Nitrates and Left Ventricular Dysfunction

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This dissertation is dedicated to my parents,
my wife Eva, and my son Hjalmar

Papers

This dissertation is based on the following papers, which are referred to in the text by their Roman numerals (I-IV):

Paper I Tingberg E, Roijer A, Thilén U, Eskilsson J, Öhlin H. Randomized, double-blind, placebo-controlled long-term study of isosorbide-5-mononitrate therapy in patients with left ventricular dysfunction after acute myocardial infarction. Am Heart J. 2003;145(1):E1.

Paper II Tingberg E, Öhlin AK, Gottsäter A, Öhlin H. Lipid peroxidation is not increased in heart failure patients on modern pharmacological therapy. Int J Cardiol. 2006;112(3):275-81.

Paper III Tingberg E, Roijer A, Thilén U, Öhlin H. Neurohumoral changes in patients with left ventricular dysfunction following acute myocardial infarction and the effect of nitrate therapy: a randomized, double-blind, placebo-controlled long-term study. J Cardiovasc Pharmacol. 2006;48:166-72.

Paper IV Tingberg E, Roijer A, Thilén U, Öhlin H. Effects of isosorbide-5-mononitrate therapy on diastolic function in patients with left ventricular dysfunction after acute myocardial infarction. Submitted.

Abstract

Use of β-blockers and inhibitors of the renin-angiotensin system (RAS) is established practice in the treatment of chronic heart failure (CHF).
Clinical studies also suggest beneficial long-term effects of nitrates in CHF. In the Veterans Administration Heart Failure Trial (V-HeFT) I, the combined use of a nitrate and hydralazine reduced the mortality rate. However, it is not possible to determine the relative importance of nitrate therapy in that study. The main objective of this thesis was to study the long-term effects of nitrate therapy in patients with evidence of left ventricular (LV) dysfunction after acute myocardial infarction (AMI) already treated with standard heart failure (HF) therapy.

Paper I. In a randomized, double-blind, placebo-controlled trial, we evaluated the effects of a 60 mg dose of isosorbide-5-mononitrate (IS-5-MN) given daily to 47 patients with clinical or echocardiographic evidence of LV dysfunction after AMI. Forty-five patients received a placebo. No significant haemodynamic effects of IS-5-MN therapy were observed. We found that nitrate therapy resulted in lower plasma atrial natriuretic peptide (P-ANP) levels and reduced the need for additional diuretics. Less LV dilatation was observed in patients with more severe LV dysfunction at baseline, indicating clinically beneficial effects, especially in patients with more reduced LV function after AMI.

Paper II. The main finding of this study was that lipid peroxidation measured by plasma malondialdehyde (MDA) and 8-isoprostane were not increased in patients with LV dysfunction treated with standard HF therapy. No positive correlations to the markers of severity of HF were found. Long-term IS-5-MN therapy did not influence P-MDA concentrations. Results from many studies suggest that oxidative stress is increased in HF, but this may not be true for patients treated with β-blockers and inhibitors of the RAS.

Paper III. Chronic nitrate therapy did not significantly affect the neurohumoral status in patients with LV dysfunction after AMI, apart from a decrease in P-ANP. Some hormones were seen to be more closely associated with diastolic dysfunction/increased volume load, viz ANP and brain natriuretic peptide (BNP),

while others were found to be more closely associated with systolic dysfunction, namely plasma renin activity (PRA), norepinephrine (NEPI) and aldosterone (Aldo). A temporal dissociation between these two groups of hormones occurred 1 year after the infarction: ANP and BNP decreased, while NEPI and Aldo showed slight increases.

Paper IV. Isosorbide-5-mononitrate treatment and diastolic function were evaluated in patients with LV dysfunction after AMI. Chronic nitrate therapy did not significantly affect diastolic function evaluated by echocardiography. Changes in diastolic parameters during 1 year after AMI indicated improved diastolic function and/or reduced pre-load. Fewer factors indicating worse prognosis were demonstrated in patients with normal or slightly reduced LV ejection fraction (LVEF) 1 month after AMI, compared with patients with moderately or severely depressed LVEF.

Key words

acute myocardial infarction, heart failure, left ventricular dysfunction, nitrates, haemodynamic measurements, echocardiography, diuretics, atrial natriuretic peptide, brain natriuretic peptide, lipid peroxidation, malondialdehyde, isoprostanes, oxidative stress, neurohumoral activation, diastolic dysfunction

Abbreviations

A	late peak transmitral flow velocity
ACE	angiotensin-converting enzyme
ADH	anti-diuretic hormone
ADH-LI	anti-diuretic hormone-like immunoreactivity
A-HeFT	African-American Heart Failure Trial
AIRE	Acute Infarction Ramipril Efficacy (study)
Aldo	aldosterone
AMI	acute myocardial infarction
ANCOVA	analysis of covariance
ANP	atrial natriuretic peptide
AT_1	angiotensin II receptor
BNP	brain natriuretic peptide
BSA	body-surface area
CAD	coronary artery disease
CCU	coronary care unit
CGRP	calcitonin gene-related peptide
CGRP-LI	calcitonin gene-related peptide-like immunoreactivity
CHF	chronic heart failure
CI	cardiac index
CK	creatine kinase
CK-MB	creatine kinase-muscle-brain (iso-enzyme)
CO	cardiac output
CONSENSUS	Cooperative North Scandinavian Enalapril Survival Study
D	dimension
DIG	Digitalis Investigators Group
dPV	peak pulmonary venous flow velocity during ventricular diastole

DT	deceleration time of E wave
E	early peak transmitral flow velocity
E/A	ratio between early and late mitral peak velocity
ECG	electrocardiogram
EDTA	ethylenediamine tetra-acetic acid
EDV	end-diastolic volume
EDVI	end-diastolic volume index
EDWS	end-diastolic wall stress
EF	ejection fraction
EPHESUS	Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study
EPI	epinephrine
ESC	European Society of Cardiology
ESV	end-systolic volume
ESVI	end-systolic volume index
FeC12	liquid ferrous chloride
GISSI	Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto micoardico
GMP	guanosine monophosphate
HF	heart failure
Hg	mercury
HPLC	high-performance liquid chromatography
HR	heart rate
HT	hypertension
IHD	ischaemic heart disease
IQR	interquartile range
IRT	isovolumetric relaxation time

ISDN	isosorbide dinitrate
IS-5-MN	isosorbide-5-mononitrate
ISIS	International Study of Infarct Survival
IVSd	interventricular septal thickness in diastole
IVST	interventricular septal thickness
LA	left atrial
LPWDd	left posterior wall diameter in diastole
LV	left ventricular
LVEDP	left ventricular end-diastolic pressure
LVEDV	left ventricular end-diastolic volume
LVEF	left ventricular ejection fraction
LVESP	left ventricular end-systolic pressure
LVFS	left ventricular fractional shortening
LVID	left ventricular internal dimension
LVIDd	left ventricular internal diameter in diastole
LV-mass	left ventricular mass
LVIDs	left ventricular internal diameter in systole
MAP	mean arterial pressure
MDA	malondialdehyde
MPAP	mean pulmonary arterial pressure
NADPH	nicotinamide adenine dinucleotide phosphate
NEPI	norepinephrine
NICE	Nitrates in Congestive Heart Failure study
NO	nitric oxide
NOS	nitric oxide synthase
NPY	neuropeptide Y
NPY-LI	neuropeptide Y- like immunoreactivity
NYHA	New York Heart Association

PAWP	pulmonary artery wedge pressure
PGF2	prostaglandin F2 alpha
PRA	plasma renin activity
PTCA	percutan transluminal angioplasty
PVR	pulmonary vascular resistance
PWT	posterior wall thickness
RALES	Randomized Aldactone Evaluation Study
RAP	right atrial pressure
RAS	renin-angiotensin system
r_s	Spearman´s rank correlation coefficient
RT	routine (blood) test
RV	right ventricular
SAVE	Survival and Ventricular Enlargement (trial)
SD	standard deviation
SH	sulfhydryl (group)
SI	stroke (volume) index
SOLVD	Studies of Left Ventricular Dysfunction trial
SP	substance P
SP-LI	substance P-like immunoreactivity
sPV	peak pulmonary venous flow velocity during ventricular systole
SVR	systemic vascular resistance
TBARS	thiobarbituric acid-reactive substance
trop T	troponin T
V-HeFT	Veterans Administration Heart Failure Trial
VIP	vasoactive intestinal peptide
VIP-LI	vasoactive intestinal peptide-like immunoreactivity

Contents

1 Introduction

1.1 Heart failure overview.

Chronic heart failure (CHF) can be defined as a state in which cardiac output (CO) fails to meet the metabolic needs of the body at a normal filling pressure. It is a manifestation or complication of many types of diseases and is caused by a compromise of either systolic or diastolic function, or both. In the early stages, haemodynamic abnormalities may be seen only during exercise, but as the disease becomes increasingly severe they will appear even at rest.

The predominant causes of CHF are coronary artery disease (CAD) and hypertension (HT).

At least 30 % of patients with CHF have preserved systolic function in the absence of significant valvular heart disease [1]. These patients are generally old and various studies have suggested that diastolic CHF may account for more than 50% of the hospitalizations for heart failure (HF) [2]. Patients with diastolic HF often have HT, diabetes or atrial fibrillation and are frequently female [3]. The mortality rates and the rates of readmission for HF in this population group with preserved ejection fraction (EF) appear to be similar to those with reduced EF [4, 5].

Left ventricular (LV) systolic function after acute myocardial infarction (AMI) has been extensively studied but it has been increasingly apparent that LV diastolic dysfunction contributes to signs and symptoms of clinical HF with or without systolic dysfunction [6, 7, 8] and has important therapeutic and prognostic implications.

1.2 How to diagnose heart failure.

Symptoms and signs

The manifestations of CHF vary widely, depending on the location in the heart (left or right ventricle or both) and on whether the HF is acute or chronic. Common symptoms are dyspnoea, orthopnea, fatigue and nocturia. Acute LV failure usually presents with rapidly worsening fatigue. There may also be prodromal symptoms indicating an underlying aetiology. To establish a clinical diagnosis the Framingham criteria for the diagnosis of congestive HF can be used [9].

Electrocardiography

Electrocardiography may show signs of acute or previous AMI, ischaemic features or arrhythmia (tachycardia, atrial fibrillation). The QRS amplitude is also affected by ventricular mass and larger complexes may occur with a history of HT.

X-rays

Chest X-rays with a postero-anterior and lateral view of the chest may demonstrate an abnormal heart shadow with hypertrophy and enlargement of the heart chambers. Pulmonary congestion can often be seen when the left atrial (LA) pressures rises to 20-25 mmHg [10]. When fluid accumulates in the interlobular septa, horizontal Kerley B lines in the lateral lower lobes can be seen which reflect such fluid. In chronic LV failure, there may be right or left sided pleural effusion.

Echocardiography

Echocardiography, an operator-dependent technique, is today one of the most frequently used methods of diagnosing cardiac diseases. It was first used by Edler

and Hertz [11] in 1954 to record movements of cardiac structures. Two-dimensional sector scanning was developed in the mid-1970s and Doppler echocardiography, a method useful in the assessment of cardiac blood flow and pressure has been used in clinical practise since the late 1970s.

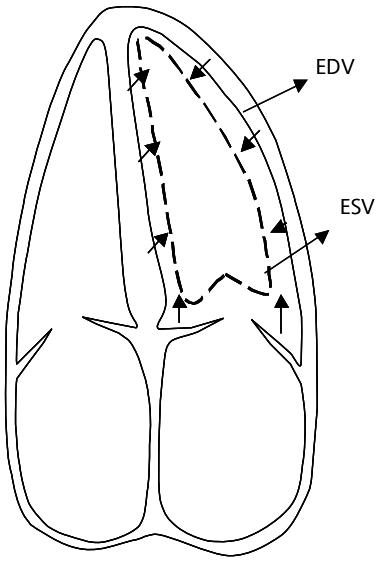

Figure 1.1. LVEF represents stroke volume as a percent of end-diastolic LV volume . Calculated according to: Stroke volume = EDV – ESV and EF = EDV-ESV/EDV x 100%.

Evaluation of ventricular systolic and diastolic function is an essential part of all echocardiographic examinations. Determination of global systolic function is based on changes in ventricular size and volume. The global systolic function of the LV can be expressed by the EF (Figure 1.1.). EF represents stroke volume as a percentage of end-diastolic (EDV) LV volume, and its determination requires measurement of LV volume, as follows:

Stroke volume = EDV – ESV and EF = EDV-ESV/EDV x 100%.

where ESV is the end-systolic volume. The volume of the LV is measured from the dimensions and area obtained from orthogonal apical views and then calculated by the modified Simpson method or disk summation method. The most commonly

used formulas to estimate LV mass are all variations of the same mathematical principle. The following equations provides a reasonable determination of LV mass in grams [12]:

$$\textbf{LV mass} = 1.04\ [(LVID + PWT + IVST)^3 - LVID^3]\ X\ 0.8 + 0.6$$

where LVID is the internal dimension, PWT the posterior wall, IVST the interventricular septal thickness, 1.04 the specific gravity of the myocardium and 0.8 the correction factor.

Another, frequently used formula is [13]:

$$\textbf{LVmass} = 1.04\ ([LVID+PWT+IVST]^3 - [LVID]^3) - 13.6\ g$$

where all measurements are performed in diastole. Dimensions and areas can be measured from the parasternal short or long-axis view. Diastolic function is assessed mainly by Doppler echocardiographic analysis of mitral inflow, tricuspid inflow, and pulmonary vein flow velocities [14]. Another method to assess the diastolic function is pulsed wave tissue Doppler imaging, where a sample volume is placed at the septal or lateral border of the mitral annulus in an apical four chamber view to record longitudinal velocities at the mitral annulus [15].

The LV filling pattern obtained by Doppler analysis of mitral inflow and pulmonary vein flow is often classified as “normal”, “impaired relaxation”, “pseudonormal” and “restrictive” (Figure 1.2.).

