependent binding of the drug to the calcium-saturated N-terminal domain of cardiac troponin C, which stabilises and prolongs the life span of the molecule (Figure 2) (14, 15, 416). Since levosimendan dissociates from cardiac troponin C at low calcium concentration (15), it does not impair relaxation (16, 417). Levosimendan has been shown to increase calcium sensitivity of contractile proteins in intact cells without altering the calcium content of sarcoplasmatic reticulum (17). The positive inotropic effect of levosimendan has been demonstrated by experiments in isolated cardiomyocytes (17), papillary muscles (16, 418), ventricular muscle strips (419) and in isolated hearts (418, 420, 421).

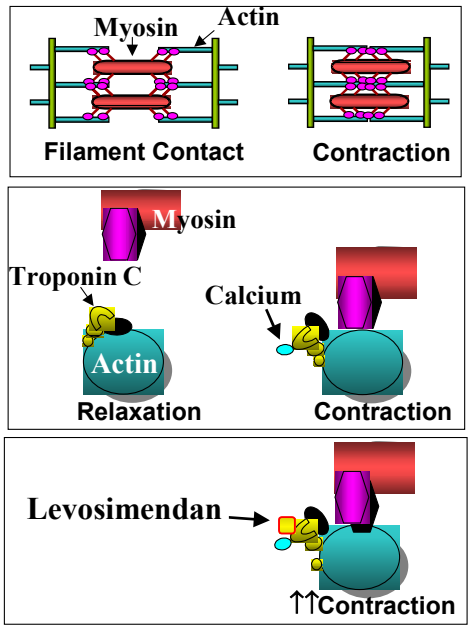


Figure 2. Calcium sensitisation of the contractile proteins in cardiac muscle by levosimendan.

Vasodilation

The second mechanism – vasodilation – is due to the opening of ATP-sensitive K-channels (K^+_{ATP} channels). This effect has been electrophysiologically demonstrated in arterial and venous preparations and in coronary arteries (19, 422). The vasodilator effects on human coronary conductance and resistance arteries have been demonstrated also in patients (423). It has also been shown that the venodilatory effect of levosimendan on the noradrenaline-constricted human saphenous vein is mediated by the opening of K^+_{ATP} channels (424). In addition, some pharmacological findings indicate that the compound may open the voltage-dependent potassium channels in coronary arteries (425).

Anti-ischaemic effect

In a dog infarction model, levosimendan – given before ischaemia - reduced infarct size by 50%. This reduction of infarct size was abolished by the K^+_{ATP} channel blocker glibenclamide. This proves that levosimendan has an anti-ischaemic effect, which is mediated *via* opening of K^+_{ATP} channels (20). Levosimendan opens the K^+_{ATP} channels also in rat ventricular myocytes (18). This effect, however, does not produce negative inotropy as it does in the case of all other potassium channel openers. In conclusion, the anti-ischaemic effect of levosimendan is mediated *via* opening of K^+_{ATP} channels. Levosimendan opened the K^+_{ATP} channels also in rat liver mitochondria, in a manner dependent on the potassium concentration (426). The effect was abolished by 5-hydroxydecanoate, a selective blocker of mitochondrial K^+_{ATP} channels. The effect of oral administration of racemic simendan on the survival of rats with healed AMI after coronary ligation has been studied. The material was divided into control and 3 treatment groups receiving simendan, milrinone or enalapril. After a follow-up period of 312 days the

infarct size-corrected mortality in control, simendan, enalapril and milrinone groups was 76%, 53%, 62% and 70%, respectively (427).

PDE inhibition

Intracellular calcium levels are not affected by levosimendan at therapeutically relevant concentrations, but several studies have shown that levosimendan also inhibits highly selectively phosphodiesterase type III activity and causes accumulation of cyclic adenosine monophosphate with resultant increased influx of calcium into the myocyte (419, 428-430). However, this effect was associated only with higher levosimendan concentrations and therefore probably does not contribute significantly to the contractility-enhancing and vasodilatory effects of levosimendan at therapeutic concentrations (21, 418).

2.6.3 Pharmacokinetics and metabolism

The terminal elimination half-life ($t_{1/2el}$) of levosimendan is about 1 hour (431-433). Levosimendan is highly bound to plasma proteins (97-98%) (431, 434). During intravenous administration the concentrations of levosimendan increase dose proportionally (435). The bioavailability of levosimendan from an oral solution is approximately 80% and it is rapidly absorbed reaching peak concentrations within 1 to 2 hours after an oral capsule (431).

Levosimendan is metabolised through glutathione conjugation at one of its nitrile groups and through subsequent amino acid cleavage and cyclication or acetylation. The main pathway is conjugation with glutathione to inactive metabolites and the minor pathway (only approximately 5% of the total levosimendan dose) is reduction in the intestine to the aminophenylpyridazinone metabolite OR-1855. The amine metabolite is further metabolized by acetylation to the N-acetylated conjugate OR-1896 (436). The enzyme responsible for the acetylation of OR-1855 to OR-1896 is suspected to be N-acetyltransferase and as this enzyme is polymorphically distributed in the population, the acetylation capacity of the individual determines the plasma levels of OR-1896 during treatment with levosimendan (437). Also an inactive hydration product, OR-1420, is formed through addition of water to one of the nitrile groups. Levosimendan is excreted via urine and faeces as conjugates and only traces of unchanged levosimendan are found in urine or faeces in experimental animals and in man (431, 438). Levosimendan metabolism is described in Figure 3.

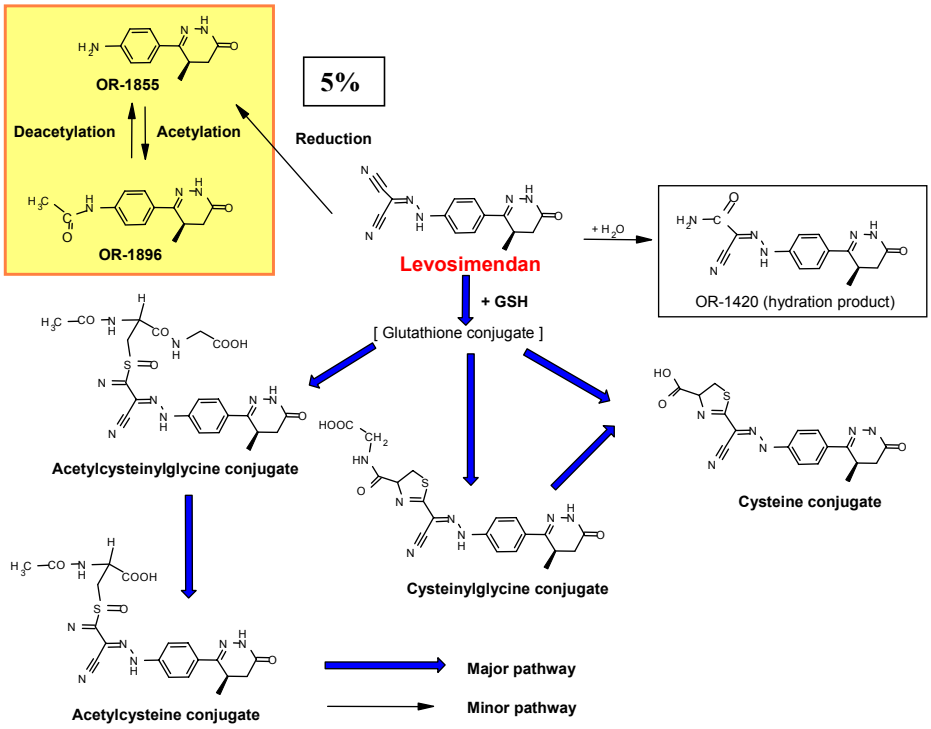


Figure 3. The metabolic pathways of levosimendan

The metabolite OR-1896 has been shown to have haemodynamic properties similar to those of the parent drug (439-441). In different experimental animals, the elimination half-life of levosimendan was 0.25 to 1.3 hours, whereas that of the metabolite OR-1896 was 4.7 to 6.5 hours (436). In humans, the mean elimination half-life values for the levosimendan metabolites OR-1855 and OR-1896 are approximately 80 hours (442, 443). The mean plasma protein binding values are 39% for OR-1855 and 42% for OR-1896 (444).

Several interaction studies have been performed with levosimendan. No pharmacodynamic or pharmacokinetic interactions have been seen with captopril, itraconazole, warfarin, isosorbide 5-mononitrate or carvedilol (434, 445-448).

2.6.4 Clinical experience with levosimendan

Studies in healthy volunteers

In studies with healthy volunteers, levosimendan has been shown to increase CO and ejection fraction dose-dependently (435). The increase in CO at low doses was due exclusively to an increase in stroke volume. No increases in heart rate have been seen with doses of up to 1 mg of intravenous levosimendan (433, 435, 449).

The enhancement in cardiac performance was attributed both to an increase in contractility, assessed by systolic time intervals (433, 447, 449) and echocardiography (435), and to a reduction in afterload, reflected by a dose-dependent decrease in systemic vascular resistance (433, 435, 449). Levosimendan increased leg blood flow with only minor increases in skin

blood flow, indicating that the increased flow was directed predominantly to the skeletal muscles (447). The effects of levosimendan on cardiac performance were maintained during light dynamic exercise (449). Systolic BP generally increased or was unchanged, and dBP has consistently been shown to decrease (433, 435, 449). The haemodynamic efficacy of levosimendan was not associated with a significant increase in myocardial oxygen consumption. In a study, utilising positron emission tomography with 11^{C}-acetate, levosimendan, in comparison with placebo, increased myocardial oxygen consumption non-significantly (+12%), whereas dobutamine increased oxygen consumption markedly (+58%) (22).

Studies in patients

Designs of main studies

Intravenous levosimendan has been studied by now in more than 3000 patients and several medium and large-scale studies have been carried out. Mostly these studies have been carried out in patients with chronic heart failure. The presence of patients with acute or chronic IHD varies between the studies. The main levosimendan studies and doses used in them are summarised in Table 11.

The therapeutic dose range of levosimendan administered over a 24-hour period was studied in a placebo-controlled, double-blind, parallel-group, randomized study including 151 patients with stable (mainly NYHA class III) heart failure of ischaemic origin, but patients with recent AMI (within 3 months before enrollment) were excluded (450).

Forced up-titration, maintenance and withdrawal of levosimendan was studied in a placebo-controlled, double-blind, parallel-group, randomized study in 146 patients hospitalized for decompensated heart failure (NYHA class III or IV) due to IHD or dilated cardiomyopathy. Patients with significant IHD (angina-limited exercise, unstable angina or AMI within 8 weeks) were excluded. The study was divided into three phases. During the first 6-hours, escalated doses of levosimendan (n=98) were compared to placebo (n=48). From 6 to 24 hours, the patients in the levosimendan group continued to receive the drug as an open-label infusion. At 24 hours, the remaining patients were randomized to continue on levosimendan (levosimendan continuation group) or placebo (levosimendan withdrawal group) in a double-blind fashion up to 48 hours (451, 452).

The haemodynamic efficacy (increase in cardiac index and decrease of PCWP) of 24-hour levosimendan infusion was compared to dobutamine in a double-blind, parallel-group, randomized trial in 203 patients with low-output heart failure in need of intravenous inotropic support (the LIDO study). Patients had either an ischaemic or non-ischaemic etiology of heart failure, however, patients with acute ischaemia were excluded also from this study (453).

The efficacy of 24-hour levosimendan infusion was compared to dobutamine and placebo in a double-blind, parallel-group, randomized trial in 299 patients with severe (NYHA IV) heart failure (the CASINO study). Patients with both ischaemic or non-ischaemic etiology of heart failure were studied (394).

The on-going REVIVE study evaluates the efficacy of 24-hour levosimendan infusion on symptoms of heart failure with a new composite endpoint, consisting of patient's subjective symptom assessment and signs of worsening symptoms (including death) during five days after starting a 24-hour study drug infusion. The study is a placebo-controlled, double-blind, parallel-group, randomized trial in patients with acutely decompensated heart failure. Patients with

either ischaemic or non-ischaemic etiology of heart failure are studied, however, patients with acute ischaemia (within 6 hours) are excluded. The pilot phase of this study (REVIVE I) has been published recently with preliminary data on 100 subjects (454).

Another on-going, double-blind, parallel-group, randomized trial, the SURVIVE study, evaluates the effect of 24-hour levosimendan infusion on survival in comparison with dobutamine in patients with decompensated heart failure and in need of intravenous inotropic support. Patients with ischaemic (including AMI) or non-ischaemic etiology of heart failure are studied. In addition, the study includes the option of re-administration of study drugs, a feature that has not been implemented in any previous trials with intravenous levosimendan (455).

Table 11. Pivotal studies with intravenous levosimendan in heart failure

Study (ref.)	N (total/ LS)	Dose and duration of LS-infusion*	Comparator	Diagnosis/ NYHA class	Primary endpoint
Dose-finding (450)	151/95	0.05-0.6 µg/kg/min for 24 h	placebo/dobutamine 6 µg/kg/min	CHF/III	haemodynamics
Dose-escalation & withdrawal (451, 452)	146/98	0.1-0.4 µg/kg/min for 24 or 48 h	placebo for 6 h	CHF/ III-IV	haemodynamics
LIDO (453)	203/103	0.1-0.2 µg/kg/min for 24 h	dobutamine 5-10 µg/kg/min	CHF/(III)-IV	haemodynamics
REVIVE** (454)	700/350	0.1-0.2 µg/kg/min for 24 h	placebo	CHF/IV	composite symptom/outcome
CASINO (394)	299/100	0.1-0.2 µg/kg/min for 24 h	placebo/dobutamine 5-10 µg/kg/min	CHF/IV	Morbidity/ mortality
SURVIVE*** (455)	event-driven	0.1-0.2 µg/kg/min for 24 h	dobutamine 5 µg/kg/min	CHF/IV or de novo HF	Mortality

N= number of patients
LS = number of levosimendan patients
**) a 10-min loading dose of 3-36 µg/kg preceded the maintenance infusions*
***) consisting of two separate studies: REVIVE I as a pilot phase and REVIVE II - a large phase III study*
****) dobutamine dose at least 5 µg/kg/min and duration at least 24 h, otherwise unlimited.*

Results

Haemodynamics

Levosimendan exerted significant, dose-dependent increases in CO, stroke volume and heart rate, and decreases in PCWP, mean blood pressure, mean pulmonary artery pressure, mean right atrial pressure and total peripheral resistance (Figure 4) (450). The effect of levosimendan on haemodynamic variables (CO, stroke volume, heart rate, and PCWP) was clearly evident already at the end of a 5-minute bolus infusion (456). There is no sign of development of tolerance with a prolonged infusion up to 48 hours (Figure 5) (451, 452). Compared to dobutamine, levosimendan produces a similar increase in CO, but profoundly greater decrease in PCWP (453). In contrast to the haemodynamic effects of dobutamine, those of levosimendan are not attenuated by concomitant beta-blocker use (453). Due to the formation of the active metabolite OR-1896, the haemodynamic effects are maintained several days after stopping levosimendan infusion (Figures 3 and 5) (442, 452, 457).

