Pathophysiological role and clinical relevance of cytokines in hypertensive heart failure

A combined clinical and experimental study

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"Vi er alle oppdagelsesreisende i livet,
hvilken vei vi så enn følger."

Fridtjof Nansen (1861 – 1930)

Abstract

Although mortality due to heart failure has decreased significantly in the last decade since the introduction of ACE-inhibitors and β-adrenergic receptor blockers in the management of heart failure, chronic heart failure is still one of the most important causes of morbidity and mortality and has a very high frequency for readmission to hospitalization because of the aggravation of heart failure. This accounts for a significantly higher health-care expenditure that is twice as much the cost for cancer. One of the most important reasons is that the present heart failure management is mostly directed at the restoration of neurohormonal imbalance, rather than targeting the primary mechanism of the disease. There is an increasing body of evidence showing that inflammation is involved in the pathophysiology of heart failure, particularly in the young. Moreover, one of the most frequently seen sub-sets of heart failure in daily clinical practice is heart failure due to hypertension, particularly in the elderly. Heart failure in the elderly is often characterized by predominance of diastolic dysfunction and comorbidity. Therefore, heart failure is heterogeneous and the underlying mechanism may differ greatly from one cause to another and between the young and the elderly.

The portfolio of cytokines includes at least tumor necrosis factor α and interleukin-6 in the cardiovascular system. These have been referred to as proinflammatory cytokines. These inflammatory mediators are known to be expressed by all nucleated cell types residing in the myocardium including myocytes, suggesting that these molecules may not only orchestrate an inflammatory response but also participate directly in the pathophysiological processes such as remodelling. Thus, it is very likely that the elaboration of cytokines represents not only the mechanism responsible for worsening of heart failure, but also the mechanism of initiating heart failure. In heart failure in younger patients, the circulating level of tumor necrosis factor α has been shown to be elevated.

However, whether this is similarly elevated in elderly heart failure patients remains poorly understood. Since majority of heart failure patients are over 65 years old, this issue becomes very important and clinically relevant. Furthermore, recent large randomized clinical trials of tumor necrosis factor antagonists in heart failure patients in the younger population showed disappointing results. Therefore, the significance of cytokine activation in heart failure remains controversial. Is cytokine activation important in heart failure? Is this equally important in heart failure irrespective of age? If so, why was tumor necrosis factor α inhibition not effective in chronic heart failure in recent clinical trials?

We hypothesize that proinflammatory cytokine activation is pathophysiologically important and clinically relevant in hypertensive heart failure. However, cytokine activation may differ between the young and the elderly. Moreover, suppression of a single cytokine in heart failure may not be a rational and effective treatment strategy because it may lead to the upregulation of other proinflammatory cytokines.

In this PhD thesis, we studied cytokine mRNA expressions in myocardium in the early stage of hypertensive heart failure in Spontaneously Hypertensive Rats with or without treatment with tumor necrosis factor α antagonist Etanercept. Moreover, we characterized circulating cytokine profile among heart failure patients in the elderly.

We have shown that there were cardiac hypertrophy in Spontaneously Hypertensive Rats with increased heart/body weight ratio, diastolic heart dysfunction as determined by the tissue Doppler, increased mRNA levels for interleukin 6 and brain natriuretic peptide whereas a decreased mRNA for the β-adrenergic receptor. Chronic treatment with etanercept in

Spontaneously Hypertensive Rats resulted in decreased relative wall thickness as well as increased cardiac reserve and higher blood pressure. In addition, interleukin-6 was further upregulated compared with placebo treatment. In human studies, our results have shown increased interleukin-6 and tumor necrosis factor α in heart failure patients compared with those in the control group. Moreover, interleukin-6, tumor necrosis factor α and vascular endothelial growth factor were significantly increased in patients who died within one year. Further logistical regression analyses showed that interleukin-6 is the only significant predictor for one-year mortality. In a subgroup of heart failure with atrial fibrillation there were significant cytokine activations, whereas in subgroups such as those with ischemia or diabetes, cytokines were less activated. Furthermore, we have shown significantly increased adiponectin levels in these elderly heart failure patients.

In conclusion, in hypertensive heart failure, both clinically and experimentally, there is increased cytokine activation. In case of heart failure in the elderly, there is different activated cytokine profile as compared with that in the younger. Moreover, it appears that it is not enough to suppress one single cytokine because the cytokine network is redundant. However, more fundamental studies are needed to understand this complex cytokine network before an appropriate anti-inflammatory therapy emerges.