The rates of myocardial relaxation and compliance change with aging, which means that different diastolic filling patterns are expected for different age groups. In the young, myocardial relaxation is swift but with aging it decreases. An impaired relaxation pattern is often the initial abnormality of diastolic filling. Whenever the ratio between early (E) and late (A) peak transmitral flow velocity (E/A ratio) is < 0.75, there is often impaired relaxation. This pattern is often seen in LV hypertrophy or ischemia/infarction. As the diastolic function changes for the worse there is a transformation from a patter of impaired relaxation to one of

restrictive filling. A restrictive pattern is present when the LV compliance is decreased and the left atrial (LA) pressure is increased. An early rapid diastolic

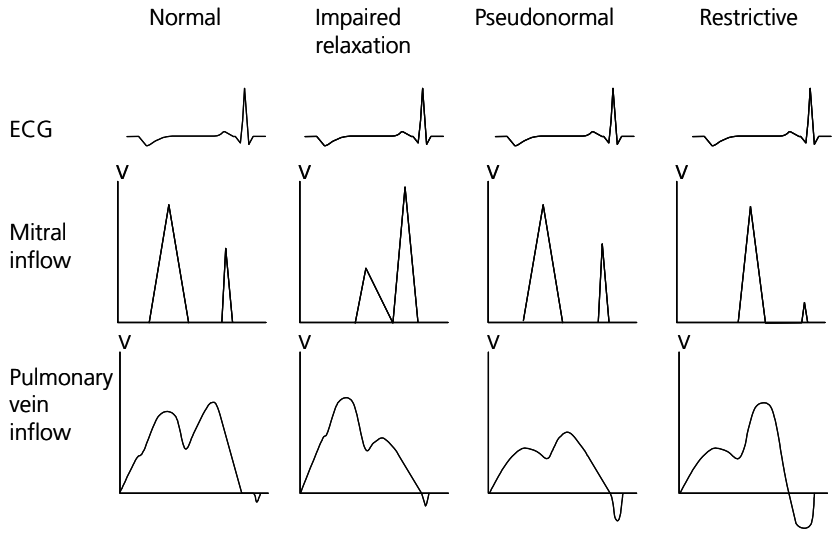

Figure 1.2. LV filling pattern obtained by Doppler analysis of mitral inflow and pulmonary vein flow is often classified as normal, impaired relaxation, pseudonormal and restrictive

filling into a non-compliant left ventricle due to a high LA-LV pressure gradient causes a rapid increase in early LV diastolic pressure, prematurely terminating the inflow.

A restrictive filling pattern is characterized by an E/A ratio greater than 2. However, myocardial relaxation continues to be impaired in patients with restrictive filling but is masked because of the rapid blood flow from left atrium to the left ventricle in early diastole. The so-called "pseudo-normal" pattern with an E/A ratio between 1 and 2 represents a moderate stage of diastolic dysfunction, with increased LV-filling pressure but not to the same extent as in restrictive dysfunction.

Is "pure" diastolic heart failure possible? In a study by Kawaguchi et al [16], LV pressure-volume relations were measured in HF patients with preserved EF and in age-matched controls. HF patients with preserved EF demonstrated a systolic-ventricular and arterial stiffening beyond that associated with aging and/or

hypertension. It has also been demonstrated that patients diagnosed with isolated diastolic dysfunction according to current guidelines, in fact have reduced longitudinal systolic function [17]. The authors concluded that tissue Doppler reveals the subtle and progressive changes that affect longitudinal systolic function.

Cardiac catheterization

Cardiac catheterization is the gold standard to assess ventricular function. Thermodilution as a method for measuring blood flow was introduced in 1954 [18]. Since the development of the flow-directed, balloon-tipped thermodilution catheter and solid-state computers to determine the CO, thermodilution has become the most widely used technique for measuring CO.

Bedside catheterization of the pulmonary artery was introduced in 1970 by Swan et al [19]. The positioning of an inflatable balloon at the tip of the catheter allows the catheter to be directed by blood flow, and vascular pressures distal to the balloon can be monitored. When the balloon occludes the pulmonary artery segment in which the catheter is placed, the so-called "pulmonary artery occlusion pressure" or "pulmonary artery wedge pressure" (PAWP) is obtained. When the catheter is equipped with a distal thermistor, determination of the CO by thermodilution technique is possible [20].

The position of the catheter tip is determined by the characteristic waveform pattern. Figure 1.3. shows a representative recording of pressures as a Swan-Ganz catheter is inserted through the right side of the heart into the pulmonary artery.
In 1976, Foresterr et al [21] defined four subsets of AMI (I-IV) based on measured hemodynamic parameters of ventricular function, PAWP and CO. Subset I consisted of patients without clinical or haemodynamic evidence of hypoperfusion or pulmonary congestion (mortality rate 3%) while subset II included patients with evidence of pulmonary congestion (PAWP > 18 mm Hg) without hypoperfusion (mortality rate 10%). Subset III consisted of patients with systemic hypoperfusion (cardiac index (CI) < 2.1 L/min/m^2) without pulmonary congestion (PAWP < 18 mmHg), mortality rate 23%, and subset IV consisted of patients with both

hypoperfusion (CI < 2.1 L/min/m^2) and pulmonary congestion (PAWP > 18 mm Hg) with an associated mortality rate of 60%. This marked relationship is however not valid for patients with CHF.

The PAWP reflects the relationship between intravascular volume and LV function. When the balloon is inflated, the blood flow in a distal segment of the pulmonary artery is occluded, creating a conduit through which LA pressure can be measured. The PAWP is a reliable index of the LA pressure even when the pulmonary vascular resistance (PVR) is elevated. In the absence of mitral valve disease, the LA pressure reflects the LV end-diastolic pressure (LVEDP).

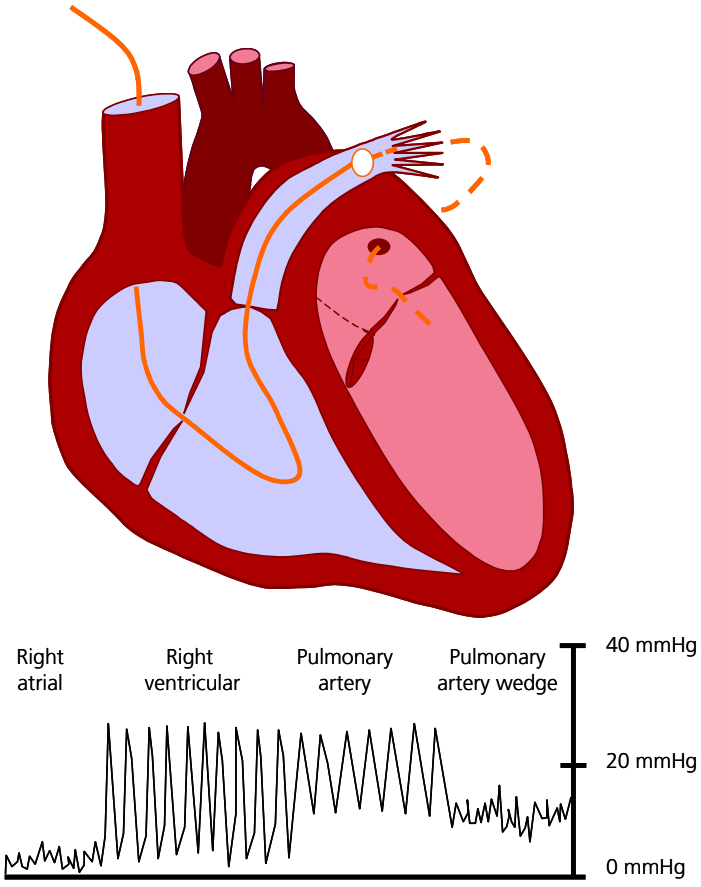

Figure 1.3. A representative recording of pressures as a Swan-Ganz catheter is inserted through the right side of the heart into the pulmonary artery.

1.3 Heart failure and neurohumoral activation

A number of neurohormones and neuro-endocrine systems may be activated in the failing heart. The haemodynamic balance may temporarily be restored by activation of the neuroendocrine system through vasoconstriction and retention of salt and water. However, this may also be harmful and further impair the function of the heart.

Two of the key systems involved are the renin-angiotensin system (RAS) and the sympathetic nervous system. The interaction between these systems is complex. Renin, synthesized in the kidneys, is increased in HF [22] but, is of no diagnostic or prognostic value in CHF because of the influence of medical treatment such as diuretics [23], angiotensin-converting enzyme (ACE) inhibitors [24], β-blockers [25], and angiotensin II blockers [24]. Elevated aldosterone is associated with a reduced EF [26] as well as with an increased mortality [27] in patients with chronic systolic HF but is also influenced by medical treatment. It is increased by activation of the RAS and consequently inhibitors of that system reduce the levels of the hormone.

Catecholamines have a number of adverse effects related to HF. Numerous studies have demonstrated that norepinephrine (NEPI) is increased in HF [28, 29] and that elevated levels indicate a worse prognosis [30]. Studies have also shown that NEPI reflects the sympathetic activity in this category of patients [31]. Anti-diuretic hormone (ADH), synthesized in the hypothalamus, is also increased in HF where it contributes to vasoconstriction and water retention [32]. The prognostic value of ADH is unclear [33].

Increased plasma levels of atrial and brain natriuretic peptides (ANP and BNP) are found in patients with CHF and are important prognostic predictors [34]. Earlier data have shown that vasodilator treatment can be titrated to reduce plasma BNP concentrations towards the normal [35]. Troughton et al [36] demonstrated that therapy guided by BNP levels reduces cardiovascular events compared with intensive, clinically guided therapy. Consequently, there is a strong trend to use BNP for monitoring of HF. There are also data demonstrating a close relationship

between plasma levels of ANP and BNP and LV dimensions and EF in patients with chronic LV systolic HF [37]. However, pharmacological treatment with a β-blocker may activate the natriuretic peptides in patients with CHF, not as part of any remodeling effect but as an effect of β-blockade in itself [38]. Brain natriuretic peptide level is also a strong predictor of cardiovascular events in patients with diastolic HF and that increased BNP levels are associated with a progressively bad prognosis [39].

Several other neurohormonal systems appear to be activated in the failing heart, both during the acute stages and during the more chronic congestive stage of HF. As an example we can mention neuropeptide Y (NPY) [25, 40, 41, 42], substance P [41], and calcitonin gene-related peptide (CGRP) [43].

1.4 Treatment of cardiac failure

Diuretics.

Diuretics offer rapid symptomatic relief in CHF as well as effective blood-volume reduction, and are easy to administer. They have disadvantages such as hypokalaemia and further activation of the neurohumoral systems in the failing heart [23]. Treatment in conjunction with other drugs such as β-blockers, ACE inhibitors and vasodilators including nitrates is of uncertain efficacy but is general practice.

Aldosterone receptor antagonists

The neurohormonal actions of Aldo (cardiac and vascular remodeling) influence the prognosis in HF [27]. The neurohormonal substudy of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) [27], demonstrated that patients with a baseline Aldo concentration above the median at 6 months had significantly greater mortality than those below the median.

The use of an ACE inhibitor (enalapril) did not confer any mortality benefit compared with placebo in patients with a baseline Aldo concentration below the median but in those with above-median values of Aldo, the 6-month mortality was markedly reduced by enalapril treatment. Other studies such as the Randomized Aldactone Evaluation Study (RALES) mortality trial [44] have shown beneficial effects of Aldo receptor blockade in patients with chronic severe HF. Eplerenone, a selective Aldo blocker, has been studied in patients with LV dysfunction after myocardial infarction [45]. Addition of this Aldo blocker to optimal medical therapy reduced the morbidity and mortality among patients with LV dysfunction and HF after AMI.

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor (AT_1) antagonists

Modulating the renin-angiotensin-aldosterone system is one way to manage patients with HF. Angiotensin-converting enzyme inhibitors are effective at reducing the mortality and morbidity of patients with asymptomatic LV dysfunction [46] and overt clinical HF [47]. Their use is based on the concept that peripheral circulating mechanisms, which maintain cardiovascular homeostasis, may overshoot, and that excessive vasoconstriction may therefore exacerbate CHF. Angiotensin-converting enzyme inhibitors influence neurohumoral factors such as renin [24] and Aldo [24] and attenuate LV remodeling after AMI [48]. Angiotensin-converting enzyme inhibitors induce regression of myocardial hypertrophy [49] and reduce peripheral vascular tone and systemic arterial pressure [50]. Treatment with ACE inhibitors is today, established as one of the corner stones of modern therapy in both acute and chronic HF [46, 47, 51, 52]. Studies such as the Acute Infarction Ramipril Efficacy (AIRE) study [47], the Survival and Ventricular Enlargement (SAVE) trial [52] and the Studies of Left Ventricular Dysfunction (SOLVD treatment) study [46] have established this. An alternative to ACE inhibitors may be AT_1 receptor antagonists [53, 54, 55] which generally

are well tolerated and significantly reduces cardiovascular deaths and hospital admissions for HF.

Beta-blockers

By acting through β_1 and β_2 receptors, the sympathetic nervous system can increase heart rate (HR) which affects the relation between myocardial demand and supply in the failing heart [56]. Norepinephrine stimulates growth and oxidative stress [57, 58] and by increasing cyclic AMP, cell death [59]. Beta-receptor blockers interfere with the action of the sympathetic nervous system and may antagonize these negative effects, preventing structural changes that occur during HF progression [60]. They may also reduce platelet aggregation and increased blood viscosity caused by adrenergic stimulation which may in turn reduce the risk of thrombosis and plaque rupture [61, 62, 63]. Studies have also suggested that β-blockers have anti-oxidant and anti-apoptotic actions [64, 65].

Long-term treatment (>3 months) with β-blockers increases CO and EF [66, 67]. Given intravenously in the acute phase of HF, β-blockers cause a reduction in blood pressure, HR, and decrease CO (unchanged stroke volume), while EF and intraventricular volumes remain unchanged [68, 69] and diastolic function improves. Since the US Carvedilol Heart Failure Trials Program [70] was presented in the early 90s, use of β-blockers has been established therapy for patients with stable HF.

Cardiac glycosides (Digitalis)

Digitalis may be used as complementary therapy in patients with symptomatic HF already on ACE inhibitors and diuretics and in patients with HF and atrial fibrillation (HR control). The Digitalis Investigators Group (DIG) study [71] investigated the effect of digitalis in patients with mild- to-moderate HF and sinus rhythm. There was no effect on mortality but digoxin reduced the number of hospitalizations for aggravated HF.

1.5 Free oxygen radicals

A free radical is any molecule that has an odd number of electrons. Free radicals are highly reactive and transient. Both organic (e.g. peroxides) and inorganic molecules (e.g., O_2^-) can be free radicals [72].