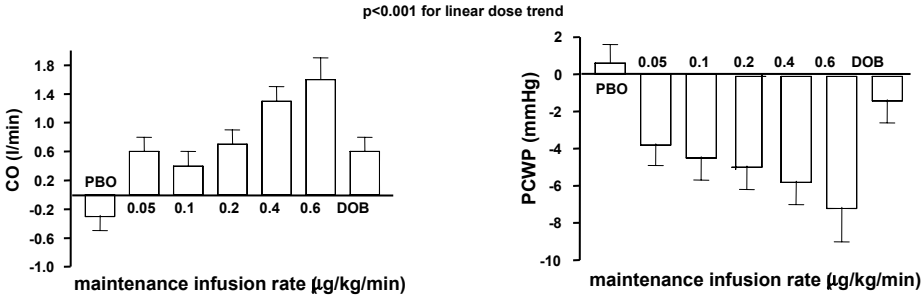


Figure 4. Change in CO and PCWP at 24 hours compared with baseline after a 24-hour infusion of 5 doses of levosimendan, placebo (PBO), or dobutamine (DOB, dose 6 µg/kg/min) in 151 patients with stable heart failure. Mean (± SEM) values are shown. P<0.001 for linear dose trend (450).

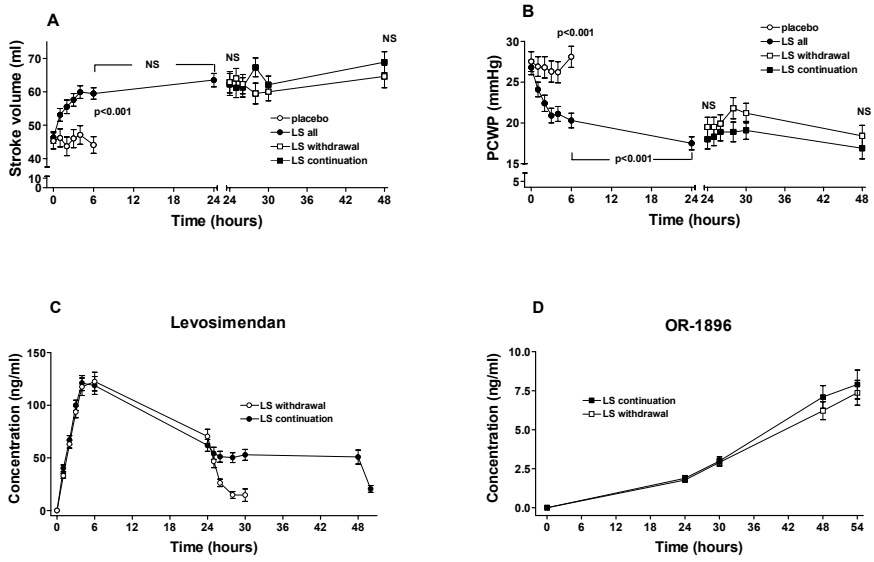


Figure 5. Mean (± SEM) changes in stroke volume (A), PCWP (B) and plasma concentration-time curves of levosimendan (C) and the metabolite OR-1896 (D) (452).

The beneficial effects of levosimendan on haemodynamics are not associated with an increase in myocardial energy consumption. This was evidenced by utilizing dynamic PET technique in 8 hospitalized patients with heart failure (NYHA III-IV) (23). The patients were given levosimendan 18 µg/kg as a loading dose followed by a continuous infusion of 0.3 µg/kg/min for about 5 hours, and placebo in a cross-over fashion. Despite increases in both CO and stroke volume, myocardial oxygen consumption was unaltered by levosimendan.

Symptoms

The effects of levosimendan on dyspnoea and fatigue have been evaluated in several trials. In the dose-escalation/withdrawal study dyspnoea improved in statistically significantly more patients treated with levosimendan (29%) than in patients receiving placebo (15%) at 6 hours after starting the treatment (p=0.037). There was a trend toward improvement also in fatigue with more patients in the levosimendan group reporting improvement (42% versus 22%, p=0.057) (451).

In the LIDO study, symptoms improved equally well in levosimendan- and dobutamine-treated patients at 24 hours. Dyspnoea improved in 68% and 59% (p=0.865) of the patients in the levosimendan and dobutamine groups, respectively. Fatigue improved in 63% and 47% (p=0.155) of patients with levosimendan and dobutamine, respectively (453).

In a pilot phase of the REVIVE study (REVIVE I), improvement was observed more frequently in levosimendan-treated patients than in patients on placebo (49% vs 33%), although the difference was not significant (p=0.229), most probably due to the small number of patients included, 100. The greater level of improvement in the clinical composite on levosimendan was supported by significant reductions in plasma BNP concentrations compared to placebo at both 24 hours and 5 days (p<0.05) (454).

Mortality and morbidity

Mortality was pre-specified as a secondary endpoint in several studies. In the LIDO study, mortality was followed prospectively for 31 days following randomisation. At 31 days, 7.8% of patients assigned to levosimendan and 17.0% assigned to dobutamine had died (p=0.049). The follow-up was retrospectively extended to 180 days and the mortality rates were 26.2% with levosimendan and 38.0% with dobutamine (p=0.029) (Figure 6).

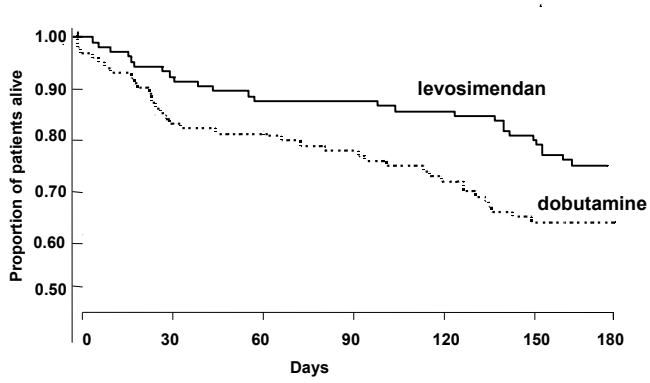


Figure 6. Kaplan-Meier curves showing all-cause mortality during 180 days after 24-hour infusion of levosimendan or dobutamine in 203 patients hospitalized with low-output heart failure (the LIDO study). Hazard ratio 0.57; 95% CI 0.34 - 0.95, p=0.029 (453).

In REVIVE I, mortality was prospectively followed for 31 and 90 days. The overall mortality rate was low, at 31 and 90 days mortality in the levosimendan group was 2% and 8%, respectively, compared with 8% and 10%, respectively, in the placebo group (454). In the CASINO study the mortality rate at 180 days in the levosimendan group (18.0%) was significantly lower than in both placebo (28.3%) (p=0.03) and dobutamine (42.0%) (p=0.0001)

groups (394). It is, however, important to consider that none of these trials were powered for mortality and therefore this positive evidence should be viewed as preliminary.

In the LIDO study, a combined mortality/morbidity analysis was undertaken for a 180-day period following randomization, utilizing the endpoint "number of days alive and out of hospital". It was revealed that patients in the levosimendan group spent significantly more days alive and out of hospital than dobutamine-treated patients (median 157 vs 133 days for levosimendan and dobutamine, respectively; p=0.027) (453).

Anti-stunning effects

The anti-stunning effects of levosimendan were evaluated in a randomized, double-blind study in patients with AMI who had undergone PTCA. The patients received levosimendan 24 μg/kg as a bolus dose (n=16) or corresponding placebo (n=8) 10 minutes after completion of the successful PTCA. The study showed that levosimendan improved the function of stunned myocardium, as shown by a reduction in the number of hypokinetic segments in the left ventricular wall compared with placebo (p=0.016) (458).

Further support for an anti-stunning effect of levosimendan comes from an open-label study in 20 patients with AMI and LVD. Various loading doses of levosimendan significantly improved contractility of the left ventricle both globally and in the infarcted region with wall motion abnormalities (459).

Effects on diastolic function

The effects of levosimendan on diastolic function were assessed using intracoronary infusions. Ten patients with heart failure received two doses without systemic effects (3.75 and 12.5 μg/min) and dextrose (control) as bolus doses. In this study, τ, the time constant of left ventricular isovolumic relaxation, was unaffected by the lower dose of levosimendan. The higher dose of levosimendan produced a decrease in τ, indicating a mild positive lusitropic effect. Levosimendan had no significant effects on left ventricular (–)dP/dt (rate of fall of intraventricular pressure), another measure of diastolic function (460).

In another randomized, placebo-controlled study, it was found that a 24-hour levosimendan infusion improves echocardiographic markers of abnormal left ventricular diastolic function (transmitral flow patterns and mitral annulus velocities, as assessed by transthoracic pulse-wave Doppler and tissue Doppler imaging, respectively) in patients with advanced heart failure (461). In a study in patients with AMI who had undergone PTCA the diastolic function was not worsened by levosimendan compared with placebo, while end-diastolic pressure-volume ratio and chamber compliance during late diastole changed similarly with levosimendan and placebo. In addition, τ was improved in the levosimendan group and impaired in the placebo group, which suggests improved diastolic function by levosimendan (458).

Use in cardiac surgery

The effects of levosimendan in patients undergoing cardiac surgery have been investigated so far in three studies. In one placebo-controlled study levosimendan was administered either as an 18 μg/kg loading dose followed by an infusion of 0.2 μg/kg/min for 6 hours, or as 36 μg/kg followed by 0.3 μg/kg/min after cardiopulmonary by-pass in patients with normal preoperative cardiac function. Both levosimendan doses increased CO and stroke volume significantly and reduced peripheral vascular resistance. Levosimendan did not affect arterial oxygenation or cause arrhythmogenic effects and was well tolerated. The lower dose of levosimendan was as

effective as the higher dose, but it was associated with less hypotension and tachycardia, and was therefore considered to be a potential dose for use in this patient population.

A placebo-controlled study with 2 bolus doses of levosimendan (8 and 24 µg/kg) in patients after cardiopulmonary by-pass showed that levosimendan transiently increased CO, reduced systemic and pulmonary vascular resistances, and did not adversely affect myocardial metabolism (462). Similarly, the bolus doses of 8 or 24 µg/kg did not increase myocardial oxygen consumption in these post-operative patients, although cardiac function improved markedly (462).

An open-label study using only a bolus dose of 24 µg/kg in patients after cardiopulmonary by-pass again showed that levosimendan transiently increased CO and stroke volume, and reduced systemic vascular resistance (463). Levosimendan did not impair isovolumic relaxation. An increase in coronary graft flow was observed.

Safety

Adverse events

Headache and hypotension have been the most frequently reported adverse events in levosimendan-treated patients in placebo-controlled studies. Their incidence has been 2 to 9% for headache and approximately 5% for hypotension (450, 451).

In the LIDO study, disturbances of heart rate and rhythm were reported with lower frequency in the levosimendan group than in the dobutamine group (3.9% versus 13.0%, respectively; p=0.023). Angina pectoris, chest pain and myocardial ischaemia was also reported less frequently with levosimendan than with dobutamine (0.0% versus 7.0%, respectively; p=0.013). Headache or migraine (13.6% versus 5.0%; p=0.052) and hypotension (8.7% versus 4.0%; p=0.252) were reported more frequently with levosimendan than with dobutamine (453).

ECG and Holter findings

The effects of levosimendan on conduction intervals have been modest. In the dose-finding study there were no changes in PQ or QRS intervals (450). The QTc interval (Bazett) was not prolonged at doses of 0.05 - 0.2 µg/kg/min, but the dose of 0.4 µg/kg/min increased heart rate by 10 bpm, and also prolonged QTc interval by 15 ms. An intracardiac electrophysiology study was conducted in 10 patients with normal cardiac function who were evaluated for rhythm disorders (464). Heart rate was kept constant by atrial pacing at various cycle lengths, corresponding to heart rates of 75 to 120 bpm. Levosimendan was given as a 10-minute loading dose of 18 µg/kg followed by a continuous infusion of 0.4 µg/kg/min for a total duration of 30-40 minutes. The QT interval was unchanged compared with baseline at all studied cycle lengths. This indicates that levosimendan does not actually affect the duration of ventricular repolarisation.

A meta-analysis of ambulatory ECG recordings was done on a total of 792 recordings pooled from 10 studies, with data from 386 patients. The recordings represented a total duration of >14 000 hours. There were no significant differences between levosimendan and placebo in the occurrence of bradyarrhythmias, atrio-ventricular block or supraventricular or ventricular arrhythmias. New occurrence of supraventricular or ventricular tachycardia or other proarrhythmia according to Morganroth criteria was similar during levosimendan and placebo administration (465).

Studies with oral levosimendan

The haemodynamic effects of an oral formulation of levosimendan were evaluated in a pilot study in 10 patients with NYHA IIIb-IV heart failure. The patients had a mean baseline PCWP of 22 mmHg and LVEF of 23%. The study was open and uncontrolled, with 3 escalating single doses. Each patient was given 1 mg as the first dose, 2 mg 6 hours later, and 4 mg 18-24 hours later. After 1 mg, PCWP decreased by 18% (p=0.002), CO increased by 22% (p=0.005), while HR increased by 3-8 bpm (Figure 7). Systolic BP did not change, while dBP dropped by about 5 mmHg. The effects after 2 mg were small, which was probably due to a meal served to the patients before drug intake, which could have delayed the absorption of the drug and altered baseline haemodynamics. After 4 mg CO increased by 27% compared to baseline (p=0.009), while HR increased by 1-3 bpm. Systolic BP increased by 7-9 mmHg, while dBP was virtually unchanged. PCWP showed only a small decrease, probably due to rather low baseline values (carry-over from previous doses). Also pulmonary artery pressure decreased slightly. However, right atrial pressure decreased substantially by 28-40% after 4 mg. All doses were well tolerated. The study suggested that oral levosimendan is haemodynamically beneficial, the effects resembling those seen after intravenous administration (466).