Key Words: hypertension, cardiac remodelling, heart failure, cytokines, interleukin 6, etanercept, adiponectin, elderly.

List of Publications

I. Haugen E, Chen J, Wikström J, Grönros J, Gan LM and Fu LXM.
Parallel gene expressions of IL-6 and BNP during cardiac hypertrophy complicated with diastolic dysfunction in spontaneously hypertensive rats.
International Journal of Cardiology. 2007; 115: 24-28

II. Haugen E, Scharin Täng M, Isic A, Andersson B, Fu LXM.
TNF-alpha-antagonist upregulates interleukin-6 in rats with hypertensive heart failure.
International Journal of Cardiology. 2007 (in press)

III. Haugen E, Gan LM, Isic A, Skommevik T, Fu LXM.
Increased IL6 but not TNF-alpha predicts mortality in heart failure population in the very elderly.
Experimental & Clinical Cardiology. 2007 (in press)

IV. Haugen E, Furukawa Y, Isic A, Fu LXM.
Increased adiponectin levels in parallel with increased NT-pro BNP in patients with severe heart failure in the elderly: a hospital cohort study.
Manuscript

List of abbrevations

ACE	angiotensin converting enzyme
AT_2	angiotensin II
βAR	β-adrenergic receptor
β -blocker	β-adrenergic receptor blocker
BMI	body mass index
BNP	brain natriuretic peptid
CAD	coronary arterial disease
cAMP	cyclic adenosine monophosphate
CHF	chronic heart failure
CRP	c-reactive protein
EF	ejection fraction
EGF	epidermal growth factor
FS	fractional shortening
GPCR	G-protein-coupled receptor
HF-psf	heart failure with preserved systolic function
IFNγ	interferon gamma
IL	interleukin
LVEF	left ventricular ejection fraction
MCP	monocyte chemotactic protein
NFκB	nuclear factor kappa B
NYHA	New York Heart Association
PKA	protein kinase A
RWT	relative wall thickness
SHR	spontaneously hypertensive rats
TNFα	tumor necrosis factor alpha
Vcf	velocity of the circumferential fiber shortening
VEGF	vascular endothelial growth factor
WKY	wistar Kyoto

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Introduction

Chronic heart failure (CHF) is a complex multi-step disorder in which a number of physiologic systems participate in its pathogenesis [Jessup 2003]. Although mortality due to heart failure has decreased significantly in the last decade since the introduction of ACE-inhibitors and β-blockers in the management of heart failure, CHF remains to be one of the most important causes for morbidity and mortality [Mann 2002]. According to Swedish National Heart Failure Registry (RiksSvikt) 2006, one year mortality was 35% for severe CHF. This accounts for a significantly higher health-care expenditure that is twice as much the cost of cancer. These data suggest that we still have not properly defined strategy in the management of CHF. The present heart failure management aims mostly at restoration of neurohormonal imbalance, rather than targeting primary mechanisms of the disease.

Heart failure in the elderly

CHF accompanied by higher comorbidity and mortality is increasing in line with advancing age. CHF in the elderly constitutes the majority of heart failure population. CHF in older adults differs in many aspects from heart failure that occurs during middle age. There is increasing prevalence of heart failure with preserved left ventricular systolic function, and a marked increase in the number of coexisting medical conditions. On average at least 40 percent of patients with CHF have preserved systolic function [Senni 1998]. The incidence of diastolic heart failure increases with age, and it is more common in older women [Ahmed 2003; McCullough 2002,]. It is well known that hypertension often leads to diastolic dysfunction without concomitant systolic dysfunction [Vasan 1999]. In the Original Framingham Heart Study hypertension was the most common risk factor for CHF. It is known that as many as 91 % of the elderly heart failure patients may have current or previous hypertension [Levy 1996].