There are many biological sources of superoxide (O_2^-). Autoxidation of small molecules such as thiols and catecholamines reduces molecular oxygen to O_2^-. Superoxide can also be produced enzymatically, for example by the action of xanthine oxidase during ischaemia. Leakage of electrons to O_2 from the cellular electron transport chains, is an important source of O_2^-

Lipid peroxidation and aldehyde formation.

A peroxidation sequence is initiated by the removal of a hydrogen (H) atom from a methylene (-CH_2-) group of fatty acids by a free radical e.g., the hydroxyl radical. The reaction leaves behind an unpaired electron on the carbon -CH-. The carbon radical undergoes molecular rearrangement to form a conjugate diene, which reacts with O_2 to form a peroxy radical.

This peroxy radical is capable of abstracting a hydrogen atom from another fatty acid, thus starting a chainreaction of peroxidation. One of the aldehydes formed in this process is malondialdehyde (MDA).This substance is widely used as an indicator of peroxidation reactions.

Recent studies support the hypothesis that free radicals may promote myocardial damage in CHF [73]and oxygen-derived radicals have been implicated as a cause of endothelial dysfunction in HF, either following reduction of the anti-oxidant tissue reserve or as a result of enhanced radical formation [74, 75].

Several clinical and experimental studies have demonstrated increased oxidative stress in CHF [73, 76, 77, 78, 79, 80, 81, 82].

1.6 Effects of nitric oxide (NO)

Organic nitrates are often used to control ischaemic pain but also, to alleviate symptoms in CHF [83].Their effect is mediated through the production of NO. Endogenous NO is synthetized by NO synthase (NOS) from L-arginine and is released from endothelial cells. This release can be stimulated by local and circulating factors such as bradykinin but also, by shear stress of the blood tream acting on the endothelial lining. Exogenous NO donors produce venodilation and reduction in cardiac pre-load and in myocardial oxygen consumption [84]. They may also have a role in controlling acute HT and mitral regurgitation and have been reported to inhibit platelet activation [85] and leukocyte adhesion [86]. Nitric oxide generated from nitrate intake may have effects on the myocardium independently of the vasodilatory effects of the substance [87, 88]. It is now accepted that nNOS-derived NO regulates myocardial inotropy and relaxation [89] and plays a crucial role in preventing adverse LV remodeling after AMI [90].
In vitro experiments have shown that NO, a free radical gas, may act as a scavenger of free radicals, inhibiting lipid peroxidation [91, 92]. On the other hand, it is conceivable that NO may act as a pro-oxidant by being a precursor to free radicals such as peroxynitrite, nitrogen dioxide and the hydroxyl radical [93, 94].

1.7 Nitrates – effects and therapeutic applications

Nitrates in medicine: a short history

Treatment with organic nitrates is the oldest known therapy for angina pectoris. In 1847 Sobrero discovered nitroglycerin (Figure 1.4.) and in 1867 Brunton [95] reported that inhalation of amyl nitrite relieved anginal pain. Some years later Murrell [96] hypothesized that nitroglycerin may be effective for treatment of angina pectoris. In 1943 was a long-acting nitrate, isosorbide dinitrate (ISDN), was synthesized in Sweden by Meldahl [97]. In 1967 Dietz [98] reported that ISDN was metabolized to isosorbide-2-mononitrate and isosorbide-5-mononitrate

Figure 1.4. Ascanio Sobrero (1812-1888), Italian chemist who discovered nitroglycerine.

(IS-5-MN), the latter with the longest elimination half-life and a 100% bio-availability.

Nitrates may be administered in various preparation forms such as nitroglycerin patches, oral sustained-release or immediate-release formulations, sublingual, buccal, and paranteral administrations.

Nitrates in patients with acute myocardial infarction (AMI)

Nitrates are often administered with a variety of other pharmacologic agents in the management of CHF. Organic nitrates have a relaxant effect on vascular smooth muscles [99], and the principle of this nitrate-induced smooth muscle relaxation is the activation of the intracellular NO receptor enzyme, soluble guanylyl cyclase, subsequent elevation of the cyclic guanosine monophosphate (GMP) levels, and activation of cyclic GMP-dependent protein kinases and/or cyclic nucleotide-gated ion channels [100]. Consequently, nitroglycerin and other organic nitrates are believed to use the same signalling mechanisms as used by NO generated by NOS [101]. Nitrates reduce pre-load and afterload and inhibit platelet aggregation [102]. They are the most frequently prescribed anti-anginal

medications for patients presenting with stable and/or unstable angina pectoris. Intravenous administration is often chosen in patients with AMI. Patients treated within the first hour of symptom onset have demonstrated a lower incidence of early cardiac death, infarct extension, or congestive HF as a combined end-point [103]. Prolonged administration of intravenous nitroglycerin has been recommended for patients with AMI on the basis of improved LV function and beneficial effects on remodeling [104]. It has also been demonstrated that intravenous nitroglycerin reduces lipid peroxidation during myocardial ischaemia and reperfusion [105].

The fourth International Study of Infarct Survival (ISIS-4) included patients who were hospitalized within 24hours of suspected AMI. All patients received three study treatments, each being randomly assigned to active medication or placebo within each treatment, viz oral captopril 50 mg twice daily or placebo, for 28 days (blinded), oral controlled-release isosorbide mononitrate 60 mg twice daily or placebo, for 28 days (blinded), intravenous magnesium sulphate or no infusion (open). Median follow-up time was 15 months. In that study, mononitrate conferred no survival advantage in the short or long term [106]. In another study, the Gruppo Italiano per lo Studio della Sopravvivenza nell´Infarto miocardico-3 (GISSI-3) was evaluated the effects of early treatment with lisinopril and transdermal glyceryl trinitrate (10 mg), single and together on 6-week mortality and ventricular function after AMI. No difference in mortality was found between patients with and without glyceryl trinitrate [107].

Nitrate therapy in patients with chronic heart failure

Since the late 1950s [108], organic nitrates have been known to have a beneficial effect on elevated pulmonary pressures. Beneficial haemodynamic effects of ISDN in patients with chronic HF were documented in the early 1970s by Franciosa et al [109] and in the 1980s by Cohn et al [83]. In these and other short-term studies in patients with chronic HF, nitrates caused a considerable degree of venodilatation and a significant decrease in right ventricular (RV) and left LV

filling pressures, while atrial dilatation was less pronounced. Heart rate remained stable, while CO usually increased mildly [110-115].

Clinical studies also suggest beneficial long-term effects of nitrates in CHF. In the Veterans Administration Vasodilator Heart Failure Trial (V-HeFT) I [116], the combined use of ISDN and hydralazine reduced the mortality rate compared with placebo. In the African-American Heart Failure Trial (A-HeFT) [117], African-American patients in New York Heart Association (NYHA) class III or IV HF with dilated ventricles were randomized to receive a fixed dose of ISDN plus hydralazine or placebo in addition to standard therapy for HF, which included neurohumoral inhibitor drugs. This study demonstrated a significantly higher mortality and hospitalization rate in the placebo group than in the group given ISDN plus hydralazine. The sole effect of nitrates only cannot be judged by the results of these studies.

Nitrate tolerance

Development of pharmacodynamic tolerance to organic nitrates has been known to happen for a long time [118]. It is usually associated with a chronic use of high doses. The duration of action and the effects of the nitrate may be diminished. A number of mechanisms such as neurohormonal counter-regulation as well as intrinsic vascular processes may explain this complex phenomenon.

Superoxide and vascular NO form peroxynitrite, which aggravates tolerance by promoting NO synthase uncoupling and inhibition of soluble guanylyl cyclase and prostacyclin synthase. Several studies have also reported that nitroglycerin is associated with increased production of reactive oxygen species by the endothelium and that this may play a role in the development of endothelial dysfunction and tolerance [119, 120, 121]. The importance of free radical production in the development of tolerance is supported by investigations in which antioxidant supplementation reduced free radical production and prevented tolerance during nitrate therapy [122, 123]. Strategies to prevent or limit nitrate tolerance may comprise fewer and smaller doses, use of shorter-acting compounds and, probably

the most important factor, nitrate-free intervals [115, 124]. Prevention of nitrate tolerance may also be minimized by concomitant medication such as an ACE-inhibitor [125, 126] or hydralazine [127]. Nitrates are predominantly used in the treatment of angina pectoris, with fairly standardized doses; however, there is no generally accepted dose for CHF. Earlier reports have demonstrated that patients on treatment with diuretics and an ACE inhibitor get pronounced effects already at oral doses of 5-20 mg [128] and that retard formulations, correctly used, give long duration of efficacy with no, or minimal, tolerance development [129].

2 Aims of the study

The aims of the study were to investigate-

the long-term effects of IS-5-MN therapy in patients with LV dysfunction after AMI with respect to invasive haemodynamics, echocardiographic measurements, lipid peroxidation, neurohumoral activation and clinical characteristics

lipid peroxidation in HF patients and the correlations between markers of oxidative stress and markers of HF severity

neurohumoral activation after AMI, and its relationship to HF severity

changes in clinical characteristics, haemodynamic parameters, echocardiographic measurements, and neurohumoral response during 1 year after AMI

differences in clinical characteristics, haemodynamics, echocardiographic measurements and neurohumoral activation between patients with LVEF <45% and patients with LVEF ≥45% after AMI

3 Material and Methods

3.1 Study population

The studies enrolled two separate populations of patients at the Department of Cardiology, University Hospital, Lund, Sweden.

Paper I

This single-centre study was performed between March 1995 and January 2000. Included were non-consecutive patients aged between 18 and 80 years who were admitted to the coronary care unit (CCU) with a definitive AMI and evidence of LV dysfunction during hospitalization.

Acute myocardial infarction was defined as typical chest pain in combination with elevated markers of myocardial injury (i.e creatine kinase (CK) and/or troponine-T (trop-T) levels greater than twice the upper limit of the local chemical laboratory reference range or equivalent increases in the CK-muscle-brain (CK-MB) iso-enzyme).

Left ventricular dysfunction was defined by means of at least one of the following features: clinical criteria (bilateral post-tussive crackles extending at least one-third of the way up the lung fields), radiographic criteria of pulmonary congestion, or LVEF $\leq$ 40% assessed by echocardiography.

Heart transplantation patients, patients with clinically severe renal, hepatic or haematologic disorders and patients with a history of cerebral transient ischaemic attacks within the preceding 6 months were excluded from the study. Patients with significant mitral or aortic valve disorders, obstructive or restrictive cardiomyopathy, unstable angina, recurrent ventricular tachycardia or ventricular fibrillation, untreated third-degree atrioventricular block, systolic supine blood pressure < 100mmHg, hypersensitivity to ramipril or IS-5-MN, or obligate need for long-acting nitrates were likewise excluded. We also excluded women of child-

bearing potential who were not using adequate contraception, as well as pregnant women and nursing mothers.

In the 93 patients meeting the inclusion criteria in the main study, treatment with an ACE inhibitor (viz. ramipril) was initiated in the hospital before the day of discharge, and later, ambulatory adjustment of the dose was allowed. If patients had previously been treated with an ACE inhibitor other than ramipril, that treatment was discontinued and replaced with ramipril. Concomitant medication with digoxin, β-blockers, calcium antagonists, aspirin and diuretics was accepted.

Five weeks (36 days, standard deviation (SD) of 9 days, range 18-59 days) after the index AMI the patients came for a randomization visit, during which a complete medical history was taken and a physical examination was performed. One patient did not use ramipril at randomization and was consequently excluded. The functional severity of HF was assessed before randomization using the NYHA classification. An electrocardiogram (ECG) was recorded and after 1 hour of rest, blood samples were taken and echocardiography and a right-heart catheterization performed. The patients were then randomly allocated to treatment with either oral IS-5-MN or placebo. The initial dose of IS-5-MN was 30 mg every morning for 7 days, followed by an increase to 60 mg daily. Apart from short-acting nitrates, non-study nitrates were avoided.

All patients, including those withdrawn from randomized treatment were seen for a clinical evaluation at 6 weeks and 3 and 6 months after the index AMI.
One year after the index AMI (335±12 days after randomization) a new evaluation was performed with the same set of tests as used at randomization. Finally, 1 month after drug withdrawal, a new echocardiographic evaluation was performed.
An overview of the study design is presented in Figure 3.1. and patient characteristics at randomization in Table 3.1.