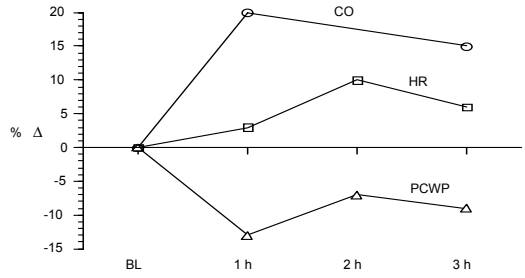


Figure 7. Effects of a single oral dose of 1 mg of levosimendan on cardiac output (CO), pulmonary capillary wedge pressure (PCWP), and heart rate (HR) in 10 patients with severe heart failure. Percent changes from baseline (BL) values are shown.

In order to determine the efficacy of multiple dosing of oral levosimendan in severely ill patients, 24 patients with end-stage heart failure, who were dependent on i.v. inotropic support, were enrolled in an open-label pilot study. Dependence on i.v. inotropic therapy was defined as inability to wean the patient from i.v. inotropic support without clinical decompensation on two separate occasions within a two-week period. The mean LVEF was 24% at baseline.

The patients were administered oral levosimendan at escalating doses to the maximum tolerated dose concomitantly with a stable dose of an i.v. inotrope for the first 2 days (Day 1-2). From then on, the i.v. inotropic agent was tapered over 3 days (Day 3-5). After successful discontinuation of the i.v. inotropic agent, the dose of oral levosimendan was reduced to one-half, and maintained at this level through Day 10 after starting levosimendan. After Day 10 the patients who were successfully weaned from i.v. inotropic therapy were allowed to continue their oral treatment with levosimendan. The weaning was considered successful if: (1) the i.v. inotrope was tapered by Day 5, and (2) the patient remained off these agents for 5 additional days (until Day 10).

Of the 24 patients enrolled, 20 were successfully weaned from i.v. inotropic support (dobutamine or milrinone). Ejection fraction was unchanged between screening and Day 10. About 50% of the patients experienced improvement or no change in their clinical signs and

symptoms of heart failure by Day 10. After Day 10, 7 patients continued on oral levosimendan more than 90 days during optional follow-up. The average duration of long-term therapy was 71 ± 65 days and average dose 1 mg two or three times daily (467).

This study suggested that oral levosimendan may be useful as a substitute for i.v. inotropic agents in patients with severe heart failure who are unable to be withdrawn from these agents, thus allowing the patients to be managed out of hospital.

3. Aims of the study

Previous clinical experience with levosimendan has suggested that levosimendan is effective and well-tolerated in patients with severe congestive heart failure. Very often, however, the decompensation of heart failure occurs as a consequence of acute or chronic ischaemia. The experience in patients with acute ischaemia has been very limited and the risk-benefit profile of the drug in this setting has not been characterised. In addition, the information about the efficacy and safety of oral levosimendan has been obtained only from open label studies and no placebo-controlled evaluation of oral levosimendan has been performed in patients with ischaemia or ischaemic heart failure.

The aim of the thesis can be summarised as follows:

1) To study the safety and efficacy of intravenous levosimendan in patients with acute IHD - AMI, complicated by left ventricular failure (Study I)

2) To characterise the pharmacodynamic effects and pharmacokinetic-pharmacodynamic interrelationships of intravenous and oral levosimendan in patients with ischaemia or ischaemic heart failure (Study II and Study III)

3) To characterise the pharmacodynamic effects of long-term administration of oral levosimendan in patients with ischaemia or ischaemic heart failure (Study III and Study IV)

4) To characterise the pharmacokinetics of oral levosimendan and its metabolites in patients with ischaemic heart failure (Study IV)

4. Subjects and methods

The four publications included in this thesis are based on three different studies. Reference to the four publications is made in the text as studies I, II, III and IV, as summarised below:

Study I (publication I)

- Study in 504 patients experiencing AMI complicated by left ventricular failure
- Four different 6-hour intravenous infusions of levosimendan were administered

Study II (publication II)

- Study in 29 patients with chronic heart failure due to IHD
- Levosimendan was administered as 6-hour intravenous infusion and as single oral dose

Study III (publication III)

- Study evaluating pharmacodynamic effects of oral levosimendan and its interactions with felodipine in 24 patients with chronic IHD
- Levosimendan was administered four times daily

Study IV (publication IV)

- Study investigating pharmacodynamics and pharmacokinetics of oral levosimendan and its metabolites in 25 patients with chronic heart failure due to IHD
- Levosimendan was administered in 4 different daily doses and 4 different dosing intervals

4.1 Study subjects

A total of 557 patients were included in this thesis (Table 12). Most of the patients participating in **study II**, participated also in **study IV**, so that the population of **study IV** represents actually a subpopulation of **study II**. Almost all patients in all studies experienced different forms of IHD, which was complicated by acute left ventricular failure or chronic heart failure (except in **study III**). **Study I** was performed in 21 centers in Russia and Latvia, while **studies II-IV** were performed in Mustamäe Hospital, Tallinn, Estonia.

Since the studies had relatively different objectives, several important differences can be noted also between the study populations. **Study I** investigated the effects of levosimendan in the acute setting, whereas **studies II-IV** evaluated the effects of the drug in patients with a chronic form of IHD or heart failure and required a clinically stable condition. Therefore, the criteria for exclusion used in **studies II-IV** were rather similar, except for the existence of heart failure, which was actually an inclusion criterion for **studies II and IV** and exclusion criterion for **study III**. Patients with bradycardia, tachycardia, history of supraventricular or ventricular tachycardia, ventricular flutter or fibrillation, second or third degree atrioventricular (AV) block, valvular disease, severe hyper- or hypotension, obesity, relevant renal or hepatic failure and chronic pulmonary or endocrinological disease were excluded from those three studies.

Study I included patients with recent AMI complicated by left ventricular failure, evidenced by pulmonary venous congestion or pulmonary oedema on chest X-ray and clinical need for inotropic therapy on the basis of symptomatic heart failure despite conventional therapy.

Specifically, the study was designed to evaluate whether the pharmacological actions of levosimendan would be well tolerated in acutely ill patients with unstable heart failure due to an AMI who had a clinical need for positive inotropic therapy. Positive inotropic agents have been reported to produce serious hypotension and worsening ischaemia in these patients, and thus, these two safety variables were selected as the primary outcome measures for this study. As in **studies II-IV**, patients with severe arrhythmic disorders, hypotension or hepatic failure were excluded from **study I**, but as to renal failure, only patients with severe renal failure were excluded. Other exclusion criteria, such as myocardial rupture or severe mitral valve insufficiency, cardiac tamponade, use of beta-adrenergic agonists within 30 minutes before the start of the study, septic shock and agonal status underline the severity and acuteness of the investigated patients.

Table 12. Demographics and baseline characteristics of the patients

	N	**Study I (504)**	**Study II (29)**	**Study III (24)**	**Study IV (25)**
Main diagnosis		AMI + LVF	CHF	IHD	CHF
Age, years (mean ± SEM)		67 ± 11	58 ± 2	57 ± 2	57 ± 4
Sex, N (%)					
Male		260 (52)	24 (83)	24 (100)	23 (92)
Female		244 (48)	5 (17)	0 (0)	2 (8)
Weight, kg		77 ± 13	76 ± 2	84 ± 1	76 ± 4
NYHA/CCS/Killip class		II-III (Killip)	III-IV (NYHA)	II (CCS)	III-IV (NYHA)
Ischaemic heart disease, N (%)		504 (100)	25 (86)	24 (100)	21 (84)
Hypertension, N (%)		338 (67)	5 (17)	5 (21)	5 (20)
Atrial fibrillation, N (%)		29 (6)	6 (21)	0 (0)	7 (24)
Gastrointestinal disease, N (%)		126 (25)	3 (10)	3 (13)	2 (8)
Pulmonary disease, N (%)		121 (24)	0 (0)	0 (0)	0 (0)
Diabetes mellitus, N (%)		97 (19)	3 (10)	1 (4)	2 (8)
Cerebrovascular disease, N (%)		72 (14)	1 (4)	0 (0)	0 (0)
HR, bpm (mean ± SEM)		82 ± 4	72 ± 3	70 ± 2	70 ± 5
SBP, mmHg (mean ± SEM)		125 ± 5	121 ± 2	140 ± 3	121 ± 6
DBP, mmHg (mean ± SEM)		75 ± 3	78 ± 2	85 ± 2	75 ± 3
Use of selected drugs					
Thrombolytics, N (%)		86 (17)	0 (0)	0 (0)	0 (0)
Nitrates, N (%)		487 (97)	22 (76)	18 (75)	19 (76)
ACE inhibitors, N (%)		237 (47)	26 (90)	0 (0)	23 (92)
Beta-blockers, N (%)		196 (39)	9 (31)	16 (67)	8 (32)
Calcium antagonists, N (%)		66 (13)	2 (7)	0 (0)	2 (8)
Diuretics, N (%)		376 (75)	14 (48)	0 (0)	18 (72)
ASA, N (%)		444 (88)	24 (83)	20 (83)	23 (92)
Heparin and analogues, N (%)		427 (85)	1 (3)	0 (0)	1 (4)
Cardiac glycosides, N (%)		66 (13)	18 (62)	0 (0)	16 (64)
I.v inotropes, N (%)		72 (14)	1 (3)	0 (0)	0 (0)
Antiarrhythmics, N (%)		129 (26)	4 (14)	1 (4)	4 (16)

Mean age in **studies II-IV** was about 57 years, but in **study I** it was higher, being about 67 years. In **studies II-IV** most of the patients were male, whereas in **study I** almost half of the patients were women. In all studies most of the patients suffered from IHD. Mean weight was rather similar in all studies. All patients were Caucasian.

The studies are different regarding severity and acuteness disease of the patients, which is reflected in the baseline characteristics. Patients in **study I** had a much more severe and acute form of the disease and also their basic health condition was more complicated, which is characterized by the higher number of concomitant diseases. For example, hypertension was present in 67% of patients in **study I**, whereas in the other studies only about 20% of patients suffered from hypertension. The incidence of gastrointestinal disease, pulmonary disease, cerebrovascular disease and diabetes was also higher in **study I**. This can partly be explained by the fact that severe forms of those diseases were exclusion criteria in **studies II-IV**, which obviously limited the frequency of also mild forms.

In all studies study treatment was administered in addition to the patients' normal medication for IHD and heart failure. Only in **study III**, which was also an interaction study with felodipine, were calcium antagonists prohibited from patients. There are some differences between the studies regarding concomitant medication such as ACE inhibitors, which were more frequently administered in **studies II and IV**. The absence of concomitant ACE inhibitors and the relatively high frequency of beta-blockers in **study III** are related to the absence of concomitant heart failure. The same applies to the use of diuretics. Nitrates and ASA were administered in similar frequency in all studies, while thrombolytics and heparin were administered solely in **study I** as treatment of AMI.

The haemodynamics at baseline were relatively similar in all studies. Heart rate was higher at baseline in **study I** and blood pressure in **study III**. Despite higher incidence of hypertensive patients in **study I**, baseline blood pressure values in that study were relatively low, which can be explained by severe left ventricular failure and depression of the pump function of the heart in those patients.

4.2 Study designs

Study I

Study I was a randomized, parallel-group, placebo-controlled and double-blind study. Five hundred and four patients were recruited between June 1996 and December 1998, and the follow-up was completed in April 2000. A computer-generated randomisation schedule, based on permuted blocks and balanced within each centre, was used to allocate patients to placebo or one of four dose regimens of levosimendan (Figure 8). The loading dose was infused over a period of 10 minutes and the continuous infusion was maintained for 5 hours and 50 minutes. All the formulations and vials were made to look identical and contained either no active ingredient or different amounts of levosimendan. The volumes infused into the patients of the five different treatment arms were also identical. Study medications were introduced *via* a peripheral vein using a calibrated infusion pump.

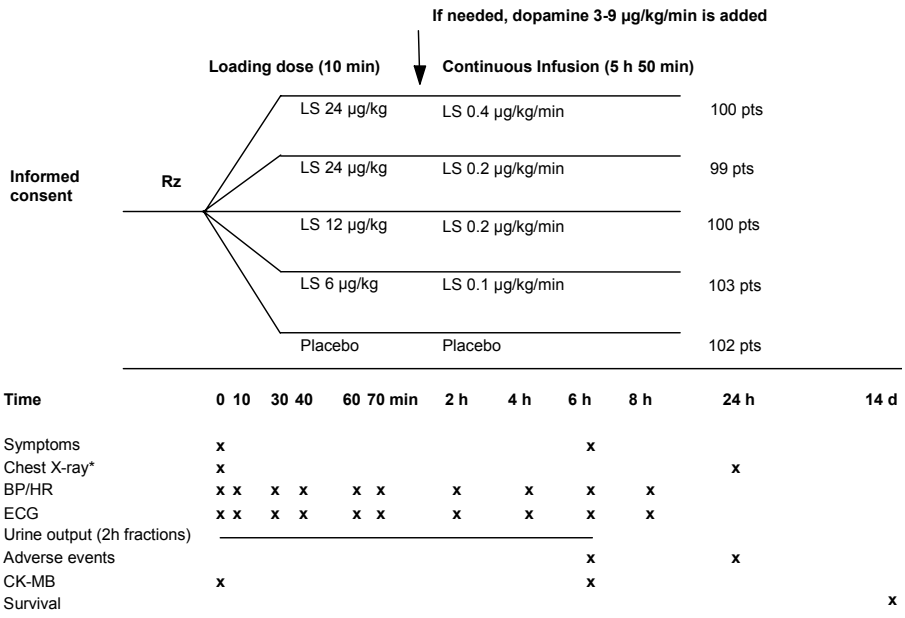


Figure 8. Design of study I

Investigators were mandated to stop the infusion of the study medication at any time if the patients experienced any of the following: symptomatic hypotension, heart rate >130 bpm sustained for 10 min, or any serious adverse event. Patients were permitted to receive all appropriate therapy for the management of both AMI and heart failure. Patients developing persistent hypotension or refractory heart failure during infusion were permitted intravenous dopamine (3-9 µg/kg/min).