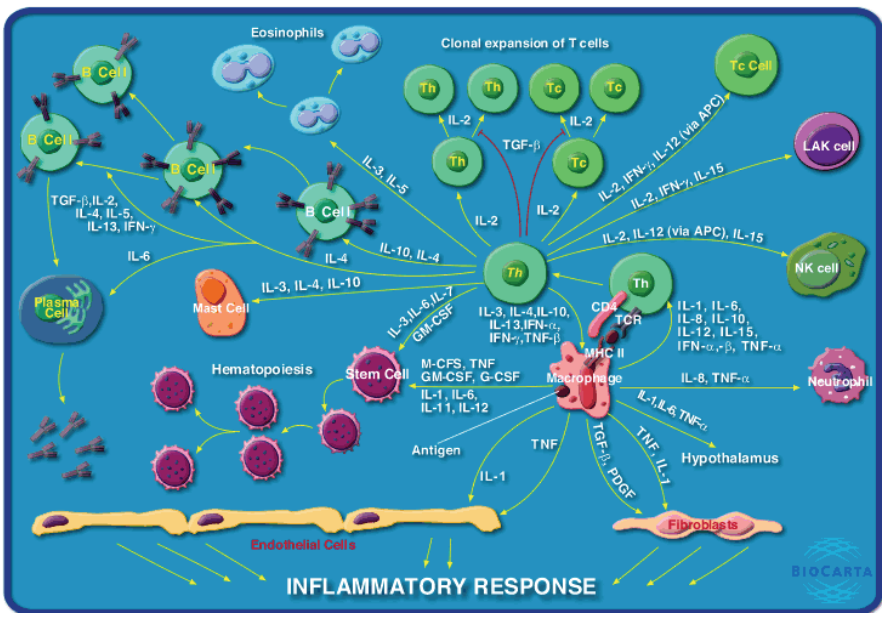
Despite high prevalence in heart failure, this elderly group has not been well studied, particularly in the octogenarian group, as compared with younger heart failure patients. Previous landmark randomised clinical trials were mostly conducted in younger systolic heart failure patients with an average age of < 63 years and a left ventricular ejection fraction of < 40%. However, in "real world" clinical practice, the majority of patients with chronic heart failure are older and the median age at presentation of new heart failure cases is often > 75 years. The lack of a representative sample of elderly patients in previous clinical trials has raised serious concerns about extrapolating the available evidence from younger population to an elderly heart failure group [Fu 2007a, b].

As a matter of fact, age-dependent structural and functional changes are seen in elderly patients, such as increases in sympathetic activity, left ventricular wall diameter, myocardial fibrosis and apoptosis, coronary sclerosis and aortic stiffness. As a consequence, both systolic and diastolic dysfunctions are more frequent in older compared to younger patients. Moreover, with age there is a significant shift in phenotype from systolic to diastolic heart failure, especially in patients with hypertension and/or diabetes as well as in women [Fu, 2007a, b]

Cytokines & cytokine network

Cytokines are a group of proteins and peptides that are used in organisms as signaling compounds. These chemical signals are similar to hormones and neurotransmitters and are used to allow one cell to communicate with another. They are important in both innate and adaptive immune responses. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory and infectious diseases. Cytokines act in networks or cascades by binding to specific membrane receptors, which then signal the cell via second messengers, often tyrosine kinases, to alter its behavior. Responses to cytokines include increasing or decreasing expressions of membrane

proteins, proliferation, and secretion of effector molecules. It is common for different cell types to secrete the same cytokine or for a single cytokine to act on several different cell types. Cytokines are redundant in their activities, i.e. similar functions can be stimulated by different cytokines. Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines. Cytokines can also act synergistically or antagonistically. Cytokines are synthesized by nearly all nucleated cells, but the predominant producers are helper T cells and macrophages. Some cytokines clearly promote inflammation and are called proinflammatory cytokines, whereas other cytokines suppress the activity of proinflammatory cytokines and are therefore called anti-inflammatory cytokines. For example IL-4, IL-10 and IL-13 are potent anti-inflammatory agents. They are anti-inflammatory cytokines by virtue of their ability to suppress genes for proinflammatory cytokines such as IL-1, IL-6, TNFα and the chemokines [Gallin 1999, Janeway 1999, Roitt 2002].



Adapted from BioCarta, Inc.

Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism [Fruebis 2001, Yamauchi 2002, Wolf 2003]. Adiponectin is exclusively secreted from adipose tissues into the bloodstream and is very abundant in plasma. Level of the hormone is inversely correlated with body mass index. Adiponectin plays a role in the suppression of the metabolic derangements that may result in type 2 diabetes, obesity, atherosclerosis and non-alcoholic fatty liver disease. In addition to its beneficial effects on both lipid and glucose metabolism, adiponectin has anti-inflammatory properties. Adiponectin can also inhibit growth factor-mediated fibroblast proliferation and prevent fibrosis [Wang 2005]. CHF is associated with altered energy homeostasis and myocardial inflammation, hypertrophy and fibrosis. Therefore adiponectin may be involved in CHF.