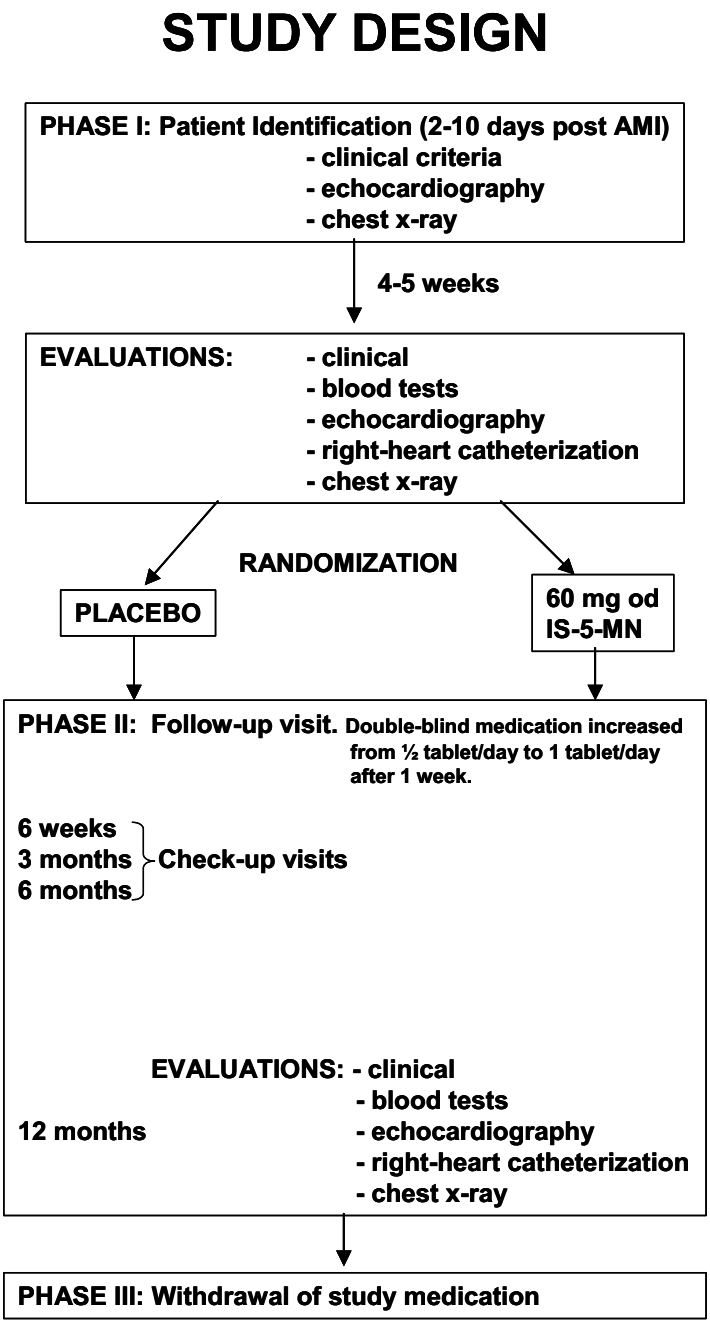

Figure 3.1. Overview of the study design

	IS-5-MN (n = 47)	Placebo (n = 45)	Total (n = 92)
Sex (%)			
Male	39 (83)	30 (67)	69 (75)
Female	8 (17)	15 (33)	23 (25)
Age (y) mean ± SD	63±10	65±9	64±10
Medical conditions before index infarction (%)			
AMI	13 (28)	9 (20)	22 (24)
CHF	5 (11)	6 (13)	11 (12)
Cardiac arythmias	2 (4)	2 (4)	4 (4)
Hypertension	15 (32)	16 (36)	31 (34)
Diabetes mellitus	14 (30)	9 (20)	23 (25)
Dyslipidemia	4 (9)	3 (7)	7 (8)
Index Infarction (%)			
Anterior	28 (60)	28 (62)	56 (61)
Inferior	6 (13)	9 (20)	15 (16)
Unclassified	13 (28)	8 (18)	21 (23)
Q-wave	34(72)	29 (64)	63 (68)
Non-Q-wave	10 (21)	15 (33)	25 (27)
Unclassified	3 (6)	1 (2)	4 (4)
Thrombolytic treatment	19 (40)	19 (42)	38 (41)
PTCA	12 (26)	11 (24)	23 (25)
Trombolytic treatment and PTCA	4 (8)	2 (5)	6 (7)
No trombolytic treatment and no PTCA	12 (26)	13 (29)	25 (27)
Concomitant medication (%)			
Aspirin	44 (94)	42 (93)	86 (93)
Warfarin	24 (51)	16 (36)	40 (43)
β-Blocker	35 (74)	30 (67)	65 (71)
Digoxin	10 (21)	7 (16)	17 (18)
Diuretic	33 (70)	29 (64)	62 (67)
Radiographic evidence of heart failure (%)			
Yes	3 (7)	6 (15)	9 (11)
No	41 (93)	33 (85)	74 (89)
Missing	3 (6)	6 (13)	9 (10)
EF≤40 % (%)			
Yes	24 (52)	16 (39)	40 (46)
No	22 (48)	25 (61)	47 (54)
Missing	1 (2)	4 (9)	5 (5)
NYHA (%)			
I	2 (4)	7 (16)	9 (10)
II	27 (58)	24 (53)	51 (55)
III	17 (36)	13 (29)	30 (33)
IV	1 (2)	1 (2)	2 (2)
Medical conditions including the index AMI (%)			
Prior PTCA	20 (43)	16 (36)	36 (39)
Prior CABG	4 (9)	2 (4)	6 (7)

Table 3.1. Patient characteristics at randomization. CHF, Congestive heart failure; NYHA, New York Heart Association; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft surgery.

Paper II

The present work included 83/92 patients from the main study (Paper I) who had available plasma MDA (P-MDA) at randomization (74 patients, had available P-MDA at randomization and after 11 months´treatment). Control subjects were 80 age-and gender-matched healthy blood donors on no medication and with no history of diabetes or lipid abnormalities.

Because of inconsistent results from previously published studies, P-MDA and plasma 8-isoprostane concentrations were studied in a second group consisting of 56 well known HF patients from the outpatient department and age- and gender-matched controls.

These patients had been previously (≤ 6 months previously) examined by a cardiologist and the diagnosis HF was based on clinical findings as well as echocardiographic and radiographic evaluation. They had all been in a stable condition for at least 1 month and had had the diagnosis HF for 1-12 years (mean 3 years). Table 3.2. describes patient characteristics in the both the patient groups studied.

	Study group I (n = 83)	Study group II (n = 56)
Sex (%)		
Male	64(77)	37(66)
Female	19(23)	19(34)
Age (y) mean ± SD	64±10	69±9
Medical conditions (%)		
Hypertension	27(33)	17(30)
Diabetes mellitus	20(24)	14(25)
Dyslipidemia	7(8)	8(14)
Cardiac arythmias	4(5)	6(11)
Prior PTCA	30(36)	19(34)
Prior CABG	5(6)	25(45)
Non-ischemic orgin of CHF (%)		
Yes	0 (0)	13 (23)
Concomitant medication (%)		
Aspirin	78(93)	56(100)
Warfarin	36(43)	14(25)
β-blocker	58(70)	39(70)
Digoxin	16(19)	19(34)
Diuretic	56(67)	38(68)
ACE inhibitor	83(100)	24(43)
A II antagonist	0(0)	32(57)
Long-acting nitrates	0(0)	15(27)
EF ≤40 % (%)		
Yes	35(42)	37(67)
No	43(52)	19(33)
Missing	5(6)	0(0)
EF (mean±SD)	42±10	37±10
NYHA (%)		
I	9(11)	17(30)
II	46(56)	22(39)
III	26(31)	17(30)
IV	2(2)	0(0)

Table 3.2. Patient characteristics in Study group I and II. AMI, Acute Myocardial Infarction; NYHA, New York Heart Association; PTCA, Percutaneous Transluminal Coronary Angioplasty; CABG, Coronary Artery Bypass Graft surgery.

Paper III

This substudy included patients from the main study (Paper I), with available plasma neurohumoral measurements at randomization and at the 12 month visit. For further information, see the relevant section in the paper.

Paper IV

The subjects in this study included patients from the main study (Paper I) with available haemodynamic and echocardiographic/Doppler measurements. Excluded from calculation of the E/A ratio were patients with atrial fibrillation, atrial flutter and pacemaker rhythm.

The characteristics of patients with LVEF < 45% and patients with LVEF ≥ 45% were compared with respect to clinical, physiological, and biochemical data.

3.2 Pharmaceutical preparation

Isosorbide-5-mononitrate Durules® PLACEBO H 513-3-1 was manufactured by AB Hässle, Pharmaceutical R&D Pharmaceutics. Mölndal, Sweden.

3.3 Haemodynamic measurements

Echocardiography and Doppler investigation

Two-dimensional, pulsed Doppler, and colour-flow Doppler echocardiographic examinations (Papers I-IV) were performed by three experienced investigators at the local echocardiographic laboratory using a Hewlett Packard Sonos 2500 (Hewlett Packard, Andover, MA, USA) (Figure 3.3.). The echocardiographers were blinded to both the treatment and the laboratory data.

Figure 3.3. Hewlett Packard Sonos 2500

Dimensions were measured from the parasternal long-axis view. The following LV dimensions were measured: left atrium/m^2 (mm/m^2); left ventricular internal diameter in diastole, LVIDd (mm); left ventricular internal diameter in systole, LVIDs (mm); inter-ventricular septal thickness in diastole, IVSd (mm); left posterior wall diameter in diastole, LPWDd (mm); left ventricular mass, LV-mass/m^2 body-surface area(BSA) (g); end-diastolic volume index, EDVI (mL/m^2 BSA); end-systolic volume index, ESVI (mL/m^2 BSA).
Left ventricular mass (gram) was calculated with the following formula [13]:

$$\textbf{LV mass} = 1.04 \times ([\text{LVIDd} + \text{IVSd} + \text{LPWDd}]^3 - [\text{LVIDd}]^3 - 13.6.$$

Left ventricular volumes (mL/BSA) and LVEF (%) were calculated by use of Simpson´s rule (method of disks) from the apical two-and four-chamber views. Pulsed Doppler recordings of the mitral and pulmonary venous flow pattern were used to evaluate diastolic function. Mitral flow velocities were recorded from the apical four-chamber view by placing the sample volume between the tips of the

mitral leaflets, while pulmonary venous flow was obtained by placing the sample volume 1 cm into the right upper pulmonary vein.

The following Doppler parameters were used to evaluate LV diastolic function: transmitral flow: E and A, E/A, and deceleration time of E wave (DT), pulmonary venous flow: peak pulmonary venous flow velocity during ventricular systole (sPV), and peak pulmonary venous flow velocity during ventricular diastole (dPV) [6]. Isovolumetric relaxation time (IRT) was obtained by simultaneous recordings of blood flow velocities in the LV inflow and outflow tracts, using continuous Doppler.

To define the LV filling pattern (Paper IV) we used the E/A ratio and the PAWP. At the time of the study, tissue Doppler imaging was not available in our echocardiographic laboratory and PAWP, measured invasively, was used instead to discriminate between a normal and a pseudonormal mitral flow pattern.
The following classification was used:

- **Normal**: E/A $\geq$ 0.75 but < 2.0 and PAWP $\leq$ 12 mmHg.
- **Impaired relaxation**: E/A < 0.75.
- **Pseudonormal**: E/A $\geq$ 0.75 but < 2.0 and PAWP > 12 mmHg.
- **Restrictive**: E/A $\geq$ 2.0.

Patients with atrial fibrillation, atrial flutter, and pacemaker rhythm were excluded from the E/A calculation.

Patients with LVEF <45% and patients with LVEF $\geq$ 45% (Paper IV) were compared with respect to clinical, physiological and biochemical data. The cut-off value for LVEF 45% was based on the European Society of Cardiology (ESC) diagnostic criteria for diastolic HF [130]. Eighty-seven out of 92 patients had echocardiographically measured LVEF at randomization. Patients in study group II (Paper II) were known HF patients from the outpatient department. Echocardiographic measurements were performed as a routine examination including LVEF evaluation.

Right-heart catheterization

For assessment of each patient´s haemodynamic condition, a right-heart thermodilution catheter was used (Swan-Ganz®, Thermodilution Paceport™ Catheter,Baxter Healthcare Corporation, Irvine, CA, USA). The catheter was introduced into the pulmonary artery allowing the following measurements: right atrial pressure (RAP); mean pulmonary arterial pressure (MPAP) and PAWP. Where possible, systemic arterial pressures (systolic, diastolic and mean arterial pressure (MAP)) were measured invasively in the radial artery, otherwise no-invasively using a sphygmomanometer. Cardiac output was determined according to the intermittent thermodilution principle and CI was expressed in L/min/m²/BSA. The following standard haemodynamic formulae were used in calculating systemic vascular resistance (SVR) and PVR (Wood units):

- **SVR** = (MAP-RAP)/CO
- **PVR** = (MPAP-PAWP)/CO

The stroke index (SI) was measured in mL/beat/m² BSA. All pressures were expressed in mmHg. Electrocardiograms were monitored continuously. Measurements were performed with the patient in the supine position after 30 minutes of rest. Where clinically possible (i.e, with PAWP at rest < 25 mm Hg) measurements were also performed during a supine leg exercise test in which a steady state was attained (i.e. stable HR for 2 minutes) at a fixed wattage load (men 50 watts and women 30 watts).

3.4 Chemical analysis

Routine blood (i.e. haematological, renal, hepatic, and blood sugar) tests (RT) were collected by venipuncture into evacuated Vacutainer tubes® and analysed at the local hospital´s clinical chemistry laboratory (Department of Clinical Chemistry, Lund University Hospital). Routine blood tests were performed (Papers I-IV) at randomization, 6 weeks, 3 months and 6 months and at the end of the study (i.e. at the 12-month visit).

Plasma MDA and plasma 8-isoprostane (P-8-isoprostane, 8-epi prostaglandin F2 alpha (PGF2α)) were measured in HF patients and age- and gender-matched controls (Paper II). After 1 hour of rest, blood samples were collected by venipuncture into evacuated tubes containing ethylenediamine tetra-acetic acid (EDTA). After centrifugation, plasma was carefully removed and stored at - 70° C until analysis (within 4 weeks). Plasma MDA concentrations were measured by high-performance liquid chromatography (HPLC) using a method described by Öhlin et al [105]. Plasma 8-isoprostane was analysed with an ACE™ Competitive Enzyme Immunoassay (Cayman Chemical Company, Ann Arbor, MI, USA) [131].

For determination of neurohumoral changes, plasma epinephrine (EPI) and NEPI, anti-diuretic hormone-like immunoreactivity (ADH-LI), Aldo, plasma renin activity (PRA), substance P-like immunoreactivity (SP-LI), neuropeptide Y-like immunoreactivity (NPY-LI), calcitonin gene-related peptide-like immunoreactivity (CGRP-LI), vasoactive intestinal peptide like immunoreactivity (VIP-LI) (Paper III), BNP) and ANP (Papers I and III) were plasma removed after centrifugation at +4°C and stored at -70 °C until analysed.

Plasma levels of SP-LI, NPY-LI, CGRP-LI and VIP-LI were measured by high-performance liquid chromatography (HPLC) with a method and procedure described by Kraiczi et al [132] and ADH-LI determined by radioimmunoassay [133]. Plasma EPI and NEPI were determined by radioenzymatic assay [134] and plasma PRA was assessed by radioimmunoassay measurement of angiotensin I generation [135]. Aldosterone was measured by competitive radioimmunoassay (Aldosterone II RIA Diagnostic Kit, ABBOTT Laboratories, Diagnostics Division, IL,USA). Plasma-atrial natriuretic peptide [136] and P-BNP [137] were assayed with a direct and specific monoclonal antibody radioimmunoassay kit.

3.5 Statistical methods

The study was empowered to detect changes in PAWP. A sample size of 90 patients was calculated to allow detection of a difference in 3 mmHg of PAWP as a

treatment effect with 90% probability and with an estimated drop-out rate of 30%. In all statistical comparisons a P-value of <0.05 was considered statistically significant.