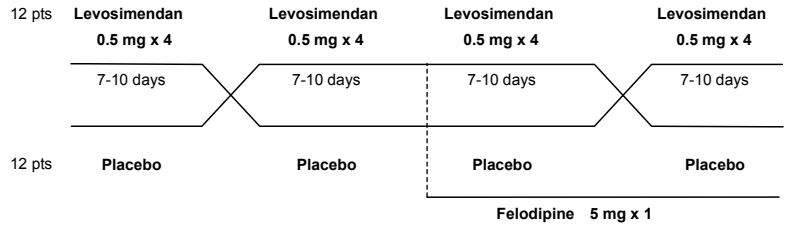
Throughout the 6-hour infusion period, patients were assessed for hypotension or myocardial ischaemia of clinical significance, symptoms of heart failure, haemodynamics, urinary output and adverse events. In addition to spontaneous reporting, an adverse event inquiry was undertaken by the investigator at the end of infusion and at 24 hours after start of infusion. Survival of the patients was evaluated at 14 days following the start of infusion. An additional, retrospective 180-day mortality follow-up was conducted after the end of the study. Blood samples drawn before the start of infusion and immediately after infusion were used to determine serum CK-MB levels. A chest X-ray was repeated within 12-30 hours after start of infusion.

Study II

Study II was a two-day open-label, non-randomized trial in 29 patients with NYHA class III-IV chronic heart failure. On the first day patients were given a 6-hour levosimendan infusion with the dose 0.2 µg/kg/min. After a 1-week washout the patients received a 2 mg single oral dose of levosimendan.

Study III

Study III was a randomized, double-blind, placebo-controlled, crossover study in 24 male patients with Canadian Cardiovascular Society (CCS) class II IHD. The study consisted of four treatment periods, each period lasting for 7-10 days. In the first period the patients received either oral levosimendan (0.5 mg) or placebo four times daily and were then crossed over to the other therapy for the second and third periods. After the third period the patients were changed back to the therapy administered in the first period. Open label felodipine, 5 mg once daily, was co-administered on the third and fourth treatment periods (Figure 9).



	Day 1,3	Day 7	Day 8,10	Day 14	Day 15-17	Day 21	Day 22,24	Day 28
Exercise test		x		x		x		x
Holter		x		x		x		x
Systolic time intervals		x		x		x		x
BP, HR, ECG	x	x	x	x	x	x	x	x
Safety laboratory		x		x		x		x

Figure 9. Design of study III

Study IV

Study IV was a randomized, parallel-group, double-blind and placebo-controlled study in 25 patients with NYHA class III-IV chronic heart failure. Each patient received an ascending dose of study medications on two consecutive two-week periods without a washout (Figure 10). Patients were randomized to three groups according to the method of sequential treatment design as follows:

- Group I ("low-dose" group, n=9) received levosimendan 2 mg x 1 for two weeks, and then 2 mg x 3 for two more weeks.
- Group II ("high-dose" group, n=8) received levosimendan 2 mg x 2 for two weeks, and then 2 mg x 4 for two more weeks.
- Group III (placebo group, n=8) received placebo during both study periods.

A safety follow-up was conducted one week after the last medication day of the second treatment period.

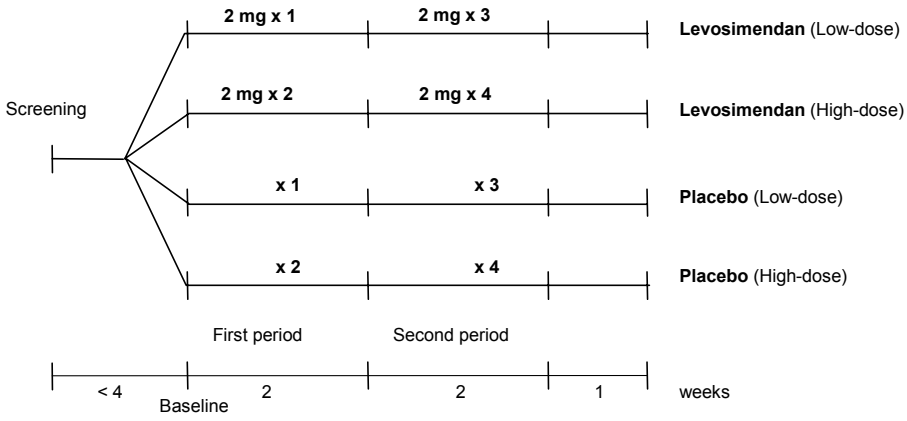


Figure 10. Design of study IV

4.3 Assessments

4.3.1 Haemodynamics

Non-invasive haemodynamic measurements were used in all studies.

Study I

Blood pressure and heart rate were measured at baseline, and at 10, 30, 40, 60 and 70 minutes and then at 2, 4 and 6 hours after the start of infusion, and finally, 2 hours after discontinuation of the study drug. For assessment of heart rate a 12-lead ECG was used at baseline and at 6 hours, while at other time points either rhythm strips or limb lead ECGs were used.

Studies II-IV

The effect of levosimendan on haemodynamics was evaluated using assessments of systolic time intervals, blood pressure and heart rate.

For determination of systolic time intervals ECG, carotid artery pulse curve and phonocardiogram were recorded simultaneously (Schiller CS-100, Schiller AG, Switzerland). Electromechanical systole (QS2) was measured from the beginning of the Q-wave in the ECG to the beginning of the aortic component of the second heart sound. Five to ten cardiac cycles were averaged using a digitizer. QS2 was corrected for heart rate according to the formula: QS2i = QS2 + 2.1 x heart rate. The digitizing of QS2 was performed by a person independent of the study team.

Blood pressure was measured by an automatic oscillometric device (Omron M4, model HEM-722C, Omron Matsusaka Co., Japan) and heart rate from ECG recordings.

In **study II**, on the first study day haemodynamics was assessed at -20 min (baseline), and at 0.5, 1, 2, 4, 6, 6.5, 7, 8 and 10 h. During the second study day QS2i-, HR- and BP-assessments were made at -20 min (baseline), and at 1, 2, 3, 4, 5, 6 and 8h.

In **study III** systolic time intervals were measured at -30 min, and at 1, 3 and 7 h, blood pressure and heart rate at –30 min, and at 1, 2, 3, 4, 5 and 6 h on first and the last day on each treatment period.

In **study IV** haemodynamics was assessed at baseline and on the last day of both treatment periods at -20 min, and at 2, 4 and 8 h.

4.3.2 Symptoms and signs

Symptoms and signs were only assessed in **study I**. Dyspnoea, fatigue, jugular venous distension, peripheral oedema and anginal pain were assessed before the administration of the study drug and at the end of the 6-hour treatment period.

The following scales were utilized for evaluation of symptoms:

- dyspnoea, fatigue and anginal pain: none, slight, moderate, marked and disabling
- jugular venous distension: none; at $\leq$45° elevation; at >45° elevation
- peripheral oedema: none, present.

Increase in symptom score was categorised as worse, decrease as better, and no change as unchanged. In the worst-rank symptom analysis, patients were considered to be worse if, in addition to the changes in actual dyspnoea and fatigue scores during the 6-hour study infusion, they (1) died; (2) developed worsening heart failure; or (3) received a new drug for the treatment of heart failure.

In order to determine the degree of pulmonary congestion, a chest x-ray was performed within 6 hours before administration of the study drug and 12-30 hours after start of infusion of study drug. Responses were ranked as:

- normal (equal distribution of blood flow in lower and upper lobes);
- pulmonary venous congestion (redistribution of pulmonary blood flow to upper lobes);
- pulmonary venous congestion with interstitial oedema;
- pulmonary venous congestion with alveolar oedema.

4.3.3 Morbidity and mortality

Morbidity and mortality as endpoints (combined risk of death and worsening heart failure) were assessed in **study I** during the first 6 hours and during 24 hours after start of infusion. Mortality alone was assessed over 14 days after start of infusion. The mortality follow-up was prolonged retrospectively to 180 days, using information from Official Inhabitants' Registries and hospital files.

The term "worsening heart failure" was predefined for characterisation of all possible major clinical events that may occur in this patient population. Patients were considered to have worsening heart failure if they experienced onset or worsening of any of the following conditions: dyspnoea, fatigue, pulmonary congestion or oedema, heart failure or cardiogenic shock.

4.3.4 Exercise test

Exercise test was used in **studies III and IV**. In **study III**, at the end of each treatment period, a symptom-limited exercise test was undertaken on a bicycle ergometer. The initial load was 50 W, which was increased by 25 W at 3-minute intervals until subjective maximum. Indications

for stopping of the exercise test were severe exhaustion, severe dyspnoea, severe angina pectoris (Borg scale 4-5 out of 10), dizziness, ST-displacement >2 mm, persistent drop in sBP, serious ventricular arrhythmias, supraventricular tachycardia or AV block. During the test and immediately before its termination heart rate, blood pressure and magnitude of changes of ST-segment (at the end of each workload and upon the termination of the test) were measured. Total exercise time and highest achieved workload were recorded. ST-depression was measured 60 ms from J-point.

In **study IV**, a six-minute walk test was performed at baseline and on the last day of each treatment period. The walk test was carried out as follows: the patient walked for 6 minutes at his/her own pace from end to end in a corridor of 30 m. The patients were not encouraged during the test, and they were allowed to take pauses, if symptoms so required. The walked distance was measured to the nearest 5 m.

4.3.5 Pharmacokinetics

Sampling

No pharmacokinetic samples were drawn in **study I**. Drug and metabolite concentrations were measured in **study III** and pharmacokinetic variables were calculated in **studies II and IV.**

On the first day in **study II** the venous blood samples for determination of levosimendan concentrations in plasma were drawn at -20 min (baseline), and at 0.5, 1, 2, 4, 6, 6.5, 7, 8 and 10 h. On day two samples were drawn at -20 min (baseline), and at 0.5, 1, 2, 3, 4, 5, 6 and 8 h.

In **study III**, samples for measurement of levosimendan concentrations were drawn at one hour after each capsule intake during the visit on the last day of each treatment period (at 1, 4 and 7 h). A plasma sample for measurement of concentrations of levosimendan metabolites was drawn on the last day of each treatment period.

In **study IV**, on the last day of each treatment period, venous blood samples for determination of levosimendan concentrations were drawn at -20 min, and at 2, 4 and 8 h. Metabolite concentrations were determined from the samples drawn at -20 min, and at 2 and 8 h and also from a sample drawn one week after the last medication day of the second treatment period (day 35).

Analyses

The concentrations of levosimendan in plasma were determined by an automated sample preparation technique with reversed-phase high performance liquid chromatography with UV detection (468). The method gave linear and interference free results over a plasma concentration range from 5 to 500 ng/ml. The within-run precision was <15% at the quantitation limit (5 ng/ml) and <5% at higher concentrations. The between-run precision was <10% at concentrations up to 50 ng/ml and <5% at higher concentrations. The concentrations of levosimendan metabolites, OR-1855 and OR-1896, in plasma were determined by liquid chromatography-tandem mass spectrometry using the selected reaction monitoring technique. The method gave linear and interference free results. The within-run precision was <11% at the quantitation limit (0.1 ng/ml) and <8% at higher concentrations. The between-run precision was <9% at concentrations up to 0.6 ng/ml and <7% at higher concentrations. Individual plasma concentration-time data of levosimendan were analyzed using a standard noncompartmental method in WinNonlin® Professional computer program (Version 3.1, Pharsight Corporation, USA).

In **study II** a model-independent method was used to determine pharmacokinetic parameters of levosimendan in plasma. Individual plasma concentration-time data of levosimendan was analysed using a standard noncompartmental method in WinNonlin® Professional computer program (Version 3.1, Pharsight Corporation, US). Peak plasma concentration (C_{max}) and time of peak plasma concentration (t_{max}) were obtained directly from the plasma concentration-time data. The terminal elimination rate constant (λ_z) was obtained directly from the regression analysis with visual inspection of the data to determine the number of points in the terminal elimination phase. Terminal elimination half-life ($t_{1/2}$) and total plasma clearance (Cl_{tot}) were calculated.

The area under the plasma concentration-time curve was calculated from the beginning of the 6-hour infusion to the last detectable concentration using the linear trapezoidal method (AUC_{last}). The area was also extrapolated to infinity (AUC_{∞}). The apparent volume of distribution based on the terminal phase (V_z) and the apparent volume of distribution at steady-state (V_{ss}) were calculated. Mean residence time (MRT) was determined.

The following pharmacokinetic parameters were calculated for levosimendan after the 2 mg single oral dose: C_{max}, t_{max}, λ_z, $t_{1/2}$, AUC_{last} and AUC_{∞}. Bioavailability of oral levosimendan was calculated using the following formula: $F_A = (D_{iv} / D_o)$ x $(AUC_{\infty,Do} / AUC_{\infty,Div})$ x 100%, where D_{iv} refers to the total dose of levosimendan, administered during the intravenous infusion and D_o refers to the 2 mg single dose of oral levosimendan.

In **study IV**, peak plasma concentration (C_{max}) and time to peak plasma concentration (t_{max}) of levosimendan were obtained directly from the plasma concentration-time data. The area under the plasma concentration-time curve was calculated from the intake of the first capsule of the day to the last detectable concentration using the linear trapezoidal method (AUC_{last}).

Pharmacokinetics of levosimendan metabolites was analysed on the last day in both study periods. C_{max} and AUC_{8h} were calculated for OR-1855 and OR-1896 using a standard noncompartmental method.

4.3.6 Safety

Ischaemia and hypotension in study I

Study I was designed as a safety study in patients with AMI complicated by left ventricular failure. The primary endpoint of the study was the proportion of patients developing hypotension or ischaemia of clinical significance as adjudicated by an independent Safety Committee. Clinically significant hypotension was defined as: (1) symptomatic hypotension (obligatory) or (2) asymptomatic drop of sBP by more than 10 mmHg (at the discretion of the investigator). Clinically significant ischaemia was defined as: (1) aggravation or a new onset of anginal pain; or (2) further depression or elevation of ST-segment by more than 1 mm in 12-lead ECG.

Clinically significant hypotension and/or ischaemia, reported by the investigator, was evaluated by the Safety Committee. For this evaluation, investigators provided the Safety Committee with the Case Records Forms, ECGs and copies of patients' hospital files (as necessary). The Safety Committee held meetings after every 100 recruited patients. Members of the Safety Committee were unaware of patient treatment allocation at the time of evaluation.

Adverse events

In addition to spontaneous reporting, an adverse event inquiry was undertaken by investigator in all studies. In **study I** adverse event follow-up was carried out during the 24 hours after start of study drug infusion. In **study II** adverse event follow-up was carried out during 24 hours after start of administration of study medication. In **studies III and IV**, adverse events were followed during the treatment periods and in **study IV** an additional follow-up was carried out also at one week after the last medication day of the second treatment period.