Inflammation and chronic heart failure

There is increasing evidence showing that inflammation is involved in the progression of heart failure mostly in younger patients [Bozkurt 2001; Deswal 1999; Deswal 2001; Mann 1994; Mann 2002, Rauchhaus 2000; Raymond 2001; Sasayama 1999; Setsuta 2004; Torre-Amione 1996; Torre-Amione 2005]. Up to now CHF has been shown to be accompanied by immune activations with proinflammatory cytokines (e.g. TNF-α, IL-1, IL-6) overexpressed both in the systemic circulation and locally in the failing myocardium particularly in the younger [Deswald 2001; Diwan 2003; Franco 1999; Levine 1990; Mann 2002; Natanson 1989; Pagani 1992; Rauchhaus 2000; Torre-Amione 1996; Torre-Amione 2005; Tracey 1986]. Sustained overexpression of inflammatory mediators contributes to the development of central and peripheral manifestations of CHF [Shan 1997]. Proinflammatory cytokines unfavourable affect left ventricular function by exerting negative inotropic effect [Meldrum 1998], inducing abnormalities in cardiac metabolism and energetics, promoting adverse

myocardial remodelling [Finkel 1992, Valgimigle 2001], and finally resulting in myocardial hypertrophy [Yokoyama 1997], necrosis and apoptosis [Krown 1996, Kubota 1997] as well as changes in the extracellular matrix [Sivasubramanian 2001]. Additionally, activation of the immune response promotes the development of endothelial dysfunction, general body wasting, skeletal muscle apoptosis, and anorexia in CHF [Anker 1999; Levine 1990; Sharma 2000; Torre-Amione 1996].

Circulating markers of inflammation, such as TNF-α, IL-6 and C-reactive protein (CRP), have been used in establishing the diagnosis and gauging prognosis in patients with heart failure in the younger. In addition, inflammatory cytokines have been investigated as targets of heart failure therapy. Although results from clinical trials directed against specific cytokine (such as TNF-α) have thus far been disappointing [Anker 2002, Chung 2003, Mann 2004], multiple studies continue to address the importance and therapeutic potential of remodulating the immune response in heart failure.

Likewise, the role of adiponectin in cardiovascular system seems paradoxical. At one hand, in animal studies, adiponectin administration protects development of systolic dysfunction after myocardial infarction [Shibata 2007]. Adiponectin had antihypertrophic effects on cardiac myocytes, and its deficiency exacerbated CHF [Liao 2005; Shibata 2004]. In humans, lower plasma adiponectin levels have been connected with increased risk of cardiovascular events [Pischon 2004] and higher incidence of type 2 diabetes mellitus. Subjects with a high adiponectin level had lower risk for myocardial infarction [Kumada 2003]. At other hand, established CHF patients with a high adiponectin level had poor prognosis [George 2006; Kistorp 2005; Tamura 2007]. Recently, Tsutamoto *et al.* found that a high plasma total adiponectin level is an independent prognostic predictor especially in CHF patients with normal body mass index (BMI)

but not with abnormal BMI [Tsutamoto 2007]. Cachexia in CHF was also shown to be associated with an increase in adiponectin concentration [McEntegart 2007].

Whereas the relationship between low adiponectin level and an increased risk of coronary arterial disease (CAD) or myocardial infarction seems to be robust, the role of plasma adiponectin in CHF appears to be more complex. Particularly in the elderly heart failure patients this has been poorly studied.

Taken together, limited information is available about whether inflammation is equally involved in the very elderly CHF patients as that in the younger and whether inflammation is involved in the development of heart failure due to hypertension. This is clinically relevant since hypertensive heart failure represents the main body of heart failure population. Moreover, heart failure is heterogeneous and the underlying mechanism may differ greatly from one cause to another and between the younger and the elderly. Therefore better understanding of pathophysiological role and clinical relevance of cytokines in CHF, particularly due to hypertension and in the elderly, may lead to better management of this disease state by adding anti-inflammatory agent if this hypothesis turned out to be the case.