Changes in quantitative variables within-group from base-line to a later visit were analysed by means of paired *t* tests. Values at a later visit were compared between the two treatment groups by means of analysis of covariance (ANCOVA) (i.e. multiple linear regression with treatment and baseline value of the variable in question as explanatory variables).

For two-group comparisons of variables measured on a continuous scale, non-parametric tests were chosen using the Mann-Whitney U-test for independent groups and the non-parametric Wilcoxon matched-pairs signed-ranks test for extremely skewed variables and/or variables with gross outliers.

Spearman´s rank correlation coefficient (r_s) was used as a measure of correlation between pairs of variables measured on a continuous scale.

Cross-tables were evaluated using χ^2-tests or Fischer´s exact test.

The Bonferroni method was used to adjust the P-values for multiple testing (Papers II and III)

For further information regarding statistical methods, see "Statistical methods" in the original articles (Papers I-IV).

3.6 Ethical considerations

The study was approved by the local Ethics Committee according to established ethical, medical and scientific standards. In case of an adverse event this was reported to the Swedish Medical Products Agency ("Läkemedelsverket"). According to study instructions an overall report concerning events was sent to the Medical Products Agency in May 1997, January 1999, December 1999 and at the end of the study. Power calculation for the study and randomization (i.e. IS-5-MN/placebo) were done by an independent statistician.

Approval was granted as follows:

- use of IS-5-MN/placebo (Papers I-IV) was approved by the Swedish Medical Products Agency/Department of Clinical Trials/licence (Approval Dnr 151:155/94);
- the Ethical Committee of Lund University (Approval Dnr LU 179-94) approved the use of IS-5-MN/placebo in the study (Papers I-IV);
- the measurement of plasma MDA (Paper II) was approved by the Ethical Committee of Lund University (Approval Dnr LU 602-98 (Paper II);
- the use of personal particulars (Papers I-IV) (Swedish Act Relating to Personal Data (LU-P3-99))was approved by the Ethical Committee of Lund University (Approval Dnr 179-94);
- the exploration of markers of lipid peroxidation and oxidative stress in HF patients (Paper II) was approved by the Ethical Committee of Lund University (Approval Dnr LU 359-02); and
- prolongation of the durability of the study-medication (IS-5-MN/placebo) was accepted by the Swedish Medical Products Agency/Department of Clinical Trials/licence 1998-09 (Approval Dnr 151:155/94).

4 Results

4.1 The effects of isosorbide-5-mononitrate (IS-5-MN) therapy in patients with left ventricular (LV) dysfunction after AMI

Overall changes in PAWP and/or other haemodynamic measurements were not significantly different between the two groups (Table 4.1.). Nitrate therapy did not significantly affect the neurohumoral status, apart from a decrease in plasma ANP levels (P = 0.041) and the need for additional diuretics (P = 0.048). Less LV dilation was observed in patients with more severe LV dysfunction (P = 0.047). Chronic treatment with IS-5-MN did not significantly affect the P-MDA levels (P = 0.71) or the LV diastolic function.

4.2 Lipid peroxidation in heart failure (HF) patients and the correlation between markers of oxidative stress and markers of HF severity

Study group I – Paper II

Relationship between plasma malondialdehyde and haemodynamic data, ejection fraction, plasma natriuretic peptides and New York Heart Association class

At baseline, right-heart catheterization data were available for 75 patients in study group I. Plasma MDA was inversely correlated to mean PAWP at rest (n = 75; r_s = –0.24; P = 0.04). No significant correlation was found between P-MDA and CI (n = 75; r_s = 0.07; P = 0.57). No correlation was found between LVEF and P-MDA (n = 78; r_s = 0.06; P = 0.63). A statistically significant negative correlation between P-BNP and P-MDA was obtained (n = 54; r_s = -0.27; P = 0.05). No significant correlation between P-ANP and P-MDA was obtained (n = 83; r_s = -0.039; P = 0.72). We found no significant correlation between NYHA class and

Parameter	IS-5-MN (n = 40) R	Placebo (n = 34) R	P* R	IS-5-MN (n = 28) E	Placebo (n = 21) E	P* E
HR (beats/min)						
Rand	67±12	65±11		98±12	101±14	
12 Month	65±11	67±12	0.32	98±11	99±12	0.88
BP (mm Hg)						
Rand	93±11	97±13		113±21	108±10	
12 Month	94±11	100±19	0.46	110±18	108±12	0.97
CI (L/min/m^2BSA)						
Rand	2.56±0.5	2.45±0.47		4.77±0.99	4.95±1.12	
12 Month	2.46±0.49	2.52±0.54	0.19	5.01±0.88	5.09±1.31	0.83
SI (mL/beat/m^2BSA)						
Rand	39.2±9.0	38.0±7.3		49.0±10.3	48.7±9.5	
12 Month	38.6±8.4	38.1±8.2	0.87	51.7±10.0	51.5±12.7	0.98
RAP (mm Hg)						
Rand	4.2±3.3	4.4±3.2		9.8±4.5	8.4±4.7	
12 Month	4.4±3.2	4.9±3.8	0.53	9.7±3.9	10.4±4.4	0.33
PAWP (mm Hg)						
Rand	11.3±6.6	11.3±7.3		27.0±9.4	23.3±9.3	
12 Month	9.8±6.1	10.7±8.1	0.51	24.3±10.3	22.1±7.9	0.9
PVR (Wood)						
Rand	1.40±0.54	1.46±0.60		1.13±0.38	1.39±0.53	
12 Month	1.58±0.74	1.61±0.71	0.99	1.36±0.55	1.33±0.63	0.065
SVR (Wood)						
Rand	18.97±5.30	20.99±4.47		11.29±3.71	10.27±2.27	
12 Month	20.18±5.80	21.40±4.86	0.7	9.98±4.04	10.29±3.40	0.54

Table 4.1. Haemodynamik measurements at randomization and 12 moth visit.Data are presented as mean ± SD. R, Values at rest; E, values at exercise; HR, heart rate; BP, mean systemic blood pressure; CI, cardiac index; BSA, body-surface area; SI, stroke index; RAP, mean right atrial pressure; PAWP, mean pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance. * P values refer to ANCOVA of final values at randomization as covariates.

P-MDA (n = 83; r_s = 0.07; P = 0.53). There were no significant differences between patients (n = 80) and their controls (n = 80) with regard to P-MDA (0.89±0.26 μmol/L v. 0.89±0.26 μmol/L; P = 0.88).

Study group II – Paper II

Relationship between plasma malondialdehyde, plasma 8-isoprostane, plasma brain natriuretic peptide and echocardiographic measurements in patients in study group II and their controls

No significant correlation between P-8-isoprostane and P-BNP or EF was demonstrated; nor did we observe any significant correlation between P-MDA and P-BNP or EF. There was no significant difference with regard to P-8-isoprostane between patients (n = 48) and their controls (n = 48) (69±32 ρmol/L v. 83±71 ρmol/L; p = 0.29).

There were no significant differences between P-MDA in study group I and P-MDA in study group II (0.89±0.26 μmol/L v. 0.83±0.28 μmol/L; P = 0.16).

4.3 Neurohumoral activation and its relationship to congestive HF severity, and the relationship between different markers of the severity of HF.

Table 4.2. shows the correlations at randomization between neurohormones and different markers of the severity of LV dysfunction. Table 4.3. shows the relationship at randomization between different markers of the severity of LV dysfunction.

	EF	PAWP	CI	NYHA
EPI	-0.19	0.21	-0.04	-0.14
NEPI	-0.29**	0.12	-0.10	0.24*
PRA	-0.25*	-0.20	-0.04	0.22*
Aldo	-0.07	-0.04	-0.22*	0.09
ADH-LI	-0.29**	-0.15	-0.07	0.22*
SP-LI	-0.10	-0.18	0.27**	-0.03
NPY-LI	-0.10	-0.16	0.02	0.03
CGRP-LI	-0.33**	-0.07	0.08	0.14
VIP-LI	-0.09	0.11	0.06	-0.01
ANP	-0.28**	0.32**	-0.04	0.17
BNP	-0.33**	0.63***	-0.02	0.19

Table 4.2. Relationship at randomization between different markers for the severity of heart failure and plasma neurohumoral factors. EF, ejection fraction; PAWP, mean pulmonary artery wedge pressure (at rest); CI, cardiac index (at rest); NYHA, New York Heart Association; EPI, epinephrine; NEPI, norepinephrine; PRA, plasma renin activity; Aldo, aldosterone; ADH-LI, antidiuretic hormone; SP-LI, substance P; NPY-LI, neuropeptide Y; CGRP-LI, calcitonin gene-related peptide; VIP-LI, vasoactive intestinal peptide; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide. Correlation coefficients are given (Spearman) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Only the positive rank correlation between BNP and PAWP remained significant after Bonferroni adjustment for multiple testing ($p<0.001$)

	BNP	ANP	NYHA	EF	PAWP	CI
BNP	1.00	0.42**	0.20	-0.48***	0.65***	0.13
ANP	0.42**	1.00	0.20	-0.32**	0.34**	-0.12
NYHA	0.20	0.20	1.00	-0.40***	0.28**	-0.16
EF	-0.48***	-0.32**	-0.40***	1.00	-0.27	0.27*
PAWP	0.65***	0.34**	0.28**	-0.27	1.00	-0.12
CI	-0.13	-0.12	-0.16	0.27*	-0.12	1.00

Table 4.3. Relationship at randomization in Study group I between different markers for the severity of left ventricular (LV) dysfunction. BNP means brain natriuretic peptide; ANP, atrial natriuretic peptide; NYHA, New York Heart Association; EF, ejection fraction; PAWP, mean pulmonary artery wedge pressure (at rerst); CI, cardiac index (at rest). Correlation coefficients are given (Spearman) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

When adjusted for multiple testing, five correlations remained significant, viz BNP-ANP and ANP-PAWP ($P<0.05$), BNP-EF and NYHA-EF ($P<0.01$), and BNP-PAWP ($P<0.001$).

4.4 Changes in haemodynamic parameters, neurohumoral response and diastolic function during 1 year after AMI

Changes in New York Heart Association class and in haemodynamic and echocardiographic measurements in the total study population

Two-dimensional echocardiographic data and invasive haemodynamic measurements were unchanged during the study period. Altogether 46 patients (53%) fell into a less severe NYHA category, 33 patients (38%) remained stable and eight patients changed into a more severe NYHA class, $P<0.0001$.

Changes in neurohumoral activation from baseline to the end of the treatment after AMI in the total study population

We found small but significant increases in EPI, NEPI, Aldo and VIP-LI and decreases in ADH-LI, NPY-LI, BNP and ANP. Data are presented in Table 4.4.

		Randomization		12 month visit		
Variable	**N**	**Median**	**IQR**	**Median**	**IQR**	**P***
EPI (nmol/L)	84	0.17	0.10-0.24	0.18	0.10-0.28	0.05
NEPI (nmol/L)	84	2.9	2.3-3.7	3.2	2.6-4.1	0.01
PRA (µg/L/h)	84	3.3	1.1-11	4.1	1.4-9.7	0.31
Aldo (nmol/L)	85	0.22	0.17-0.28	0.24	0.18-0.33	0.03
ADH-LI (pmol/L)	86	0.9	0.3-1.5	0.6	0.3-1.3	0.006
SP-LI (pmol/L)	82	1.4	0.9-2.1	1.2	0.9-1.7	0.18
NPY-LI (pmol/L)	85	133	123-154	131	114-146	0.001
CGRP-LI (pmol/L)	84	21	14-30	20	14-26	0.49
VIP-LI (pmol/L)	86	7.5	6.0-8.0	8.0	7.0-10	0.0005
BNP (pmol/L)	56	31	19-57	12	6-30	<0.0001
ANP (pmol/L)	86	69	51-95	60	44-79	0.0039

Table 4.4. Plasma neurohumoral measurements in total study population at randomization and 12 month visit. Data are presented as median and IQR, inter quartile range. EPI, epinephrine; NEPI, norepinephrine; PRA, renin activity; ADH-LI, antidiuretic hormone; Aldo, aldosterone; SP-LI, substance P; NPY-LI, neuropeptide Y; CGRP-LI, calcitonin gene-related peptide; VIP-LI, vasoactive intestinal peptide; BNP, brain natriuretic peptide; ANP, atrial natriuretic peptide. P* values refer to Wilcoxon matched pairs signed rank sum test.

Changes in diastolic function in the total study population

We found significant increases in IRT and sPV/dPV, and decreases in the E/A ratio. Data are presented in Table 4.5. The observed change in the mitral filling pattern indicates improved LV diastolic function.

Variable	N	Randomization	12-month visit	P*
DT (ms)	77	197±48	209±66	0.27
IRT (ms)	51	99±23	110±25	0.008
E (m/s)	78	0.70±0.20	0.65±0.20	0.04
A (m/s)	75	0.61±0.19	0.69±0.19	0.0006
E/A ratio	73	1.30±0.73	1.04±0.54	0.0005
sPV (m/s)	67	0.58±0.16	0.54±0.17	0.02
dPV (m/s)	68	0.54±0.17	0.47±0.17	0.001
sPV/dPV	67	1.16±0.37	1.25±0.43	0.04

Table 4.5. Echocardiographic diastolic measurements in total study population at randomization and at the 12 month visit (mean ± SD). DT, deceleration time; IRT, isovolumetric relaxation time; E, peak mitral flow velocity in early diastole; A, peak mitral flow velocity at atrial contraction; E/A ratio, ratio between early and late mitral peak flow velocity; sPV, peak pulmonary venous flow velocity during ventricular systole; dPV, peak pulmonary venous flow velocity during ventricular diastole.