Safety laboratory variables

For assessments of safety laboratory variables, in **studies II-IV** the local hospital laboratory was used, while in **study I** a central laboratory analyzed the samples.

In **study I** samples for determination of CK-MB were taken at baseline and at the end of the 6-hour infusion. In **study II**, samples were taken on both study days at baseline and at 24 h. In **studies III and IV** samples were taken at baseline and at the end of treatment periods.

In **studies II-IV** the following haematological and biochemical markers were determined: blood (B)-Erythrocytes, B-Glucose, B-Haematocrit, B-Haemoglobin, B-Leukocytes, B-Thrombocytes, serum (S)-Alanine aminotransferase, S-Alkaline phosphatase, S-Aspartate aminotransferase, S-C-reactive protein, S-Creatinine, S-Gammaglutamyltransferase, S-Potassium, S-Sodium, urine (U)-Glucose, U-Protein.

Holter monitoring

For detection of clinically relevant arrhythmic and ischaemic disorders 24-hour ambulatory ECG recording (Holter) was undertaken in **studies II-IV**. The recordings were measured using the Holter Recorder Marquette device (Series 8500, Marquette Electronics, Inc., Milwaukee, WI, USA). In **study II** Holter recordings were carried out at baseline and during both study days. In **studies III and IV** recordings were carried out at baseline and on the last day of each treatment period.

The occurrence of relevant arrhythmic disorders such as ventricular fibrillation, sustained ventricular tachycardia (duration >30 seconds, frequency >120 bpm) and runs of ventricular and supraventricular tachycardia (duration ≥ 3 beats, frequency >120 bpm) and episodes of bradyarrhythmia were registered. For evaluation of ischaemia, ST-segment deviations were represented in a 24-hour trend diagram and verified with a 50-mm/s electrocardiogram printout. Typical ischaemic change was defined as a transient horizontal or descending ST-depression ≥ 0.1 mV (measured 80 ms from J-point) that lasted at least 1 minute. Transient ST-elevations were evaluated in the same manner with the precondition that they did not occur in leads with pathological Q-waves. The time between episodes had to be at least 1 minute. The total duration and number of ischaemic episodes and the longest ischaemic episode were recorded.

4.4 Statistical methods

In all studies statistical analyses were based on the intention-to-treat principle, performed at a two-sided 0.05 level of significance. Analyses were carried out using SAS 6.12 statistical software (SAS Institute Inc., Cary, NC, US) for Windows.

Study I

Baseline characteristics were summarised using appropriate descriptive statistics; values for each characteristic were compared among the five treatment groups by using Analysis of Variance (ANOVA) with effects for treatment, centre and treatment by centre interaction or the non-parametric Cochran-Mantel-Haenszel (CMH) test, controlling for centre. In analyses for primary endpoint the differences between the five treatment groups and between the placebo group and pooled levosimendan group the proportions of patients were tested using the CMH row mean scores test, controlling for centre. The relationship between dose and frequency of event(s) was evaluated using the CMH non-zero correlation test.

Combined risk of death and worsening heart failure was expressed using a time-to-event model. The log-rank test was used for detecting differences between placebo and pooled levosimendan groups. Cumulative survival curves for placebo and pooled levosimendan groups were constructed by the Kaplan-Meier method and the differences between the curves were tested for significance using the Cox proportional hazards model. Survival time in the model was calculated as the difference in days from the start of infusion to the event or to the last follow-up date. The relationship between dose and frequency of event(s) was evaluated using the CMH non-zero correlation test, controlling for centre.

Changes in symptoms, jugular venous distension, peripheral oedema, urinary output, pulmonary congestion and CK-MB values were evaluated using ANOVA methods or by use of CMH row mean scores test, controlling for centre. The frequency of adverse events in the five treatment groups was compared using Fisher's exact test. Dose-relations of adverse events were tested using the CMH non-zero correlation test, controlling for centre.

Study II

Changes in haemodynamics were evaluated by repeated measures ANOVA. Descriptive concentration-effect loops between QS2i, heart rate, sBP, dBP and levosimendan concentrations as a function of time were constructed. Descriptive statistics was applied for the pharmacokinetic parameters, safety laboratory parameters and for Holter recordings.

Study III

Results of haemodynamics and Holter recordings on the last day of each treatment period were evaluated statistically by univariate repeated measures ANOVA models, which included both random and fixed effects (MIXED-procedure). The possible carry-over effect was evaluated by comparing the two sequences (LS and PL vs PL and LS; LS+FD and PL+FD vs PL+FD and LS+FD) using the two-sample t-test.

Study IV

The changes in haemodynamics and in the 6-minute walk test from baseline to 2 hours on the last treatment day of the study period were compared between the three treatment groups in the two study periods separately using ANOVA with effect for treatment. Descriptive statistics was applied for the safety laboratory and pharmacokinetic parameters. The relationship between levosimendan dose and metabolite concentrations was tested using the paired t-test. The

relationship between the pharmacokinetic parameters of levosimendan and its metabolites and daily dose were examined by using linear regression analyses. Changes in ECG parameters were compared between the three treatment groups in the two study periods separately using one-sample t-test and ANOVA methods and changes in Holter parameters by using the Cochran-Mantel-Haenszel test.

4.5 Ethics

The studies were conducted according to the Declaration of Helsinki of the World Medical Assembly and its amendments. The study protocols and any relevant amendments were reviewed and approved by the local Ethics Committees and Regulatory Authorities. Written informed consent was obtained from all patients prior to inclusion in the study.

5. Results

5.1 Efficacy

5.1.1 Haemodynamics

Study I

At 30 minutes following the start of treatment, there were similar minor changes in sBP (p=0.84) and dBP in all treatment groups (p=0.20). In contrast, at the end of the 6-hour double-blind treatment period, levosimendan produced dose-dependent decreases in systolic and dBP (p=0.001). The changes in blood pressure at 6 hours were relatively modest with the three lowest infusion rates of levosimendan (placebo-corrected decreases in systolic and dBP of up to 4 and 2 mmHg, respectively). The highest infusion rate of levosimendan (24 µg/kg + 0.4 µg/kg/min), however, produced placebo-corrected decreases in sBP and dBP of up to 7 mmHg (Table 13).

Levosimendan increased heart rate dose-dependently at both 30 minutes and 6 hours (p=0.001). At 30 minutes, patients who received a 6 or 12 µg/kg loading dose had only small increases in heart rate (1-2 bpm), whereas patients who received the largest loading dose (24 µg/kg) showed 6-7 bpm increases in heart rate (corrected for placebo). Similarly, at 6 hours, the changes in heart rate were relatively modest with the three lowest infusion rates of levosimendan (up to 4 bpm). In contrast, the highest infusion rate of levosimendan (24 µg/kg + 0.4 µg/kg/min) produced an increase of over 11 bpm in heart rate (Table 13).

Table 13. Baseline values and changes in blood pressure and heart rate at 30 minutes and at 6 hours (end of infusion). Mean (SEM) are given.

	Placebo (n=102)	LS 6 µg/kg + 0.1 µg/kg/min (n=103)	LS 12 µg/kg + 0.2 µg/kg/min (n=100)	LS 24 µg/kg + 0.2 µg/kg/min (n=99)	LS 24 µg/kg + 0.4 µg/kg/min (n=100)
sBP (mmHg)					
baseline	124 (2.1)	123 (1.9)	128 (2.0)	125 (2.2)	126 (1.9)
Δ 30 min	-1.6	-3.0	-1.7	-2.1	-3.0
Δ 6 hours*	-1.3	-2.1	-4.2	-5.4	-7.9
dBP (mmHg)					
baseline	77 (1.3)	75 (1.1)	76 (1.3)	75 (1.3)	75 (1.3)
Δ 30 min	-2.4	-3.4	-3.0	-4.5	-3.8
Δ 6 hours†	-2.5	-3.1	-4.3	-4.6	-8.0
HR (bpm)					
baseline	84 (1.6)	82 (1.4)	82 (1.8)	85 (1.7)	80 (1.7)
Δ 30 min†	-1.5	0.5	1.1	4.7	5.2
Δ 6 hours†	0.0	2.0	3.7	3.9	11.4

*p=0.012 for dose-relation; † p=0.001 for dose-relation

Study II

Both sBP and dBP decreased from baseline at the end of the 6-hour infusion. Heart rate remained stable, after both the 6-hour infusion and 2 mg oral dose. Changes in blood pressure after intake of the 2 mg capsule were smaller than after the 6-hour infusion. QS2i shortened both after the 6-hour infusion and after intake of the 2 mg capsule (Table 14).

Table 14. Baseline values and changes in blood pressure, heart rate and QS2i. Mean (SEM) are given.

	SBP (mmHg)	DBP (mmHg)	HR (bpm)	QS2i (ms)
6-hour infusion (0.2 µg/kg/min)				
baseline	120 (3.9)	78 (2.1)	68 (2.6)	515 (6.5)
Δ 6 hours	-7.1	-5.7	+1.6	-9.0
p-value	0.004	0.0004	0.33	0.007
2 mg single oral dose				
baseline	118 (3.1)	77 (2.2)	68 (2.3)	532 (6.3)
Δ 2 hours	-3.4	-2.0	+0.2	-7.2
p-value	0.06	0.09	0.91	0.006

The concentration-effect loops for QS2i, sBP and dBP after both 6-hour infusion and 2 mg single oral dose showed clear counter-clockwise hysteresis (Figure 11).

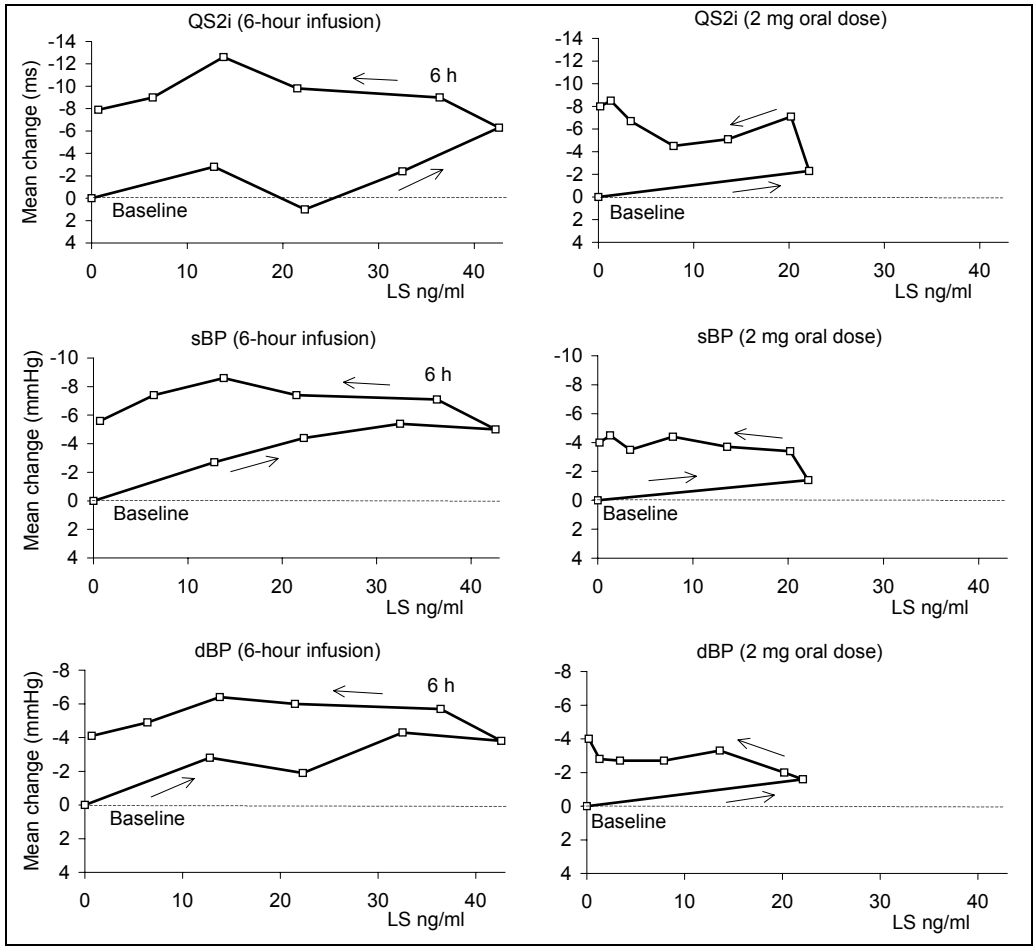


Figure 11. Concentration-effect loops for QS2i, sBP and dBP after 6-hour intravenous infusion and 2 mg single oral dose of levosimendan.

Study III

Oral levosimendan, administered 0.5 mg four times daily increased heart rate in comparison with placebo and felodipine, while felodipine decreased sBP in comparison with both levosimendan and placebo. The differences between treatments regarding heart rate and sBP were statistically significant ($p<0.001$ and $p=0.003$, respectively). Changes in dBP were small ($p=0.26$).

Levosimendan shortened QS2i by 10 ms (95% CI [-15, -4]), compared with placebo, indicating a moderate positive inotropic effect. The difference between treatments was statistically significant ($p<0.001$). The results were similar when levosimendan was administered concomitantly with felodipine. Pairwise comparisons of haemodynamic parameters on the last day of the treatment period are presented in Table 15.

Table 15. Pairwise comparisons of QS2i, heart rate and blood pressure on the last day of the treatment period. Dose of oral levosimendan 0.5 mg four times daily, and that of felodipine 5 mg daily.

		Mean difference	95% CI
QS2i (ms)	LS vs PL	-10	[-15; -4]
	FD vs PL	+1	[-5; +6]
	LS + FD vs LS	+3	[-3; +8]
	LS + FD vs FD	-8	[-14; -2]
HR (bpm)	LS vs PL	+6	[+3; +9]
	FD vs PL	+2	[-1; +6]
	LS + FD vs LS	+3	[0; +6]
	LS + FD vs FD	+7	[+3; +10]
sBP (mmHg)	LS vs PL	+2	[-2; +5]
	FD vs PL	-4	[-8; -1]
	LS + FD vs LS	-5	[-8; -2]
	LS + FD vs FD	+1	[-2; +5]
dBP (mmHg)	LS vs PL	0	[-1; +2]
	FD vs PL	-1	[-3; +1]
	LS + FD vs LS	-2	[-3; 0]
	LS + FD vs FD	0	[-2; +2]

Study IV

Levosimendan given at 2 mg daily did not cause an increase in heart rate after 14 days of administration, the effect being similar to placebo. After 4-8 mg daily doses of levosimendan heart rate increased by 8-11 bpm. Interestingly, no further increase in heart rate was seen in the high-dose group when the daily dose was doubled. The differences between the treatment groups regarding change in heart rate after both first and second treatment periods were nearly significant (p=0.09 and p=0.05, respectively) (Table 16).