Aims of the study

1) To study whether there is increased cytokine expression in the myocardium in experimental hypertensive heart failure in rats;

2) To study effects of TNF-α-antagonist on cardiac structure, function and its underlying mechanism in experimental hypertensive heart failure in rats;

3) To study whether there are increased cytokine activations in the very elderly heart failure patients;

4) To study whether increased cytokine activation has prognostic significance in the very elderly heart failure patients.

Materials and Methods

Experimental hypertensive heart failure model

Spontaneously hypertensive rats (SHR) have been frequently used as an animal model of genetic hypertension which develops heart failure with aging, similar to man [Mitchell 1997]. Absolute left ventricular dimensions in the SHR increase out of proportion to body growth, consistent with concentric hypertrophy [Pfeffer 1979]. Postnatal growth of the left ventricle of rat occurs by hypertrophy of myofibers and hyperplasia of connective tissue elements. The consistent pattern of a long period of stable hypertrophy followed by a transition to heart failure provides a useful model to study mechanism of heart failure with aging, particularly from hypertension to diastolic dysfunction [Pfeffer 1985]. Moreover, SHR with heart failure mimics hypertension-induced heart failure in humans due to changes in relevant cardiac hemodynamic and neurohormonal parameters during the transition from compensated hypertrophy to heart failure [Mirsky 1983]. Therefore, SHR has been regarded as a very useful model.

In paper 1, male 12-week-old SHR (n=10) and age-matched Wistar rats (Wky, n=10) were used. All animals were fed with standard rat pellets and tap water ad libitum and housed in cages in groups of 3 animals, at 26 °C with 60% humidity. At the end of the study, 26 weeks old, all rats underwent echocardiography, heart and blood were collected for analysis. Heart biopsies for mRNA analysis were frozen in RNA later whereas the rest of the heart tissues were frozen immediately. Serum and plasma were stored at -80 °C.

In paper 2, male 12-week-old WKY (n=30) rats and SHR (n=30) were used. From the age of 14 to 26 weeks, rats in each group were treated subcutaneously twice a week with either: (1) etanercept at a dose of 0.3 mg/kg for the first two weeks and 0.15 mg/kg for the following ten weeks; or (2) NaCl as placebo [Anker 2003]. That is the same doses as used in RENAISSANCE and RECOVER [Mann

2004], and for the daily management of rheumatoid arthritis and Crohn´s disease. At 26 weeks of age, all rats underwent echocardiography and blood pressure was measured with the tail cuff method. Heart tissues and blood samples were collected for later analysis in the same way as in paper 1.

Patients and healthy controls

Patients with CHF, New York Heart Assosiation (NYHA) III-IV, who were admitted at Heart Failure Unit, Dept. of Medicine, Sahlgrenska University Hospital/Sahlgrenska, Gothenburg, Sweden, were recruited to participate in the present study during 2004-2005 (paper 3 and 4). The diagnosis of CHF was based on definition of European Society of Cardiology [Swedberg 2005]. For systolic heart failure, left ventricular ejection fraction (LVEF) assessed by conventional echocardiography must be <40%. For heart failure with preserved systolic function (HF-PSF), two criteria must be fulfilled: 1) EF > 50% by echocardiography and 2) B-type natriuretic peptide (BNP) (Biosite, USA) > 400 pg/ml or NT-pro BNP (Roche, Switzerland) > 1000 pg/ml. In Paper 3, main part of study patients has reduced LVEF (n=54). The reason for this is due to the fact that at the time of study start, BNP/NTpro BNP were not widely used at heart failure diagnostic and, moreover, diastolic function was difficult to be measured by conventional echocardiography in 42% of elderly CHF patients due to presence of atrial fibrillation [Jensen et al., 2007]. In order to avoid bias in diagnosis, only reduced LVEF were included. In Paper 4, both systolic and diastolic heart failure were included (n=92). In addition, NT-pro BNP was determined in all patients. Healthy control subjects were age- and gender-matched to the heart failure patients (n=70). All healthy subjects were examined by physical examinations, blood analyses including NTpro BNP (< 300 pg/ml) and echocardiography.

Echocardiography at rest and during stress

Echocardiography in humans was conducted conventionally.