4.5 Differences between patients with left ventricular ejection fraction (LVEF) <45% and patients with LVEF ≥ 45% in a group of patients with left ventricular dysfunction after myocardial infarction

Patients in the group with lower LVEF were older (65±10 years v 60±9 years; P = 0.017) and had a higher incidence of prior myocardial infarctions (30% v 3%; P = 0.003) and diabetes (30% v 10%; P = 0.03), a more profound functional impairment (in terms of the NYHA classification) (P = 0.01), reduced CI (2.4±0.4 L/min/m^2 BSA v 2.7±0.5 L/min/m^2 BSA; P = 0.0045), and larger LA (23.7±3.0 mm/m^2 v 22.1±3.1 mm/m^2; P = 0.03) and LV dimensions (LVIDd 58.5±7.8 mm v 52.4±4.9 mm; P = 0.0001, and LVIDs 43.3±9.5 mm v 34.5±6.2 mm; P = <0.0001). They also had significantly elevated plasma NEPI (3.45±1.50 nmol/L v 2.73±0.98nmol/L; P = 0.027), ANP (89±47 v 74±51; P = 0.017), BNP (52±32 pmol/L v 21±17 pmol/L; P = 0.0001) and ADH levels (1.28±1.06 pmol/L v

0.99±1.52; P =0.028) compared with the LVEF ≥ 45 % group. However, there was no significant difference in diastolic filling pattern (P = 0.65) and only a non-significant trend towards higher PAWP in patients with LVEF < 45% (11.8±7.4 mmHg v 9.1±5.5 mmHg, P = 0.10)

Changes in the parameters mentioned above (randomization – 12-month visit) were not significantly different between patients with LVEF < 45% and patients with LVEF ≥ 45%.

5 Discussion

5.1 Effects of nitrates

Why was no haemodynamic effect of IS-5-MN detected?

Chronic treatment with IS-5-MN given orally once daily for 11 months to patients with LV dysfunction following AMI did not significantly influence the haemodynamic response, echocardiographic measurements, lipid peroxidation or neurohumoral activation, apart from a decrease in ANP. There are several possible explanations for these negative findings

IS-5-MN dosage

Nitrates are predominantly used in the management of CAD with fairly standardized doses. They are frequently given not only to patients with stable angina pectoris, but also to those with unstable angina, AMI and HF. With all modes of administration, the acute use causes a marked venodilation and a substantial decrease in PAWP and RAP [110, 114, 115].

In chronic HF, there is no generally accepted dose [138, 139]. A once-daily sustained dose of IS-5-MN was used in our study on the based on earlier reports demonstrating that CHF-patients on treatment with diuretics and an ACE inhibitor show a pronounced effect already at oral doses of 5-20 mg [128] and that retard formulations, correctly used, give long duration of efficacy [129].

Consequently, we used a moderate dose of a sustained-release formula of IS-5-MN. For obvious reasons, results from this study cannot be generalized to other nitrate doses or modes of administration.

Mild-moderate LV dysfunction in most patients

Most patients included in the present study demonstrated only mildly or moderately reduced LVEF at randomization, which probably influenced the possibility of detecting significant changes in our material. In a pre-specified subgroup of patients with elevated PAWP at rest (≥12mmHg), a significant haemodynamic increase in CI and SI during exercise was demonstrated with nitrate therapy, while in a group of patients with LVEF ≤40%, the EDVI increased significantly more in the placebo group than it did in the IS-5-MN group.
These subgroup results should be interpreted with caution, but may suggest that nitrates exert beneficial effects in patients with severe LV dysfunction.

Concomitant therapy

It is possible that concomitant pharmacological treatment with drugs affecting the haemodynamic status made it more difficult to demonstrate the effects of IS-5-MN therapy. Treatment with ACE inhibitors and β-blockers is today an established approach to managing LV dysfunction. However, both these regimens influence the haemodynamic status in patients with HF. Angiotensin- converting enzyme inhibitors have acute and long-term beneficial haemodynamic effects in the failing heart [140, 141, 142, 143]. Long-term treatment (>3 months) with β-blockers increases CO and EF [66, 67] but given intravenously in the acute phase of HF, they cause a reduction in blood pressure and HR, and decrease CO while EF and intraventricular volumes remain unchanged [68, 69].

Nitrate tolerance

A once-daily sustained formula of IS-5-MN was used on the basis of earlier data showing that treatment with an appropriate dosing regimen is an effective method for preventing nitrate tolerance seen during continuous administration [115, 124]. It has also been demonstrated that retard formulations, correctly used, give long

duration of efficacy with no, or minimal, tolerance development with respect to antianginal efficacy [129]. Franciosa and Cohn [144] studied the effects of ISDN, 40 mg four times daily or placebo for 30 months in patients with chronic LV failure. The PAWP remained decreased after 3 months treatment. Moreover, an acute response to ISDN could still be elicited. Chronic therapy also significantly decreased arterial blood pressure and resistance. The authors concluded that there was no evidence of nitrate tolerance. Leier et al [145] also studied ISDN 40 mg given orally four times daily for 12 weeks in patients with congestive HF. The investigators concluded that chronic ISDN therapy was associated with persistent venous vascular and overall cardiovascular effects even though tolerance to nitrate administration apparently developed in arterial vascular smooth muscle. The effects of a calcium channel blocker and IS-5-MN for 4 weeks in patients with chronic cardiac failure were evaluated in a study by Hutton et al [146]. They found that treatment with a calcium channel blocker produced sustained ahemodynamic improvement and that a combination of a calcium channel blocker and a nitrate produced further benefit.

Since oxidative stress is important for tolerance and cross-tolerance, antioxidants or drugs, which are able to reduce oxidative stress may play an important role to prevent development of tolerance.

Co-treatment with ACE inhibitors [125, 126] and/or angiotensin II receptor antagonists [147] may inhibit tolerance. In one study Mehra et al [125] demonstrated that treatment with ramipril prevented nitrate tolerance to ISDN. In another, Katz et al [148] that captopril or enalapril prevented tolerance in normal subjects, suggesting that the sulfhydryl (SH) group in ACE inhibitors is not responsible for tolerance prevention. However, the clinical data are contradictory [149] which may be explained by different inclusion criteria and different functional tests to verify tolerance development.

Nitrate tolerance in patients with CHF may be prevented by β-blockers with anti-oxidant properties, such as carvedilol, but not with metoprolol [150].
Favourable interactions between nitrates and hydralazine have been demonstrated in 2 large trials, in the V-HeFT) [116] and in the A-HeFT) [117]. However, it is

not possible to determine the relative importance of the nitrate therapy in those studies, but prevention of tolerance to haemodynamic effects of nitrates with concomitant use of hydralazine in patients with chronic HF has been demonstrated in a study by Gogia et al [127].

Several studies have demonstrated that tolerance to the haemodynamic effects of nitroglycerin can be avoided using a nitrate-free interval or eccentric dosing approaches [114, 151]. On the other hand, endothelial dysfunction has been reported to persist despite such approaches[152].

To summarize, ways of decreasing or preventing tolerance include lowering plasma nitrate concentrations, and giving less frequent doses with prolonged nitrate-free intervals. In patients with ischaemic heart disease (IHD), these strategies may increase the risk for ischaemia and serious clinical events. Concomitant HF medications may modulate nitrate tolerance development.

Nitrate therapy is associated with an increase in intravascular volume and activation of neurohumoral counter-regulatory mechanisms, regarded as pseudotolerance. This has led to the hypothesis that increased volume and filling pressures would counteract the nitrate- induced decrease in pre-load, thus causing tolerance. However, plasma volume expansion may be induced, at least in part, by increased oxygen-free radical production [153].

We could not demonstrate any neurohumoral activation in our study that may have caused nitrate tolerance.

Today, there is little dispute that tolerance of nitrates occurs, particularly when they are the sole medication in the treatment of angina or CHF [124]. To prevent or at least reduce the development of tolerance, we combined a moderate, retard preparation dose of IS-5-MN with an ACE inhibitor (ramipril).

However, nitrate tolerance cannot be excluded. We did not study the acute effects of IS-5-MN before randomization or at the end of the study.

Sample size

The study was designed to detect haemodynamic changes (in PAWP) and not changes in hormonal or echocardiographic measurements. A sample size of 90 patients was calculated to allow detection of a difference in 3 mmHg of PAWP as a treatment effect with 90% probability and with an estimated drop-out rate of 30%. Thus, there was a 10% risk of not detecting a true treatment effect with regard to LV filling pressure.

Nitrates and oxidative stress

Increased formation of reactive oxygen species secondary to nicotinamide adenine dinucleotide phosphate (NADPH) oxidase(s) formation is an important cause of nitroglycerin tolerance [101]. However, we could not demonstrate any increase in plasma levels of MDA, a marker of oxidative stress. We have previously shown that an infusion of nitroglycerin can reduce P-MDA in patients with AMI treated with thrombolysis [105]. In the present study no effect of long-term oral treatment with a nitrate could be demonstrated (Paper II). These findings are in agreement with those of Jurt and co-workers [154] who found a differential effect of penta-erythritol tetranitrate and nitroglycerin on the development of tolerance and evidence of lipid peroxidation. In that study 30 healthy volunteers were randomized to a continuous infusion of nitroglycerin or to penta-erythritol tetranitrate given orally three times a day for 7 days. The nitroglycerin infusion was associated with a significant increase in cytotoxic aldehydes and isoprostenes while penta-erythritol tetranitrate was not [154]. Keimer et al [155] found no increase in urinary isoprostanes or 3-nitrotyrosine after 5 days of oral administration of ISDN or penta-erythritol tetranitrate in healthy volunteers. Our results add to previous reports the finding that nitrate therapy given to HF patients for several months does not appear to affect P-MDA concentrations.

IS-5-MN and neurohumoral activation

We observed a significant decrease in P-ANP in the nitrate group compared with the placebo group, while P-BNP was reduced similarly in both groups (Paper I). However, P-BNP was only analysed in a small subgroup of patients, which may have influenced the results. In a comprehensive analysis of neurohumoral activation we did not observe any effect of IS-5-MN on plasma levels of EPI, NEPI, Aldo, ADH, PRA, SP, NPY, CGRP or VIP. (Paper III)

The possible associations between nitrate therapy and neurohumoral activation have been evaluated in previous studies, but to our knowledge, the effects of nitrates on peptides such as SP, NPY, CGRP and VIP have not been previously investigated.

The haemodynamic and hormonal responses to 24 hours of therapy with transdermal nitroglycerin were studied in nine patients with severe CHF and in nine normal subjects [156]. In the CHF patients, RV and left LV filling pressures were decreased, but plasma levels of NEPI and PRA were unchanged.

In normal subjects, peripheral vasodilatation was accompanied by sympathetic activation, reflected by an increase in HR and plasma NEPI.

Similar results have been reported using transdermal nitroglycerin (90mg) for 24 hours [157] and after using a single dose of ISDN (40 mg) [158].

In the Nitrates in Congestive Heart Failure (NICE) study [138], IS-5-MN (50 mg once daily) or placebo was administered to CHF patients (n = 136) in NYHA class II – III for 12 weeks. All of the patients received captopril and the majority received furosemide in addition. No IS-5-MN effect was seen on plasma NEPI and ANP.

In summary, previous studies with different patient characteristics, route of administration and duration of therapy have not been able to demonstrate any effect of nitrate therapy on NEPI or PRA levels. We obtained concordant results in our study with a longer duration of treatment. Our finding of reduced P-ANP levels as a result of nitrate therapy is inconsistent with the NICE outcomes.

Nitrates and diastole

We did not observe any effect of IS-5-MN on diastolic function (Paper IV). In a study by Kiraly et al [159] long-term (i.e. 6 months) transdermal nitrate treatment (0.2 mg/h) after a myocardial infarction was associated with changes in pulmonary venous flow and EF, indicating better preserved diastolic and systolic functions of the left ventricle in response to nitrate treatment. There were no statistical differences in E/A and DT when the nitrate-treated group were compared with the control group. The authors suggested that (a) the transmitral inflow pattern was not a reliable indicator because of relatively preserved (i.e. only mildly reduced) LV systolic function (EF); (b) the pulmonary venous flow indices are more sensitive indicators of diastolic function compared with E/A or DT [15]; and (c) nitrates exert a more pronounced effect on the pre-load than on the afterload.

The study by Kiraly et al [159] differs from our study in several respects, namely with regard to the concomitant medication, the route of administration (transdermal v oral), the shorter duration of treatment and the number of HF patients in the study population.

Nitrates – different sites of action?

In the Introduction, we discussed the direct effects of NO on the myocardium, stating that nitrates may have effects independent of the vasodilatory effects of the substances [87, 88]. Hill et al [160] have shown that nitroglycerin elicits protective effects against infarction (late preconditioning) despite development of nitrate tolerance. In a pre-specified subgroup of patients with LVEF ≤40% we found that the EDVI increased significantly more in the placebo group than in the IS-5-MN group (Paper I). This finding was of borderline significance. Also the statistical significance was not adjusted for multiple testing and this should therefore be regarded merely as hypothesis generating. However, our results are in agreement with a study by Mahmarian et al [161] and with results from an animal study

[162]. Both studies showed that nitrates prevent remodeling after AMI. There are consequently good reasons to further study the effects of nitrate therapy on remodeling and preconditioning.

5.2 Lipid peroxidation

We studied plasma levels of MDA in two groups of patients with CHF and found no increase compared with healthy controls (Paper II). In one of the groups we also analysed P-8-isoprostane but could demonstrate no increase. For none of these markers could a positive correlation between the plasma levels and the severity of HF be demonstrated. In study group I we observed a negative correlation between P-MDA and P-BNP, which may have been a chance finding as it could not be reproduced in study group II.

Our results are divergent from many previous reports which have consistently demonstrated increases in various markers of oxidative stress in patients with HF, such as plasma and urinary MDA [76, 77], breath pentane [78], glutathione peroxidase [75], plasma lipid peroxides [163], and urinary [79] and pericardial isoprostanes [80]. In some of these studies positive correlations between the plasma level of these markers and indices of CHF severity such as NYHA class [73, 77], ventricular dimensions [80], exercise capacity [164] and LVEF [163] have been shown.

Several differences exist between our study and those previously reported. In our first subgroup all patients had CAD while most other investigations included patients with HF of different origin. However, Keith et al [73] and McMurray et al [165] found increased P-MDA levels both in patients with HF of ischaemic origin and in patients with other causes of HF.