Changes in blood pressure were small. Systolic BP decreased by 2 mmHg after the 2 mg daily dose and by 1 mmHg after the 6 mg daily dose compared with the baseline. After the 4 mg daily dose sBP increased by 5 mmHg and after the 8 mg daily dose it decreased by 2 mmHg. In the placebo group sBP decreased by 5 mmHg. The differences between the treatment groups regarding change in sBP after both first and second treatment periods were not significant (p=0.15 and p=0.40, respectively) (Table 16).

Small changes were seen also regarding dBP. Diastolic BP decreased by 1 mmHg after the 2 mg daily dose and by 6 mmHg after the 6 mg daily dose compared with baseline. After the 4 mg daily dose dBP increased by 1 mmHg and after the 8 mg daily dose it decreased by 2 mmHg. In the placebo group dBP increased by 6 mmHg. The differences between the treatment groups regarding change in dBP after both first and second treatment periods were not significant (p=0.78 and p=0.18, respectively) (Table 16).

QS2i shortened by 6 ms after the 2 mg daily dose and by 11 ms after the 6 mg daily dose compared with baseline. After the 4 mg daily dose QS2i shortened by 23 ms and after the 8 mg daily dose by 28 ms. In the placebo group QS2i shortened after 14 days by 8 and after 28 days by 2 ms compared with baseline. There was a statistically significant difference in the change in QS2i between treatment groups (p=0.02) at day 28, which is attributable to the difference between the high-dose group and the placebo group (p=0.005). After first treatment period no significant difference between the treatment groups was observed (p=0.11) (Table 16).

Table 16. Haemodynamic results after oral administration of levosimendan in study IV.

	Low-dose (n=9)			High-dose (n=8)			Placebo (n=8)		
	Baseline	2mgx1	2mgx3	Baseline	2mgx2	2 mgx4	Baseline	placebo	placebo
QS2i (ms)	533 ± 6*	527 ± 10	522 ± 4	538 ± 11	515 ± 13	510 ± 8	548 ± 7	540 ± 15	546 ± 16
HR (bpm)	71 ± 4	70 ± 4	81 ± 4	73 ± 5	81 ± 5	81 ± 6	66 ± 5	67 ± 7	71 ± 8
sBP (mmHg)	122 ± 5	120 ± 5	121 ± 5	123 ± 8	128 ± 8	121 ± 6	117 ± 4	112 ± 5	112 ± 4
dBP (mmHg)	78 ± 1	77 ± 2	72 ± 3	77 ± 4	78 ± 4	75 ± 4	71 ± 3	77 ± 4	77 ± 4

5.1.2 Symptoms and signs

Symptoms and signs were assessed in **study I** only.

Dyspnoea and fatigue

In **study I**, there were no differences between the five treatment groups in the degree to which dyspnoea had improved or worsened when recorded at the end of the study drug infusion, assessed either by the physician (p=0.41) or by the patient (p=0.46). There was a difference between the five treatment groups in the degree to which fatigue had improved or worsened when recorded at the end of study drug infusion, when assessed by the physician (p=0.04), but not when assessed by the patient (p=0.10). In the worst-rank symptom analysis, however, patients treated with levosimendan experienced worsening dyspnoea and fatigue less frequently than patients receiving placebo (Table 17).

Table 17. Results of worst-rank symptom analysis in study I

	Levosimendan (N=402)	Placebo (N=102)	p-value
By patient			
Worsening dyspnoea (%)	11.0	16.7	0.06
Worsening fatigue (%)	10.8	16.7	0.05
By physician			
Worsening dyspnoea (%)	10.8	17.0	0.04
Worsening fatigue (%)	10.6	17.0	0.05

Other symptoms and signs

In **study I** there were no differences among the five treatment groups regarding change of overall clinical status, chest pain, jugular venous distension or peripheral oedema. There were more patients in the levosimendan groups (10.3-14.7%) than in the placebo group (8.3%), who at the end of the study drug infusion did not have signs of left ventricular failure in the X-ray. However, the degree to which pulmonary congestion changed at the end of the study drug infusion was not significant between treatment groups (p=0.94). There were no differences between the treatments regarding change in urinary output during study infusion (p=0.51).

New treatment for heart failure

The prescribing of new medications for heart failure by the physician was utilized in **study I** as an additional surrogate measure of the efficacy of levosimendan. Analysis revealed that during the 6-hour infusion, patients assigned to placebo had a higher frequency than patients assigned to levosimendan (all groups combined) of the use of new medications for heart failure

(intravenous positive inotropic agents, vasodilators and diuretics), 13.7% vs. 7.2% (p=0.003) (Table 18).

Table 18. Administration of new heart failure treatment in different dosing groups in study I

	New i.v. inotropic therapy		New i.v. vasodilators		New i.v. diuretics		Any new i.v. heart failure therapy	
	N	%	N	%	N	%	N	%
Placebo	4	3.9	8	7.8	4	3.9	14	13.7
6 µg/kg + 0.1 µg/kg/min	2	1.9	4	3.9	4	3.9	8	7.8
12 µg/kg + 0.2 µg/kg/min	1	1.0	6	6.0	3	3.0	7	7.0
24 µg/kg + 0.2 µg/kg/min	1	1.0	5	5.1	1	1.0	7	7.1
24 µg/kg + 0.4 µg/kg/min	0	0.0	5	5.1	3	3.0	7	7.1

5.1.3 Morbidity and mortality

Morbidity and mortality as endpoints were assessed in **study I**.

In **study I** the combined risk of death and worsening heart failure was lower among patients treated with levosimendan than among patients receiving placebo both during the 6-hour infusion period (2.0% vs 5.9%, respectively; p=0.03), and during 24 hours after start of infusion (4.0% vs 8.8%, respectively; p=0.04). Three patients, one patient treated with levosimendan and two patients receiving placebo, experienced sudden death during the study (due to ventricular fibrillation), but were successfully resuscitated. When these patients are included in the analysis of morbidity and mortality, patients treated with levosimendan died, were resuscitated from sudden death or experienced worsening heart failure more frequently during the first 24 hours than patients assigned to placebo (4.2% vs 10.8%, p=0.01).

The reduction of all-cause mortality among patients receiving levosimendan compared with placebo was seen already during the 6-hour study infusion. For the 14-day period after start of treatment all-cause mortality among levosimendan-treated patients was significantly lower than with placebo (11.7% vs 19.6%, respectively; 0.56 [95% CI 0.33-0.95]; p=0.03); this difference was seen also when the follow-up was extended to 180 days (22.6% vs 31.4%, respectively; 0.67 [0.45-1.00]; p=0.05) (Figure 12).

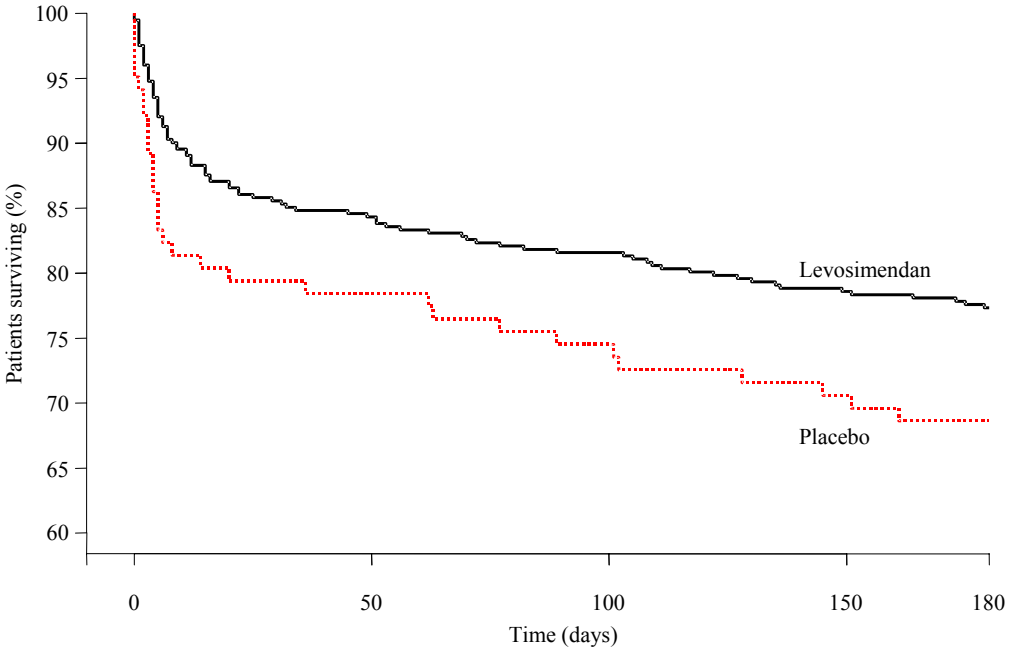


Figure 12. Overall survival during 180 days after start of infusion in study I

There was generally no relationship between the dose of levosimendan and mortality or combined risk of death and worsening heart failure. The only significant dose-relationship was seen regarding mortality during the 6-hour infusion (Table 19).

Table 19. Incidence of death and worsening heart failure after administration of intravenous levosimendan in study I

Endpoint	Placebo (n=102)	LS 6 µg/kg + 0.1 µg/kg/min (n=103)	LS 12 µg/kg + 0.2 µg/kg/min (n=100)	LS 24 µg/kg + 0.2 µg/kg/min (n=99)	LS 24 µg/kg + 0.4 µg/kg/min (n=100)	p-value*
Death or WHF at 6 hours (%)	5.9	2.9	2.0	1.0	2.0	0.09
Death or WHF at 24 hours (%)	8.8	5.8	3.0	3.0	4.0	0.09
Mortality at 6 hours (%)	3.9	1.9	1.0	0.0	0.0	0.02
Mortality at 24 hours (%)	4.9	3.9	1.0	1.0	2.0	0.13
Mortality at 72 hours (%)	9.8	5.8	3.0	5.1	5.0	0.17
Mortality at 14 days (%)	19.6	12.6	10.0	13.1	11.0	0.11
Mortality at 180 days (%)	31.4	26.2	16.0	27.3	21.0	0.09

*For dose-relation (CMH non-zero correlation test)

5.1.4 Exercise test

Levosimendan did not affect exercise capacity in two performed studies. As to the duration of the exercise test, in **study III** there was no significant difference between the treatments (p=0.09). In **study IV**, the 6-minute walk test did not show differences compared with the baseline between the groups after the first (p=0.81) or the second treatment period (p=0.33), even though improvement was highest in the high-dose levosimendan group.

5.2 Pharmacokinetics

In **study II**, the mean ± SEM levosimendan concentration in plasma at 30 minutes after start of the levosimendan 6-hour infusion was 12.8 ± 1.1 ng/ml and steady state concentrations of levosimendan were reached within 4 hours. At 4 hours the mean concentration was 42.6 ± 3.2 ng/ml. The half-life was approximately 1 hour. The drug was eliminated fast, the mean AUC_{last} was 230 ng·h/ml and it represented most of the total AUC extrapolated to infinity (236 ng·h/ml). At 30 minutes after administration of the levosimendan 2 mg oral capsule, levosimendan concentrations were detected in most patients. The mean ± SEM plasma concentration was 16.1 ± 3.4 ng/ml. The mean concentration increased up to 1 h postdose, being 22.1 ± 3.3 ng/ml. The mean AUC_{last} was 70.5 ng·h/ml and it represented most of the total AUC extrapolated to infinity (76.1 ng·h/ml). The bioavailability of oral levosimendan was 77 ± 5%.

In **study III**, the plasma concentrations of levosimendan and its metabolites were measured after each of the four 7- to 10-day treatment periods (Figure 9). The levosimendan concentration in plasma after 2 mg of levosimendan was 9.9 ± 4.5 ng/ml (mean ± SD) and it was 8.6 ± 2.7 ng/ml when levosimendan was administered concomitantly with 5 mg of felodipine. The mean plasma concentration of the metabolite OR-1855 was 3.0 ± 0.8 ng/ml when levosimendan was administered alone and 3.5 ± 0.8 ng/ml when levosimendan was administered concomitantly with felodipine. The plasma concentrations of the metabolite OR-1896 were 3.3 ± 0.8 ng/ml and 2.9 ± 0.6 ng/ml with levosimendan alone and together with felodipine, respectively.

In **study IV**, concentrations of both levosimendan and its metabolites were measured. Levosimendan pharmacokinetics was determined after the first 2 mg dose on the last day of a 14-day treatment period. The mean C_{max} for levosimendan was 38 ng/ml in patients receiving levosimendan 2 mg daily while it was about 25 ng/ml in those who received levosimendan 4-8 mg daily (Table 20). This difference was related to an exceptionally high plasma concentration in one patient (95.9 ng/ml) in the low-dose group. However, if this patient were excluded from the analysis, the mean C_{max} for this treatment period would be similar to that seen after the other periods. The mean t_{max} varied between 2.2-2.3 hours. No statistically significant difference was found in levosimendan pharmacokinetics between the medication periods and daily dose.

Table 20. Pharmacokinetic parameters of levosimendan on Day 14 after multiple dosing of 2 mg oral levosimendan (one dosing interval) in study IV (mean ± SEM).

	Low-dose (n=8)			High-dose (n=8)		
	2mgx1	2mgx3	p-value*	2 mgx2	2mgx4	p-value*
C_{max} (ng/ml)	38 ± 10*	25 ± 7	0.31	25 ± 6	25 ± 5	0.98
t_{max} (h)	2.2 ± 0.3	2.3 ± 0.2	0.99	2.3 ± 0.3	2.3 ± 0.3	0.98
AUC_{last} (ng·h/ml)	91 ± 24	57 ± 17	0.31	56 ± 13	56 ± 11	0.98

* P-values show the relationships between the dose and $C_{max,}$ t_{max} or AUC_{last}
C_{max} - peak concentration
t_{max} – time of maximum concentration
AUC - area under curve

On day 14 the mean OR-1855 concentration in plasma was about 5 ng/ml in patients who received levosimendan 2 mg daily and about 15 ng/ml in the same patients when the dose was increased to 6 mg daily. The mean OR-1855 concentration in patients who received 4 mg daily was about 8 ng/ml and 17 ng/ml in the same patients when the dose was increased to 8 mg daily. The mean OR-1896 concentration on Day 14 was about 6 ng/ml after 2 mg levosimendan daily and about 17 ng/ml when the daily dose was increased to 6 mg. The mean OR-1896 concentration was about 9 ng/ml after levosimendan 4 mg daily and 17 ng/ml in the same patients when they received 8 mg daily. The mean C_{max} and AUC of both metabolites increased linearly within the patient groups (Table 21, Figure 13).