For animal studies, echocardiography was performed using a high-frequency 12-MHz phased array transducer (P12-5, Philip Medical System, Best) connected to a HDI 5000 ultrasound system (ATL, Philip Medical System). Briefly, animals were anaesthetized with 1.6–2.7% isoflurane (Abbot Scandinavia AB, Solna, Sweden) through a nose cone. Their chests were shaved, and they were placed on an electrical heating pad to maintain normothermia during the examination. Short axis 2-D views of the left ventricle at the papillary muscle level were used to obtain M-mode targeted recordings. Posterior end-diastolic wall thickness and left ventricular internal dimensions were measured using the leading edge method of the American Society of Echocardiography. Fractional shortening (FS), relative wall thickness (RWT) and velocity of the circumferential fiber shortening (Vcf) were measured. Cardiac reserve was studied by infusion of dobutamine (Dobutrex®, Eli Lilly Sweden AB) into the tail vein at 20 μg/kg/min [Täng 2007]. All measurements were performed averaging at least three consecutive cardiac cycles and were carried out by the observer (blinded to this study) using an imaging analysis system (EchoPac ™, GE Vingmed Ultrasound A/S, Horten, Norway) with digitally acquired data.

Cytokine gene expression in myocardial tissues

Total RNA was isolated from myocardium using SV total RNA Isolation System (Promega, Madison, WI, USA) according to the manufacturer's recommendations. Reverse transcriptase reaction using TaqMan High capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) was performed for cDNA synthesis. The cycling parameters were 25°C for 10 min and 37°C for 2 h.

Real time RT-PCR analyses were used to determine mRNA expressions of IL-2, IL-6, IFNγ, NFkb, TNFα and were performed with TaqMan Assay-on-Demand on ABI 7700 Sequence Detection System (ABI), according to the manufacturer's recommendations.

The expression data were normalized to an endogenous control β-glucuronidase. The reactions for IL-2, IL-6, IFNγ, NFkb and TNFα were analyzed in triplicate and the expression levels were calculated according to the formula $2^{-\Delta CT}$, where ΔCT is the difference in threshold cycle (CT) values between the target and the endogenous control. The logarithm of the RNA concentration was calculated from standard curve. The expression was determined as the ratio of the RNA_{target}/RNA_{GusB}.

Cytokine levels in sera from heart failure patients and healthy controls

Cytokines (IL1a, IL1b, IL 2, IL 4, IL 6, IL 8, IL 10, TNFα, IFNγ, EGF, VEGF and MCP) in patients were measured by a protein array chip technology using the Evidence Analyser, which is a fully automated system from Randox Laboratories Ltd (Cat. No EV 3508, Randox Laboratories Ltd, Antrim, United Kingdom).

Plasma adiponectin was determined by a commercially available Enzyme-Linked Immunoabsorbent Assay (Otsuka Life Science Initiative, Tokyo, Japan).

Cardiac stress markers

Real time RT-PCR analyses were used to determine mRNA expressions of BNP in rat heart tissues according to the manufacturer's recommendations as stated above.

BNP/NT-pro BNP in the blood samples from heart failure patients and healthy controls were measured conventionally (Biosite, USA; Roche, Switzerland).

βAR gene expression in myocardial tissues

The βAR is one of the G-protein-coupled receptors that mediate physiological responses to noradrenalin and adrenaline. The existence of β_1AR and β_2AR in the human heart has been shown at both mRNA and protein levels [Ungerer 1993, Bristow 1993]. The predominant subtype in the heart is the β_1AR with a β_1/β_2 ratio of 60:40 in the atrium and 70:30 in the ventricle [Brodde 1991]. The dampening of receptor stimuli is one of the most important regulatory mechanisms and this process is called receptor desensitization [Hausdorff 1990]. The chronic stimulation of agonists will result in the downregulation of the β_1AR receptors. Real time RT-PCR was used to determine mRNA expression of β_1AR in myocardial tissues.

Main Results

Characterization of experimental hypertensive heart failure (paper I and II)

All rats were alive at the end of the study with no apparent fluid retention indicating absence of severe heart failure. The heart weight/body weight ratio was significant higher in SHR indicating cardiac hypertrophy in SHR. The data were confirmed by echocardiographic examinations which showed increased ratio left ventricular mass/body weight, increased relative wall thickness and simultaneous decrease in left ventricular dimension in SHR.

There was marked diastolic dysfunction in SHR as compared with WKY as shown by tissue Doppler, whereas only less difference in systolic function was found between groups (Table 1). Moreover, the SHR group displayed downregulated myocardial β_1AR and upregulated myocardial brain natriuretic peptide (BNP) at mRNA levels.