We also included a substantial number of patients with normal or slightly depressed EF. In studygroup II we even included patients with non-ischaemic origin of CHF. Consequently, several patients with predominantly diastolic dysfunction were included, which may explain the insignificant relationship between LVEF and NYHA in our study. The average EF in our first study (group)

was 42% while in the second study (group) it was 37%. By contrast, mean EF was 32% in the study by Keith et al [73] and 28% in a study by Diaz-Velez et al [77]. In the study by McMurray et al [165], mean LV fractional shortening (LVFS) was 14% and 13.1% in IHD patients and non-IHD patients, respectively, which indicates a severely depressed EF. Even though we included many patients with minimally depressed EF, a substantial portion of our patients had signs of severe HF. If oxidative stress would have been confined to such patients, this should have been detected. We also investigated the relationship between P-MDA and 8-isoprostane concentrations on the one hand and several markers of HF severity such as EF, CI, PAWP, P-BNP level and NYHA class on the other hand, but found no evidence of a positive correlation between the markers of oxidative stress and markers of HF severity (Paper II). The number of patients in our study should have been sufficient to detect changes in P-MDA and 8-isoprostane. In fact, our study is one of the largest addressing the role of oxidative stress in HF.

Other explanations for our negative findings could be differences in diagnostic methods [76, 77], duration of HF and the timing of lipid peroxidation assessment [73, 74, 76, 77, 79, 80, 163, 164, 166]. Differences in analytical methods seem unlikely as we used a similar HPLC method for the assay of P-MDA as employed by Diaz-Velez et al [77]. We have also used that method in previous studies and demonstrated increased P-MDA levels in patients treated with thrombolysis for AMI [105] and patients receiving 5-fluorouracil as anti-cancer treatment (to be published). The analysis of P-MDA is sensitive for haemolysis, which explains the reduced number of paired samples in our study. In the present study, P-8-isoprostane was analysed with a commercially available assay and using this method we have previously demonstrated an increase in P-8-isoprostane level in patients with CAD and high plasma homocysteine [167].

Mallat et al [80] found increased levels of isoprostanes in the pericardium of patients with HF and the levels correlated with increased severity of the disease. Cracowski et al [79] report increased levels of urinary isoprostanes in CHF patients. It is possible that the concentrations of isoprostanes may vary between different body fluids, making it more difficult to detect increased plasma levels in

CHF patients. Such a divergence in plasma and urinary levels of isoprostanes was found by Feillet-Coudray et al [168] who report increased urine and plasma levels of MDA in diabetics. Isoprostanes were also increased in urine but not in plasma. On the contrary, a strong trend towards a decrease in plasma levels was found.

A third explanation for our negative finding of oxidative stress was the pharmacological treatment of HF. All patients in our study received an ACE inhibitor or an AT_1 blocker and the majority were on β-blockers. In all other studies the percentages of patients on ACE inhibitors were lower. The use of a β-blocker has either not been reported elsewhere or was substantially lower than in our study.

Kukin et al [166] report that in their study, both metoprolol and carvedilol reduced levels of thiobarbituric acid-reactive substance (TBARS), a MDA-like material, in patients with HF. Lysko, et al [169] found that carvedilol but not metoprolol prevented an increase in TBARSs in cells exposed to oxidative stress (FeC12/ascorbate) in vitro, suggesting a direct scavenging effect of carvedilol.

Work from the Mayo Clinic [170] showed that a low dose of angiotensin II given as a continuous infusion for 7-30 days can increase systemic blood pressure. The pressor response was accompanied by increased oxidative stress, as measured by an increase in plasma levels of isoprostanes. Plasma angiotensin II levels are increased in CHF, which probably leads to increased oxidative stress. Consequently one beneficial effect of ACE inhibitors or AT_1 blockers may be reduced oxidative stress. Numerous studies have demonstrated an anti-oxidative effect of various ACE inhibitors in cell cultures, animal studies and humans [171].

5.3 Neurohumoral activation and its relationship to CHF severity

Levels of neurohormones and peptides in patients were not compared with those of controls which precluded a formal statistical analysis (Paper III). However, if the medians and interquartile range (IQR) are compared with the laboratory reference values it becomes apparent that a substantial proportion of the patients

had raised plasma levels of BNP, NEPI, PRA and NPY, while the levels of the other analysed substances were within the reference ranges in most patients.
The inclusion criteria were similar to those in the AIRE study [172], which included patients with low LVEF and/or clinical evidence of HF regardless of LVEF values. We found that the correlation between LVEF and PAWP was weak (-0.27) (Paper II), and statistically insignificant. Therefore it seemed essential to relate neurohumoral activation not only to echocardiographic measurements of systolic dysfunction and remodelling but also to invasive measurements of filling pressures.

Numerous studies have demonstrated that NEPI is increased in HF [28, 29] and that elevated levels signal a worse prognosis [30]. In our study NEPI was significantly correlated with LVEF and NYHA class but not with PAWP.
When studying the effect of beta-receptor antagonists on neurohumoral activation in patients with CHF following AMI [25], a significant association was found between NEPI and LVEF but not between NEPI and NYHA class.

Few patients in our study demonstrated increased EPI values and we found no correlations between EPI and markers of HF severity, which is consistent with results reported previously [25], showing no increase in EPI in CHF patients following AMI.

The majority of patients had elevated levels of PRA, which may be explained in part by the use of ramipril and diuretics. Plasma renin activity is well known to be influenced by medical treatment. Diuretics [23] and ACE inhibitors [24] are known to increase PRA levels while β-blockers [26] decrease PRA levels. Therefore, the effect of medication may partially account for the significant correlation between PRA and LVEF and NYHA class in our study.

Elevated Aldo is associated with a reduced EF [26] as well as with increased mortality [27] in patients with chronic systolic HF, but is also influenced by medical treatment. Aldosterone is increased by activation of the RAS and consequently inhibitors of that system such as ACE inhibitors [24], AT_1 blockers and β-blockers [25] reduce plasma levels of the hormone. Few patients in our study had elevated levels that may reflect the use of ramipril in particular and β-blockers.

Anti-diuretic hormone/vasopressin is increased in severe HF [32]. In our study moderate HF was predominant and may explain the low ADH levels generally detected. Nevertheless, ADH was significantly associated with LVEF and NYHA class like NEPI and PRA.

Neuropeptide Y exhibits a wide spectrum of central and peripheral activities and is the most abundant peptide present in the mammalian brain. In the periphery, NPY is generally found co-stored and co-released with NEPI [173]. In the cardiovascular-system, it acts as a potent vasoconstrictor [174].

Nearly half of the patients in our study had increased levels of NPY which is consistent with previously published reports on increased levels in CHF [25, 40, 41, 42]. However, we did not observe any significant correlations with markers of HF severity. A similar lack of correlations with EF and CI has been reported by other investigators [40]. On the other hand, Persson et al [25] found significant correlations with wall motion index and fractional shortening.
In one CHF-study [41] increased catecholamine levels were accompanied by NPY release already in mild HF, without a further increase in cardiac failure classified as NYHA class III-IV.

The majority of the circulating level of SP is generally considered to be derived from perivascular nerves, where it may participate in the regulation of vascular tone [116] and may act as a vasodilator, counteracting vasoconstrictive factors in CHF [41]. Few patients in our study had elevated levels and surprisingly, we demonstrated that levels of SP correlated positively with CI. These findings do not support previously published results showing increased plasma levels in moderate and severe CHF [41]. In one study in HF patients on ACE inhibitor therapy, SP levels were higher than in patients not treated with ACE inhibitors [175].

Calcitonin gene-related peptide is a potent vasodilator [43] synthesized and released from small, capsaicin-sensitive sensory nerves. It has potentially beneficial effects in CHF by virtue of its potential inotropic action and vasodilation [43]. Taquet et al [176] found decreased levels of CGRP in CHF patients compared with healthy controls; by contrast Edvinsson et al [41] report unchanged levels. Our laboratory did not provide any lower reference limits which precluded any

evaluation of decreased plasma levels in our study. However, a significant negative correlation with LVEF was found suggesting that CGRP is released in severe failure, possibly as a compensatory measure for vasoconstriction.

Vasoactive intestinal peptide was originally isolated from intestinal extracts and has been shown to be a potent vasodilator. It is widely distributed in the peripheral and central nervous systems and counteracts the vasoconstrictive effects of the sympathetic system and the RAS. It has a positive inotropic effect and increases HR and may also play a role in the regulation of coronary artery flow [177].
With regard to VIP levels in CHF patients, in one study in humans [178], it was reported that the plasma concentration of VIP was not higher than normal in a whole group of CHF patients and did not correlate with the echocardiographic data. Moreover, other investigators [41] also found no change in VIP levels in CHF patients. Few patients in our study had increased levels and without a lower reference limit we were unable to assess whether a tendency towards decreased values exists in CHF patients. However, no correlation to markers of CHF severity was found in our study.

5.4 Changes in haemodynamics, echocardiographic measurements and neurohumoral activation in the total study population from randomization to the 12-month visit

Changes in haemodynamics and neurohumoral activation from pre- to post-treatment following AMI

Atrial natriuretic peptide and BNP correlated significantly with LVEF and PAWP (Paper II), and both natriuretic peptides decreased during follow-up. Iwanaga et al [179] have shown that BNP levels reflect LV end-diastolic wall stress (EDWS) more than EF, LVEDP, or LV end-systolic pressure (LVESP). The main determinants of EDWS are LVEDP, LVID and wall thickness. Pulmonary artery wedge pressure reflects LVEDP, but no significant change in PAWP was observed in the present study. However, we cannot exclude the possibility that a small

change in PAWP was not detected by catheterization. This is supported by the fact that the change in P-BNP correlated significantly with the change in PAWP. A decrease in LVID could be another possible explanation for our results, but this is not supported by echocardiographic findings of unchanged LV end-diastolic volume (LVEDV). Finally, reduced EDWS could also be the effect of remodeling resulting in increased wall thickness or reduced myocardial ischaemia.

By contrast, NEPI, and Aldo, which both correlated with markers of systolic dysfunction but not with PAWP, showed slight but statistically significant increases during the study (Paper III). The increase in EPI was of borderline significance. The stimuli responsible for activation of the sympathetic nervous system in HF are yet to be determined. However, sympathetic activation appears to be linked to the structural and functional changes in the heart during cardiac remodeling [180]. Therefore, our finding of slight increases in EPI, NEPI and Aldo may represent a small and slow, but continuous impairment of the LV function and an ongoing remodeling, which the echocardiographic studies were not sensitive enough to detect.

Brain natriuretic peptide levels predict morbidity and mortality in patients with HF [34]. Troughton et al [36] demonstrated that therapy guided by BNP levels reduces cardiovascular events compared with intensive, clinically guided therapy. Consequently, there is a strong trend to use BNP for monitoring of HF therapy. However, our results suggest that BNP levels do not reflect all important pathophysiological mechanisms in HF: decreased EDWS may coexist with a continuous remodeling and impairment of LV function.
Consequently, use of neurohormones other than BNP for monitoring of HF therapy should be explored.

As previously mentioned, NPY is generally found to be co-stored and co-released with NEPI [173]. Nevertheless, we found a temporal dissociation between these two hormones: NEPI increased after 11 months while NPY decreased. Persson et al [25] also report a temporal dissociation between NEPI and NPY, but in contrast to our results, in their study NEPI decreased and NPY increased. Whereas NEPI is mainly released during low-frequency nerve stimulation, NPY

release requires nerve activation at a higher frequency [181]. Therefore, our results may indicate a change in mode of sympathetic activation during the study. However, an improved clearance of NPY via hepato-mesenteric circulation is an alternative explanation for the decrease in NPY [25].

In our study VIP increased only slightly; however the rise was highly significant. A transient increase in VIP at the time of an AMI has been reported by Caiola et al [182]. This was followed by an abrupt decrease below normal values after 24 hours. In that study, VIP reached its lowest concentration 48 hours after the onset of symptoms of AMI, and gradually returned to the normal concentration by day 14.

Explanations for the increase in VIP in our study are purely speculative. The increase may indicate an improvement in myocardial function with less ischaemia and reduced filling pressure or it may represent a counter-regulatory mechanism against reduced systolic function, or a vasoconstriction.

Changes in diastolic function during 1 year after AMI

We analyzed changes in diastolic function in the total patient population since we did not demonstrate any significant differences in diastolic function between patients on IS-5-MN therapy and patients on placebo. It has been shown that in approximately one-third of patients with HF after AMI, diastolic dysfunction is the probable cause of the signs and symptoms of HF [8, 183]. Several authors have demonstrated that assessment of LV filling provides independent prognostic information post-AMI [184, 185, 186, 187, 188].

We found changes in diastolic parameters, indicating improved diastolic function from randomization to 12 months after the index infarction, and/or reduced preload in spite of unchanged systolic function (LVEF, CI, SI etc). However, the PAWP was not significantly reduced in the total study population during the study period (Paper III), although several changes in diastolic measurements were correlated to changes in PAWP.

Both P-ANP and P-BNP decreased (Paper I, II) which also indicates a long-term reduction in LV pre-load [8]. Consequently, as previously stated, we cannot exclude the possibility that a small change in PAWP was not detected by catheterization.

5.5 Differences between patients with LVEF <45% and patients with LVEF ≥45%

In the present study most patients demonstrated only mildly or moderately reduced LVEF at randomization. However, 79% of all included patients had an LVEF≤40% during hospitalization for the index AMI, compared with LVEF 46% at randomization. Consequently, some patients with normal or only slightly reduced LVEF at randomization had moderately reduced LVEF during hospitalization. Therefore, HF signs during hospitalization for the index AMI were not entirely due to diastolic dysfunction in the group of patients with LVEF ≥45% at randomization.

We found that patients with symptoms and signs of HF during hospitalization but normal or slightly reduced LVEF 1 month later showed signs of better systolic function and smaller LV and LA dimensions, less functional impairment (according to the NYHA classification) and less pronounced neurohumoral activation compared with patients with LVEF <45% at randomization. There was a non-significant trend towards lower PAWP in patients with preserved LVEF. No significant difference in the mitral filling pattern between the two groups could be demonstrated.

The current study was too small to provide a clinical prognosis, but patients with signs of HF and LVEF ≥45 % 1 month after suffering AMI seemed to have a more favourable pattern in terms of prognostic markers, than patients with a more reduced LVEF, despite the fact that a substantial part of the latter category did no show any clinical signs of HF during hospitalization (Paper I). Similar findings were reported by Kitzman et al [189]. They found that patients with isolated diastolic HF had similar but less severe pathophysiologic characteristics compared

with patients with typical systolic HF. The latter group had higher levels of BNP and a reduced ventilatory anaerobic threshold and tended to have impaired quality of life in the Minnesota Living with Heart Failure Questionnaire [190] compared with the group with isolated diastolic HF. However, peak oxygen consumption and NEPI levels did not differ significantly between the two groups.