Table 21. Pharmacokinetic parameters of levosimendan metabolites OR-1855 and OR-1896 after multiple dosing of 2 mg oral levosimendan in study IV (mean ± SEM).

	Low-dose (n=9)		High-dose (n=7)		p-value*
	2mgx1	2mgx3	2mgx2	2mgx4	
OR-1855 C_{max} (ng/ml)	5.2 ± 0.9*	15.3 ± 2.6	7.7 ± 1.6	17.1 ± 4.5	0.0006
OR-1855 $AUC_{0\text{-}8h}$ (ng·h/ml)	40 ± 8	113 ± 20	59 ± 13	120 ± 32	0.002
OR-1896 C_{max} (ng/ml)	6.1 ± 1.4	16.9 ± 4.4	8.9 ±1.9	17.4 ± 2.3	0.0009
OR-1896 $AUC_{0\text{-}8h}$ (ng·h/ml)	47 ± 11	126 ± 34	65 ± 13	128 ± 18	0.001

* P-values show the relationship between the dose and C_{max} or $AUC_{0\text{-}8h.}$
C_{max} - peak concentration
AUC - area under curve

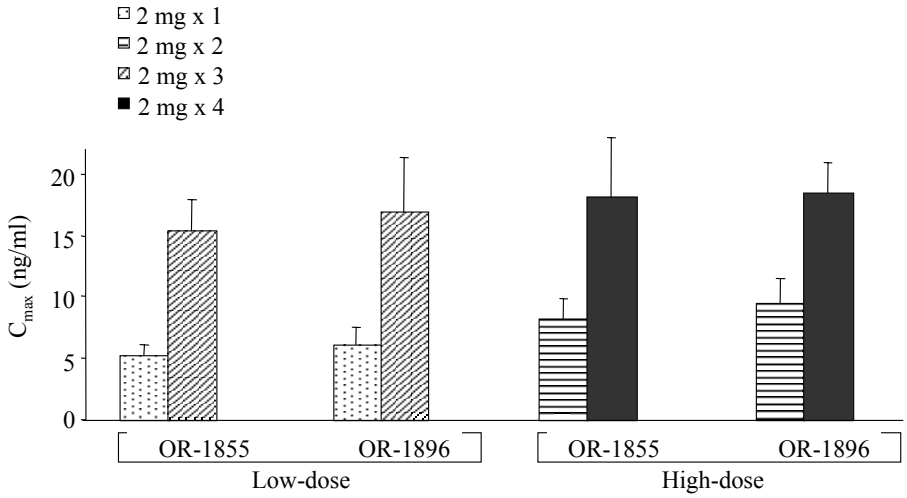


Figure 13. Plasma levels of levosimendan metabolites OR-1855 and OR-1896 in different dose groups after 14 days of treatment with oral levosimendan.

5.3 Safety

*Hypotension and ischaemia in **study I***

In **study I**, the primary variable was hypotension or ischaemia of clinical significance. Thus, it was primarily a safety study and a dedicated Safety Committee evaluated all cases of hypotension or ischaemia in a blinded fashion.

Of the 504 randomized patients, 65 (12.9%) were deemed by the Safety Committee to have experienced ischaemic or hypotensive events that were "clinically significant". The distribution of these 65 patients among the five treatment groups is shown in Table 22.

Table 22. Incidence of clinically significant hypotension and ischaemia after administration of intravenous levosimendan in study I

	Placebo (n=102)	LS 6 µg/kg + 0.1 µg/kg/min (n=103)	LS 12 µg/kg + 0.2 µg/kg/min (n=100)	LS 24 µg/kg + 0.2 µg/kg/min (n=99)	LS 24 µg/kg + 0.4 µg/kg/min (n=100)
Hypotension only	5 (4.9%)	7 (6.8%)	4 (4.0%)	5 (5.1%)	9 (9.0%)
Ischaemia only	4 (3.9%)	0 (0.0%)	7 (7.0%)	5 (5.1%)	8 (8.0%)
Hypotension and ischaemia	2 (2.0%)	4 (3.9%)	1 (1.0%)	2 (2.0%)	2 (2.0%)
Hypotension and/or ischaemia	11 (10.8%)	11 (10.7%)	12 (12.0%)	12 (12.1%)	19 (19.0%)

Overall, there were no significant differences among the five treatment groups in the proportion of patients that experienced the primary endpoint (p=0.32). When all four levosimendan groups were combined and compared with placebo, the proportions of patients who experienced clinically significant hypotension and/or ischaemia in the placebo and levosimendan groups were similar (10.8% vs 13.4%, respectively, p=0.46). There was, however, a weak relationship between the dose of levosimendan and the risk of hypotension and/or ischaemia (p=0.05), which was attributable to a higher frequency (19.0%) of ischaemia and hypotension among

patients who received the highest levosimendan infusion rate (24 µg/kg + 0.4 µg/kg/min), since the number of events in patients in other levosimendan groups were nearly identical to the number of events in patients receiving placebo.

Adverse events

In **study I**, adverse events were reported during the study infusion in 17.6% of the patients assigned to placebo and 23.4% of the patients assigned to levosimendan (p=0.23). The distribution of the most common adverse events (other than hypotension, ischaemia and worsening heart failure) is presented in Table 23. The highest frequency of arrhythmic disorders with levosimendan was seen with the infusion rate 0.4 µg/kg/min and the difference between treatment groups regarding sinus tachycardia was statistically significant. The incidence of myocardial ruptures was the highest among patients receiving placebo.

Table 23. Adverse events with a frequency ≥ 1% during the 6-hour study drug infusion in study I

Event	Placebo	LS 6 µg/kg + 0.1 µg/kg/min	LS 12 µg/kg + 0.2 µg/kg/min	LS 24 µg/kg + 0.2 µg/kg/min	LS 24 µg/kg + 0.4 µg/kg/min	p-value
Ventricular extrasystoles	1 (1.0%)	3 (2.9%)	1 (1.0%)	4 (4.0%)	6 (6.0%)	0.19
Atrial fibrillation	2 (2.0%)	1 (1.0%)	4 (4.0%)	3 (3.0%)	3 (3.0%)	0.65
Other atrial arrhythmia	1 (1.0%)	1 (1.0%)	1 (1.0%)	1 (1.0%)	2 (2.0%)	0.95
Sinus tachycardia	2 (2.0%)	0 (0.0%)	0 (0.0%)	3 (3.0%)	5 (5.0%)	0.03
Headache	1 (1.0%)	2 (1.9%)	3 (3.0%)	1 (1.0%)	1 (1.0%)	0.79
Agitation	2 (2.0%)	2 (1.9%)	1 (1.0%)	2 (2.0%)	0 (0.0%)	0.73
Hypertension	1 (1.0%)	1 (1.0%)	3 (3.0%)	0 (0.0%)	0 (0.0%)	0.20
Nausea	0 (0.0%)	1 (1.0%)	1 (1.0%)	2 (2.0%)	1 (1.0%)	0.61
Myocardial rupture	4 (3.9%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.03

In **study II**, five patients out of 29 experienced adverse events during the whole course of the study. The most common adverse events were hypotension and ventricular extrasystoles. None of those adverse events led to premature discontinuation of the study drug infusion. One patient died suddenly four days after receiving a 2 mg single oral dose. One patient was hospitalised due to suspected increased blood digoxin level, but no confirmatory blood test was performed.

In **study III**, levosimendan was well tolerated and the adverse events reported during the study were considered to be either mild or moderate. One patient experienced dizziness and one patient reported headache. No adverse events were reported during the concomitant administration of levosimendan and felodipine. One serious adverse event led to premature discontinuation of the study in one patient receiving felodipine and placebo. The patient was hospitalised with anginal pain, hypotension and ventricular extrasystoles. The event resolved and no myocardial injury was detected.

In **study IV**, five patients out of 25 experienced adverse events during the study. All events were seen among patients receiving different doses of levosimendan. One patient experienced

atrial fibrillation when receiving the 6 mg daily dose of levosimendan. Another patient, receiving levosimendan 6 mg daily, discontinued the study due to sinus tachycardia and angina pectoris. However, no rise of cardiac enzymes was seen and no myocardial injury was detected.

Safety laboratory parameters

In **study I** serum CK-MB was measured at baseline and at the end of study infusion. There were no significant differences between the five treatment groups in the change in CK-MB at 6 hours (p=0.09). In **studies II-IV** there were only minor changes in the laboratory parameters. The only notable changes were small decreases in serum potassium and blood haemoglobin, hematocrit and leukocytes with levosimendan.

Holter monitoring

24-hour ECG recording (Holter) was carried out in **studies II-IV**. In **study II**, during and after the 6-hour infusion some increase in the number of ventricular extrasystoles per hour in comparison with screening was observed (74 vs 104), except after intake of the 2 mg oral capsule (74 vs 64). Number of runs + VTs per 24 hours decreased compared with baseline both after infusion and after intake of the 2 mg capsule (6 vs 2 and 3, respectively). In **study III** the incidence of clinically significant arrhythmias was small and no differences regarding the incidence of arrhythmias were observed between treatments, neither was there any significant difference between treatments regarding the number or total duration of episodes of ST-segment changes (p=0.52 and p=0.48, respectively). In **study IV**, there were no differences between treatment groups regarding arrhythmic disorders after either treatment period.

6. Discussion

6.1 Study population

The study populations in the four studies differed as to severity and acuteness of IHD. Study I enrolled patients with acute recent ischaemia and severe heart failure, whereas study III included patients with stable IHD and studies II and IV patients with stable heart failure of ischaemic origin. This is explained by the studies having different aims. While study I primarily evaluated the safety of the short-term infusion of levosimendan in the acute setting, studies II-IV evaluated mainly the pharmacodynamics and pharmacokinetics of the drug in the chronic setting.

Intravenous inotropic drugs are indicated in patients with peripheral hypoperfusion with or without pulmonary congestion or with oedema refractory to diuretics and vasodilators. As this condition often occurs due to acute ischaemia, especially after AMI, the highly symptomatic patient population in study I is relevant also in terms of clinical practice. In studies II and IV the patient population consisted of ischaemic NYHA class III-IV heart failure patients. This patient population has been considered to gain the most benefit from treatment with positive inotropic drug as seen from the DIG trial, where the greatest effect was seen among class III-IV heart failure patients (371). The patients included in study III represent the largest group of IHD patients, for whom anti-ischaemic treatment is most commonly targeted. Therefore, one can conclude that the patient population included in these trials was clinically relevant for evaluation of the treatment effects of levosimendan.

The population of the studies consisted mainly of male patients, except the study I population, where almost half of the patients were women. This is noteworthy, since the patient population in most clinical studies consists predominantly of male patients. The patients in that study had also the worst prognosis, the severest clinical condition and highest incidence of different concomitant diseases such as hypertension and diabetes.

6.2 Background therapy

In all studies the study treatment was administered in addition to the patients' normal medication for IHD and heart failure. The use of different treatments reflects the severity of disease, but also the data that were available at time the studies were conducted. There are some important differences between the studies regarding concomitant treatment. For example, the frequency of use of ACE inhibitors and beta-blockers was quite low in study I (47% and 39%, respectively), despite the fact that they have been recommended for these patients by cardiology societies. One possible explanation for the low frequency of administration of beta-blockers is concomitant severe left ventricular failure in patients with AMI, which may have caused some reluctance to administer these drugs since no conclusive evidence about the usefulness of beta-blockers in patients with pulmonary congestion or oedema was available at the time the study was performed. It is also possible that the low frequency of use of ACE inhibitors was due to the relatively low blood pressure at baseline in certain patients. The use of thrombolytics in study I was lower than the use reported in epidemiological trials with patients from Western Europe (469, 470). It is interesting, however, that in study I these concomitant treatments were administered in rather similar frequency as in the USA (471, 472).

In studies II and IV ACE inhibitors were administered to almost all patients, but beta-blockers only to one third. Nitrates, ASA and calcium antagonists were administered in relatively similar frequency in all studies, except in study III, in which the administration of calcium antagonists

other than felodipine was excluded. Anticoagulants (heparin) were administered solely as treatment of AMI, in study I.

6.3 Haemodynamic effects

Haemodynamics was assessed in all studies using non-invasive methods. In addition to assessments used in everyday clinical practice – blood pressure and heart rate - also systolic time intervals were used for assessment of inotropic effect.

Blood pressure decreased after intravenous administration of levosimendan in similar fashion in studies I and II with the maintenance infusion rate of 0.2 μg/kg/min. Despite different baseline conditions the blood pressure response was rather similar in these two studies. The decrease in sBP at 6 hours was relatively similar (5 mmHg in study I and 7 mmHg in study II) and so was the incidence of hypotension (4.5% in study I and 6.8% in study II). The lower incidence of hypotension in study I may be related to the somewhat higher baseline values than in study II; on the other hand, the patients in study I were more unstable. It is noteworthy that the administration of the loading dose in study I did not cause an increase in the incidence of hypotension compared to study II, indicating that the administration of a 10-minute loading dose does not involve a safety risk, even in acute patients.

After administration of oral levosimendan the overall changes in blood pressure were small. The changes compared with placebo or baseline varied from a decrease of 3 mmHg to an increase of 5 mmHg. Study II provided the possibility to make a rather direct comparison with respect to change in blood pressure between the oral and intravenous formulations of levosimendan. Comparison of the decrease in sBP after intravenous and oral dosing revealed about two times greater decrease after intravenous administration, which was probably caused by the two times higher levosimendan plasma levels. Considering that the daily doses of oral levosimendan varied from 2 to 8 mg, it can be concluded that oral levosimendan administered in single doses up to 2 mg and daily doses up to 8 mg does not produce a clinically relevant change in blood pressure.