Table 1: Summary of echocardiographic data (mean ± SD)

	WKY	SHR
FS %	34.8 ± 10.8	40.2 ± 7.9
EF %	60.5 ± 14.6	68.5 ± 9.6
Septal tissue Doppler (systole) cm/s	4.3 ± 1.2	3.3 ± 0.6 *
Septal tissue Doppler (diastole) cm/s	7.7 ± 2.7	3.8 ± 0.8 **
Lateral tissue Doppler (systole) cm/s	4.1 ± 0.8	3.8 ± 0.7
Later tissue Doppler (diastole) cm/s	6.9 ± 1.6	3.6 ± 0,9 **

* p<0,05, **p<0,01; FS: Fractional shortening; EF: Ejection fraction

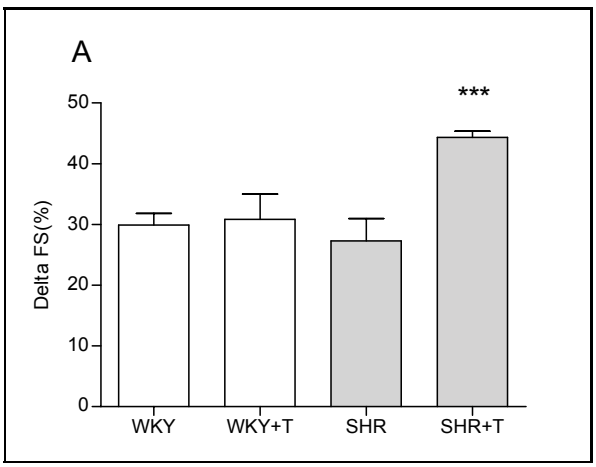
Cytokine gene expressions in experimental hypertensive heart failure (paper I and II)

By using RT-PCR, mRNAs encoding for cytokines were determined in heart tissues. Increased mRNA for the proinflammatory cytokine IL-6 whereas decreased mRNAs for IL-2, IFNγ and NFkb were found in SHR as compared with WKY.

Effect of chronic treatment of etanercept in experimental hypertensive heart failure (paper II)

The heart/body weight ratios were higher in SHR than in WKY, following either placebo or etanercept treatment. Blood pressure was significantly higher in SHR than WKY following placebo treatment. Chronic etanercept treatment significantly increased blood pressure in SHR but not in WKY. Using echocardiography there were no significant differences in FS at rest or in stress (assessed by changes in FS and Vcf during dobutamine infusion) in SHR and WKY following placebo treatment. Chronic etanercept treatment significantly increased cardiac reserve in SHR but not in WKY (Fig). Relative wall thickness increased in the SHR group compared with WKY following placebo treatment. Etanercept significantly decreased relative wall thickness in SHR and in WKY.

Figure I:



IL6 mRNA expression was elevated in heart tissue from SHR than WKY following placebo treatment, and etanercept treatment increased the expression in both groups. Expression of BNP mRNA was higher in heart tissue from SHR than from WKY, following either placebo or etanercept treatment. β_1AR mRNA expression was lower in SHR than WKY following placebo treatment; etanercept treatment did not affect β_1AR mRNA levels in SHR, but slightly increased the levels in WKY. Expression of TNFα mRNA was the same in SHR and WKY following placebo, and was not affected by etanercept treatment. AT2 and IFNγ remained unchanged in either group with or without treatment.

Cytokine levels in elderly patients with heart failure (paper III and IV)

Our results have shown increased IL-1b, IL-6, IL-8, IL-10, TNF-α and EGF in sera from heart failure patients compared with the control group (table 2). Moreover, IL-6, IL-10 and TNF-α were significantly increased in those patients who did not survive within one year. In a subgroup of heart failure with atrial fibrillation (paroxysmal, persistent or permanent) there were significant increases in IL-2, IL-4, IL-6, IL-8, IL-10 and TNF-α. However in subgroups such as coronary heart diseases and diabetes cytokines were less activated. No significant difference was found either in gender or among different medications.

Figure 1:

Comparison of effect of a TNFα- antagonist between rest and dobutamine stress assessed by echocardiography in SHR and WKY with or without treatment (T). Data is presented as mean ± SEM. FS: Fractional shortening;

**** $p<0.001$ vs. untreated for each group*

Among those 54 patients with CHF, 13 died within one year. Univariate analyses of totally 37 available risk factors demonstrated that IL6 and IL8 are significant risk factors. Other factors such as VEGF and MCP have tendency to be significant. Further multivariate analyses, after adjustment for imbalance in other risk factors (so called independent risk factor), showed that only IL6 is significant prognostic indicator for one–year mortality. However it is not linear relationship between IL6 level and one-year mortality. By dividing 54 patients in quartiles, there is significant increased mortality in IL6 >10 compared with those < 10.