6 Conclusions

We concluded that long-term IS-5-MN therapy in patients with LV dysfunction after AMI already treated with standard HF therapy-

- does not affect haemodynamic measurements;
- contributes to decreased LV dilatation in patients with more severe LV dysfunction;
- does not affect LV diastolic function;
- reduces the need for additional diuretics;
- does not affect lipid peroxidation measured by plasma MDA; and
- does not significantly affect the neurohumoral status, apart from a decrease in plasma ANP.

We also concluded that-

- lipid peroxidation is not increased in patients with LV dysfunction treated with standard HF therapy;
- there is no significant correlation between markers of oxidative stress and markers of HF severity; and
- there are significant correlations between neurohumoral activation and markers of the severity of HF (BNP-ANP, ANP-PAWP, BNP-EF and BNP-PAWP).

In our study population, during 1 year after AMI there was an improvement in NYHA class, an increase in catecholamines (EPI, NEPI), Aldo and VIP, and a decrease in ANP, BNP, ADH and NPY.

Finally, the observed changes in mitral filling pattern during 1 year after AMI indicate improved LV diastolic function. We concluded that prognostic factors are more favourable in patients with normal or slightly depressed LVEF than in patients with more severely depressed LVEF.

7 Populärvetenskaplig sammanfattning (Summary in Swedish)

7.1 Bakgrund

Nyligen genomförda undersökningar pekar på att mellan en och två procent av den vuxna befolkningen lider av *hjärtsvikt* (hjärtinsufficiens/hjärtinkompensation), en oförmåga hos hjärtat att med ett normalt fyllnadstryck förse kroppens olika organ med blod.

Vanligtvis är orsaken primärt *kardiell* (utgående från hjärtat) där *ischemisk hjärtsjukdom* (kranskärlssjukdom) utgör den största gruppen antingen orsakad av en hjärtinfarkt, där den kvarvarande hjärtmuskeln är otillräcklig för att upprätthålla en adekvat pumpförmåga, eller orsakad av en fortlöpande hjärtmuskelskada orsakad av kronisk syrebrist i hjärtmuskel på basen av kranskärlsförträngningar. Andra orsaker kan vara högt blodtryck (hypertoni), klaffel, hjärtmissbildningar, primära hjärtmuskelsjukdomar (kardiomyopatier), infektioner eller hjärtrytmrubbningar vilka alla kan bidra till att skador uppkommer på hjärtmuskeln. Till detta kommer en mängd andra orsaker såsom sköldkörtelsjukdom, blodbrist och elakartade tumör- sjukdomar.

Hjärtsvikten kan i princip indelas i en *akut* eller *kronisk* form samt i *systolisk* eller *diastolisk* form. *Systolisk* svikt innebär att hjärtats vänsterkammare har en försämrad funktion att dra ihop sig/pumpa ut blod. Isolerad *diastolisk* svikt, vilket föreligger hos ca 50 % av hjärtsvikts- patienterna, innebär istället en störning av hjärtats möjlighet att fylla sig.

Effekter på cirkulationen

Hjärtsviktens effekter på cirkulationen riktar sig både framåt och bakåt i blodströmmens riktning varför man kan beskriva två former av svikt, *forward* respektive *backward failure.*

Ett sviktande hjärta måste generera ett betydligt högre fyllnadstryck för att producera en lika stor slagvolym som ett friskt hjärta. Denna tryckstegring ses även i hjärtats förmak vars tryck måste vara högre än det i kamrarna för att dessa skall kunna fyllas under hjärtats ”avslappningsfas”.

Om hjärtats vänstra kammare sviktar sker en tryckökning i lungcirkulationen och vid högerkammarsvikt i vensystemet som ett tecken på *backward failure*. Om en uttalat nedsatt pumpförmåga föreligger kan en tillräcklig slagvolym inte åstadkommas trots ett högt fyllnadstryck utan såväl *forward* som *backward failure* föreligger.

Kompensationsmekanismer vid hjärtsvikt

För att motverka de negativa effekterna av en sviktande hjärtpumpförmåga aktiveras ett antal försvarssystem av cirkulerande substanser (*neurohumoral aktivering*) vilka påverkar såväl cirkulationen som hjärtat självt (*dilatation*/utvidgning och *hypertrofi*/förtjockning).

Neurohumoral aktivering

Kroppen kan kompensera och bemästra den sviktande hjärtpumpförmågan genom neurohumoral aktivering. I tidigt skede är detta till nytta men på sikt leder det till negativa effekter på cirkulations- systemet. När hjärtat inte förmår upprätthålla cirkulationen och blodtrycket sviktar sker en kompensatorisk pulsökning jämte frisättning av *stresshormon* från binjurarna (*adrenalin* och *noradrenalin*) som påverkar såväl hjärta som kärl.

Från hypothalamus ökar frisättningen av anti-diuretiskt-hormon (*ADH*) och från njurarna *renin* varvid *renin-angiotensin-aldosteronsystemet (RAAS*) aktiveras. I cirkulationen omvandlar renin angiotensinogen till angiotensin I. Det cirkulerande enzymet *angiotensin-converting enzyme* (*ACE*) kan i sin tur omvandla angiotensin I till angiotensin II som i sin tur påverkar frisättningen av *aldosteron* och ADH.

En *neurohumoral* aktivering är nu etablerad där kärlsammandragning i kombination med salt och vätskeansamling och direkt hormonell påverkan på hjärtat leder till en *remodeling* ,som innebär att hjärtmuskeln förtjockas och bindväv inlagras. När hjärtat tillväxer räcker inte de små blodkärlen till för att förse muskeln med syre och näring, vilket kan leda till *celldöd.* Celldöd kan också inträffa som en följd av ”programmerad” celldöd (*apoptos*)
En negativ spiral skapas med *neurohumoral aktivering – muskeltillväxt – celldöd – neurohumoral aktivering*, vilket leder till en alltmer försämrad hjärtfunktion.

Farmakologisk behandling av hjärtsvikt

Syftet med all behandling är att öka livskvaliten och förbättra överlevnaden. Den farmakologiska behandlingen av hjärtsvikt innefattar i princip *diuretika* (vätskedrivande), *beta-blockerare* samt *ACE-hämmare* och/eller *Angiotensin II antagonister*. Behandling med ACE-hämmare är etablerad efter att ett stort antal studier (CONSENSUS, SAVE, SOLVD) visat minskad dödlighet och ett minskat behov av sjukhusvård hos patienter med hjärtsvikt.

När det gäller övriga läkemedel har bl.a *långverkande nitrater* prövats som tillägg till övrig terapi. Den dominerande användningen av nitrater har varit vid behandling av *kärlkramp* men kliniska studier (V-HeFT) har visat att höga nitratdoser i kombination med *hydralazin* minskar dödligheten vid hjärtsvikt. Den enskilda betydelsen av nitrater är dock oklar även om kliniska erfarenheter talar för att långverkande nitrater minskar symptomen hos hjärtsviktspatienter som redan behandlas med vätskedrivande och/eller ACE-hämmare.

Huvudsyftet med denna vetenskapliga studie var att utvärdera *långtidseffekterna* av *isosorbid-5-mononitrat* (*IS-5-MN*) hos patienter med *nedsatt hjärtpumpförmåga* efter genomgången *hjärtinfarkt* och som redan behandlas med *sedvanliga hjärtsviktsläkemedel*.

Detta gjordes genom *följande frågeställningar*:

- Hur påverkar IS-5-MN *hemodynamiken* (pumpförmågan)?
- IS-5-MN och *hormonella/endokrina effekter*?
- IS-5-MN – *systoliska* o *distoliska effekter*?
- IS-5-MN – effekter på *oxidativ stress*?

7.2 Material och Metoder

För att utvärdera effekten av nitrater till patienter med hjärtsvikt studerade vi *två grupper av patienter*, dels en grupp av *92 män och kvinnor* som i nära anslutning till en hjärtinfarkt uppvisade tecken på nedsatt hjärtpumpförmåga eller hjärtsvikt, dels en grupp *hjärtsviktspatienter* som kontrollerades *på en hjärtmottagning*.

I gruppen med de *92 patienterna* definierades hjärtsvikt genom kliniska tecken, röntgenologiska tecken eller som en nedsatt hjärtfunktion vid en *hjärtultraljudsundersökning* (*ekokardiografi**)

En månad efter hjärtinfarkten randomiserades patienterna till behandling med *IS-5-MN eller placebo* (icke aktivt ämne). För att utvärdera hjärtfunktionen utfördes i samband med randomiseringen *ekokardiografi**, *hjärtkateterisering**, ekg*-kontroll samt omfattande *blodprovskontroll****. Under det följande året genomfördes upprepade *kliniska utvärderingar* samt *blodprovstagningar*. Efter cirka *11 månader* kallades till ett *slutbesök* i studien och undersökningar enligt samma princip som vid randomiseringsbesöket. I samband med detta utsattes behandlingen med IS-5-MN eller nitrater och en slutlig ultraljudsundersökning planerades en månad senare.

För att analysera huruvida den *oxidativa stressen* (angrepp från fria radikaler) vilken anses bidra till hjärtskada, är ökad hos patienter med hjärtsvikt studerades även en grupp *hjärtsviktspatienter* på *hjärtmottagningen*. Som referensmaterial

utfördes motsvarande analyser även på en grupp *friska blodgivare.* Här utvärderades nivåerna av *malondialdehyd* (*MDA*) samt *isoprostaner*.

*Ekokardiografi/Dopplerundersökning**

Ett sätt att objektivt värdera hjärtats anatomi och funktion. I denna studie undersöktes gruppen av 92 patienter vid 4 tillfällen. 1. under vårdtiden för den akuta hjärtinfarkten, 2. vid inkluderingsbesöket en månad senare, 3. efter 1 år. I samband med 12 månaders besöket avslutades behandlingen (IS-5-MN/Placebo) varefter patienterna kallades för en avslutande ekokardiografisk undersökning en månad senare (4). Resultaten från samtliga undersökningstillfällen dokumenterades enligt ett förutbestämt protokoll.

I gruppen av hjärtsviktspatienter som ingick i analysen avseende oxidativ stress inhämtades tillgängliga ekokardiografiska data från hjärtmottagningen.

*Hjärtkateterisering***

I gruppen av de 92 patienterna med nedsatt hjärtpumpförmåga efter hjärtinfarkt genomfördes s.k högersidig hjärtkateterisering vid 2 tillfällen. Dels vid randomiseringsbesöket dels 11 månader senare i samband med att patienten avslutade studien. Undersökningen utfördes med hjälp av en Swan-Ganz kateter som via ett kärl på halsen alternativt i armvecket fördes in till hjärtat. Mätningar utfördes i vila, men om så var möjligt även under belastning i form av bencykling i liggande.

*Blodprovskontroller****

Under studien analyserades blodprover av mer rutinkaraktär vid det kliniskt kemiska laboratoriet medan prover rörande förekomsten av oxidativ stress (MDA, isoprostaner) samt neurohumoral aktivering analyserades vid forsknings-laboratorium.

Rutinblodprover analyserades vid inkludering, efter 6 veckor, 3, 6 och 12 månader och analyser avseende neurohumoral aktivering vid randomisering och vid slutbesök.

Övrigt

Patienter i studien kontrollerades i samband med regelbundna *mottagningsbesök*. Vid behov *justerades* aktuella *läkemedel* och vid behov utfördes *kompletterande undersökningar.*

7.3 Resultat

Effekten av ett långverkande nitroglycerinpreparat studerades således under ett år hos en grupp patienter med nedsatt hjärtpumpförmåga efter hjärtinfarkt. Jämfört med gruppen som erhöll placebo noterades inga säkra skillnader avseende värdena som uppmättes vid *hjärtkateterisering*. Vid *ultraljudsundersökning* fann man att bland de patienter, som vid studiestarten uppvisat den sämsta hjärtfunktionen, fick de nitroglycerinbehandlade en mindre ökning av sin diastoliska hjärtvolym under året. Att behandla med IS-5-MN minskade även behovet av *vätskedrivande läkemedel* samt sänkte koncentrationen av *atrial natriuretic peptide* (*ANP*), tydande på en avlastning av hjärtat. En mer detaljerad analys avseende den neurohumorala aktiveringen visade inga signifikanta (säkra) skillnader mellan dem som behandlades med nitroglycerin eller dem som erhöll placebo (förutom ANP).
I gruppen som helhet fanns ett samband mellan hjärtats diastoliska funktion och nivån av *ANP* samt *brain natriuretic peptide* (*BNP*). När det gällde den systoliska funktionen fanns ett samband med *renin* samt stresshormonen *adrenlin/noradrenalin*. Ett år efter hjärtinfarkten hade i gruppen som helhet nivåerna av *ANP* och *BNP* sjunkit medan *noradrenalin* och *aldosteron* gått upp något.

Hjärtats diastoliska funktion förbättrades i gruppen som helhet men de som behandlades med IS-5-MN uppvisade inga säkra skillnader mot de som fick

placebo. När det gällde prognostiska faktorer sågs flest ogynnsamma (mer neurohumoral aktivering, större hjärtrum etc) i gruppen med den mest nedsatta pumpförmågan.

När nivåerna av *MDA* samt *isoprostaner* analyserades hos hjärtsviktspatienter och kontroller fann vi inga skillnader mellan grupperna. Att behandla med nitroglycerin påverkade inte nivåerna av MDA.

7.4 Slutsatser

- *Blodflöden och blodtryck* påverkas inte av behandling med IS-5-MN.
- Behovet av *vätskedrivande* läkemedel minskar av behandling med IS-5-MN
- Behandling med IS-5-MN minskar *hjärttillväxten* hos dem med sämst hjärtfunktion
- Behandling med IS-5-MN sänker nivåerna av *ANP* som tecken på mindre hjärtbelastning
- Fler markörer än *BNP* för monitorering av hjärtsviktspatienter bör studeras
- Hos hjärtsviktspatienter med måttliga besvär och som behandlas med moderna hjärtsviktsläkemedel förbättras den *diastoliska funktionen* under året efter en hjärtinfarkt.
- De patienter som hade sämst hjärtpumpförmåga hade också fler *andra negativa prognostiska markörer*
- Hos hjärtsviktspatienter som behandlas med moderna hjärtsviktsläkemedel ses inga tecken på ökad *oxidativ stress.*

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