Levosimendan exhibited a chronotropic effect in all studies. After 6-hour infusion the increase in HR was approximately 2-4 bpm with a 0.2 μg/kg/min dose, but after the 0.4 μg/kg/min dose the increase in HR was 11 bpm, which may increase the risk of arrhythmic events. After administration of oral levosimendan an increase in HR was seen only after multiple dosing, but not after administration of a single dose, suggesting that the chronotropic effect of levosimendan at these doses is most probably connected with the accumulation of the active metabolite OR-1896. A similar observation has been made also in a previous study with 24-hour intravenous infusion (442). It seems that plasma concentrations of levosimendan higher than those achieved after a single oral dose of 2 mg are needed to reveal the chronotropic effect of levosimendan. Since the heart rate is the most significant easily measurable change in haemodynamics after administration of oral levosimendan, it can aid in establishing safe dose regimens.

Systolic time intervals, mainly heart rate-corrected electromechanical systole (QS2i), which has been considered fairly specific and load-independent variable, were used for assessment of the inotropic effects of levosimendan and its metabolites (473). The inotropic effect observed after 6-hour infusion of 0.2 μg/kg/min levosimendan was rather moderate. The most commonly studied daily dose of oral levosimendan was 2 mg, administered altogether to 60 patients. The shortening of QS2i in these studies varied from 6-10 ms, which is similar to the change seen after 6-hour infusion. The concentration-effect loop curves for QS2i (inotropy) and blood pressure (vasodilation) showed counter-clockwise hysteresis, meaning that the time of the peak

response lagged behind the time of the peak drug concentration. This can be explained by the time the drug requires to distribute to its cardiac site of action (equilibration delay). These loop curves also revealed that the vasodilatory effect appears before inotropy, which is important from the clinical perspective.

The inotropic response was rather similar when levosimendan was administered concomitantly with felodipine as it was with levosimendan alone, indicating that the administration of a calcium antagonist does not abolish the calcium sensitizing effect.

While study II concentrated on the inotropic effect of just levosimendan, the inotropic effects of its metabolites also were assessed in study IV. The different daily doses in study IV produced linearly increasing metabolite levels, but the effects of the different metabolite levels on the index of inotropy were not so clear-cut. For example, the result for the 2 mg dose was similar after it was administered as a single dose and after longer administration, where the contribution of the active metabolite could be expected. It seems that the metabolite concentration of levosimendan after the 2 mg daily dose did not reach a level that would have produced significant additional inotropic effect. In favor of such an explanation speaks also the lack of carry-over effects as regards inotropy in study III.

When daily doses higher than 2 mg were administered, the total inotropic effect increased, but no dose response was observed, as shown by the effect being more pronounced with the 4 mg than with the 6 mg daily dose and by QS2i not shortening significantly further in the second 14-day administration period from that seen after the first 14-day administration. The evident lack of dose response may have occurred due to the small number of patients in the treatment group, but it is also possible that some form of tolerance to the inotropic action of the metabolite is developed. The latter consideration needs further clarification, as the metabolite levels were significantly higher after the second than after the first period and should have resulted in higher inotropic responses.

6.4 Symptoms

The assessment of symptomatic improvement is always challenging and easily open to criticism. Indeed there is very little well-controlled data in acute ischaemic patients in that respect. One reason is probably the absence of validated and well-recognised methods. Also the severity of clinical condition complicates the assessment, since, although the studied drug itself may have a real effect in relieving symptoms, quite a large proportion of severely ill patients receiving placebo may also improve as they will almost always receive other haemodynamically active treatments together with the study treatment (474). Therefore, the proper evaluation of symptoms should take into account several aspects of the clinical course of the patients, such as newly administered concomitant treatments, important clinical events and deaths.

In study I the “worst-rank” assessment method was applied for evaluation of dyspnoea and fatigue and it showed that patients in the placebo group worsened more often than patients in the levosimendan group. One important contribution to this result, in addition to mortality, was also a significant reduction in the need for additional heart failure therapy, as would be expected for an effective therapy for the failing heart. It is important that this result was observed in a situation in which haemodynamics was not invasively monitored, i.e. investigators had no knowledge of the cardiac output or filling pressures at the time of the assessments. Indeed, in the recent VMAC study with nesiritide, an improvement in dyspnoea was observed only in patients who were invasively monitored, but not in those who were not,

indicating that physician's knowledge of haemodynamics might affect the symptom evaluation of the patient (475).

6.5 Morbidity and mortality

An endpoint combining morbidity and mortality - "death and worsening heart failure" – was used in study I. This endpoint combines the most important clinical events that may occur in this patient population. The difference in favor of levosimendan was seen early, already during the 6-hour infusion period and also 24 hours after randomization. The positive effect of levosimendan in this endpoint is remarkable, since no other intravenous inotropic drug has ever achieved a similar result in any clinical study.

The most striking result of the study was the lower all-cause mortality among patients receiving levosimendan. The difference between levosimendan and placebo was seen already at 6 hours and the benefit continued up to 180 days after infusion. From Kaplan-Meier curves it is evident that the risk reduction attributable to levosimendan was actually achieved during the first 14 days of follow-up. The Kaplan-Meier curves are parallel after 14 days indicating no further additional survival benefit after that time.

There are many possible mechanisms how levosimendan could improve the prognosis of acute ischaemic patients. First, the cardioprotective effect may be related to the potassium-channel opening mechanism, for example *via* coronarodilation and possibly *via* the ischaemic preconditioning mechanism. K^{+}_{ATP} openers are agents that induce or enhance ischaemic preconditioning and may thereby decrease myocardial injury and cell death (96, 423). Levosimendan has also been shown to improve the function of the stunned myocardium in acute myocardial ischaemia (458). A recent study has shown that levosimendan protects the myocardium from reactive oxygen species-mediated apoptosis, which can also explain the observed beneficial effects of levosimendan (476). This is probably related to the calcium sensitising effect of the drug as a decreased sensitivity of the myofibrils to calcium appears to be responsible for the contractile depression of the myocardium. An intriguing observation was the decrease in the incidence of myocardial ruptures among levosimendan-treated patients. This result can be explained by a recent result of a clinical study, where levosimendan decreased myocardial wall stress, which can contribute to the reduction of frequency of myocardial rupture among patients with AMI (423). A similar result has been observed in a previous beta-blocker trial (166).

The mortality results should also be considered in the context of pharmacokinetics. One previous study evaluating the effects of a 24-hour infusion of levosimendan, at the rate 0.2 μg/kg/min, showed that levosimendan metabolites were first detected in plasma approximately at the end of infusion and were seen in plasma during the next 10-14 days (442). After the 6-hour infusion in study I the administered amount of levosimendan would be four times lower than after 24-hour infusion, and therefore one could predict that also the metabolite concentration would be four times lower, but the accumulation-elimination curve would be similar to that seen after 24-hour infusion. This indicates that the positive long-term effect of levosimendan regarding mortality is probably due to the positive effect of its metabolite(s), whereas the short-term benefit may be related to the cardioprotective effect of the parent drug. The active metabolite OR-1896 has been shown to be a calcium sensitizer, but no information about its effects on potassium channels is available. This indicates that a calcium sensitisation effect *per se* may be involved in the improvement of prognosis. The effect of intravenous levosimendan should therefore be viewed as a dual effect: the short-term effect of levosimendan and the long-term effect of the metabolite(s).

When evaluating the mortality results in study I, one should take into account that the study was not prospectively designed and powered to show a difference in mortality. Two currently ongoing large randomized prospective trials in patients experiencing severe decompensated heart failure should conclusively show whether levosimendan has a positive effect on the short- and long-term outcomes of these patients (454, 455).

6.6 Safety

Levosimendan was rather well-tolerated in all studies. A higher frequency of hypotension, ischaemia and also arrhythmic events were seen in comparison with placebo only with the highest, 0.4 μg/kg/min infusion rate.

In studies with oral levosimendan the frequency of adverse events was small and did not differ from that seen in comparative treatment groups. It seems, however, that daily doses higher than 4 mg may not be well tolerated during long-term administration, mainly due to the increase in heart rate.

No significant changes were seen in most laboratory values. Oral levosimendan decreased haemoglobin, hematocrit and leukocytes after approximately 1-month treatment by 7-9%. The exact mechanism of these changes is unknown. Decrease in serum potassium was similar to that seen in earlier studies.

24-hour ECG recording (Holter) was carried out in studies II-IV. Some beneficial anti-ischaemic effects were seen in study III, since the Holter recordings during the administration of levosimendan showed reduction of both duration and number of ST-depression and -elevation episodes compared with placebo and the effect was similar to that seen with felodipine. In study II there were no changes in frequency of malignant arrhythmic events compared with baseline after either the infusion or the single oral administration. No differences between treatment groups were observed in study IV. However, taking into account the heart rate increase after daily doses of 6 and 8 mg it is still possible that with these doses also the frequency arrhythmic events would have increased, had the study treatment groups included more patients. Indeed, the increased frequency of ventricular arrhythmias has been considered one of the contributing factors to increased mortality with inotropic agents (477). Therefore, one can conclude that daily doses of oral levosimendan beyond 4 mg are probably too high for clinical use.

6.7 Feasibility of levosimendan in long-term treatment

The feasibility of the long-term administration of levosimendan revolves around its complex pharmacokinetics and the long-lasting haemodynamic effects resulting from its metabolites. The key issue in terms of the clinical use of the drug seems to be the reaching of such metabolite plasma levels that would be clinically effective, but also safe in the long term. Study I showed indirectly that the plasma level of levosimendan achieved after a 6-hour infusion would be safe in long term as all doses decreased the incidence of worsening heart failure and mortality compared with placebo. However, due to increased incidence of hypotension and ischaemia during infusion (short-term safety) with the infusion rate 0.4 μg/kg/min, this rate is not suitable for therapeutic use in patients with acute ischaemia. The results showed indirectly also the favorable safety profile of the metabolite levels after all doses of levosimendan.

As intravenous levosimendan was administered only once and for only 6 hours, the possible effects of repeated and longer administration of levosimendan are not known. Of special interest would be the situation in which a new levosimendan infusion would be administered

within 14 days of the previous infusion, i.e. on top of the existing pharmacodynamic effects of the metabolites. Knowledge about the effects of intermittent administration would have a great importance, since acute patients may often need repeated administration of the drug due to severely compromised haemodynamics. Such a study including a re-administration component in its design will hopefully provide information about this issue (455).

The clinical effects of also oral levosimendan are closely related to the pharmacokinetics of the drug, and specifically to the effects of the metabolites. Taking into account the half-life of the metabolites (70-80 h), it can be considered that steady-state plasma concentrations of metabolites will be achieved after approximately 14 days of treatment. Since single doses of 1 or 2 mg have been shown to produce significant haemodynamic and inotropic effects, the appropriate daily dose for long-term administration is closely related to the metabolite level which is achieved when these doses are administered long-term. To find the dose which is effective and safe during long-term administration is of crucial importance, since the long half-life of the metabolites makes their effects (also possible adverse ones) long-lasting. It seems that administration of oral levosimendan at the dose of 2 mg was safe also on top of existing metabolite levels and did not cause unexpected adverse effects. At the same time it was revealed that metabolite levels above 9 ng/ml have a significant chronotropic effect. Taking this into account, one can consider that the administration of approximately 2 mg once or twice daily as a single dose of levosimendan may be optimal for both efficacy and safety. In any case, the effects of levosimendan after long-term oral administration need further clarification.

7. Limitations of the studies

The 14-day mortality follow-up in study I was rather short with respect to safety. Although mortality was studied, adverse events were not comprehensively followed during the follow-up period. Also data about concomitant medication administered during the follow-up would have added value to the observed mortality result. In addition, the sample size of the trial was too small to provide conclusive evidence about the morbidity/mortality effects of intravenous levosimendan in the studied population.

Study II was an open label study without a control group. Although a placebo group was not necessary for the evaluation of PK-PD interrelationships of levosimendan, it would have had value for the evaluation of safety. The PK-PD interrelationships of levosimendan, but not its metabolites' were assessed. The latter would have substantially increased the value of the current results.

Study III was carried out using a cross-over design. In spite of the fact that a cross-over design is rather often used in interaction trials, the decision to use it in a trial with a drug having long-lasting metabolites was not the optimal one. Although no carry-over effects were seen, the design could have been improved by adding wash-out periods after each treatment period. A 14-day period (instead of 7-10 days) would have been more optimal, since the steady-state of metabolites would have been reached by day 14.

In study IV the two-week treatment periods were unfortunately too short to see any improvements regarding exercise capacity. However, the study provided important information regarding PK-PD relationships of different doses, which is needed for dose selection in long-term administration.

8. Summary and conclusions

The purpose of the present study was to investigate the effects of levosimendan in patients with ischaemic heart disease with or without concomitant heart failure. Intravenous levosimendan was studied in patients with acute or chronic ischemia, complicated by severe heart failure. In addition the study provided information about the effects of long-term oral administration of levosimendan in chronic ischaemia and ischaemic heart failure settings. Altogether 557 patients were studied in this thesis, 504 of them experiencing acute and 53 chronic ischaemia.

The main conclusions of the study:

1) Levosimendan, administered as 6-hour intravenous infusion in doses of 0.1 or 0.2 μg/kg/min, did not increase clinically significant ischaemia or hypotension in patients with AMI complicated by severe left ventricular failure. Levosimendan decreased also the incidence of worsening heart failure and reduced both short- and long-term mortality. The infusion rate 0.4 μg/kg/min was associated with an increased risk of clinically significant hypotension and ischaemia and is therefore not suitable for use in acute ischaemic patients.

2) The inotropic and vasodilatory effects of oral levosimendan resembled those seen after intravenous administration. These pharmacodynamic effects appeared almost simultaneously, although the vasodilatory effect was seen earlier after intravenous than after oral administration. Chronotropic effects of intravenous levosimendan in doses of 0.1 or 0.2 μg/kg/min were small, whereas the dose 0.4 μg/kg/min had significant chronotropic effects and increased also the frequency of arrhythmic events.

3) Oral levosimendan administered in doses of 2 or 4 mg daily was well tolerated and had moderate inotropic and chronotropic effects in patients with ischaemia or ischaemic heart failure. The results indicate that the inotropic effect of the calcium sensitizer is not attenuated by ischaemia or treatment with a calcium antagonist. The doses beyond 4 mg daily possessed significant chronotropic effects and are therefore not suitable for clinical use. The vasodilatory effects of oral levosimendan after long-term administration were small. Oral levosimendan did not increase exercise capacity in ischaemic patients whether they had concomitant heart failure or not. The overall effect of the drug on ischaemia is neutral.

4) Pharmacokinetics of oral levosimendan is independent of the dosing interval. The formation of levosimendan metabolites increases linearly with the daily dose. The chronotropic effects after long-term administration of daily doses beyond 4 mg are related to the formation of the metabolites.

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