Table 2: Cytokine profile in CHF patients and controls

	CHF	Control
IL 2	4,39 ± 4,49	5,78 ± 9,00
IL 4	0,50 ± 1,69	0,40 ± 1,25
IL 6	10,59 ± 15,01 ***	1,52 ± 0,94
IL 8	15,92 ± 14,21 ***	8,21 ± 5,48
IL 10	1,02 ± 3,65 *	0,24 ± 1,05
VEGF	256,90 ± 237,66	281,17 ± 230,37
IFNγ	3,53 ± 4,45	4,45 ± 4,07
TNFα	4,96 ± 5,43 **	1,30 ± 1,57
IL1a	0,61 ± 1,85	0,42 ± 1,15
IL1b	1,40 ± 6,42 *	0,47 ± 1,27
MCP	380,47 ± 196,59	413,66 ± 156,96
EGF	23,10 ± 33,01 ***	14,68 ± 14,43

*: p < 0,05 ;**: p <0,01 ;***: p < 0,001 compared with controls; Unit: pg/ml

Adiponectin levels were significantly increased in parallel with increased NT-pro BNP levels in sera from heart failure patients for those more than 70 years old as compared with those in control group (figure 2). No difference was

seen in those younger than 70 years compared with those healthy controls. There was higher adiponectin level in non-ischemic heart failure as compared with that with ischemic cause. There was higher adiponectin level in those died as compared with those survived. Univariable regression analysis showed that adiponectin was predictor for mortality. However when multivariable analysis was applied, adiponectin was not significant.

Figure 2:

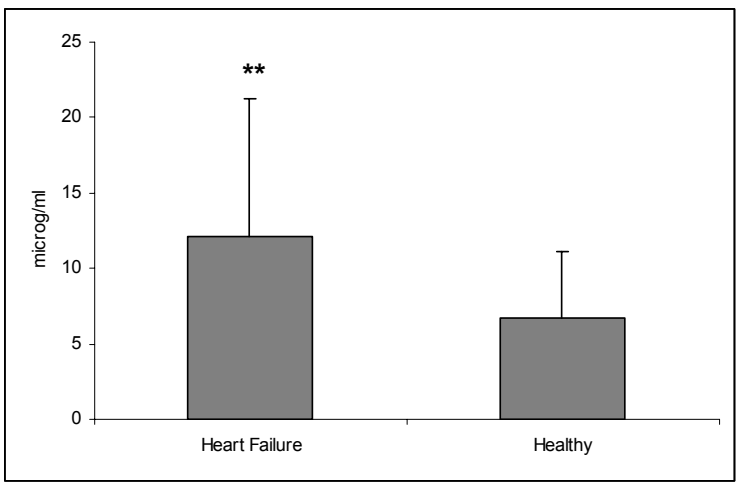


Figure 2: *Increased adiponectin level in chronic heart failure. Data is presented as mean ± SD. ** p < 0,01 as compared with control group.*

Discussion

Hypertensive heart failure in rats and humans

In the present study we have shown that SHR developed cardiac hypertrophy complicated with diastolic heart dysfunction, as shown by decreased relaxation velocity, relatively preserved systolic function by tissue Doppler in hypertrophied hearts in SHR. This was further supported by increased mRNA for BNP as a biomarker for heart dysfunction and decreased mRNA for β_1AR as a marker for desensitization process in myocardial tissues in SHR. As stated above, down-regulation of β_1AR is an important characteristic in heart failure after sustained sympathetic activation. Therefore, it is evident that SHR rats have developed diastolic heart dysfunction as early stage of heart failure due to hypertension in the present study.

One of the most frequently seen subset of heart failure in the elderly is heart failure due to hypertension which is often characterized by predominance of diastolic dysfunction due to relaxation disturbance and increased filling pressure in left ventricles. It is well known that hypertension is one of main causes for heart failure, particularly in the elderly. Almost 91% of heart failure patients have current or previous hypertension [Levy 1996]. In current study, patients with remaining hypertension are around 46%. However this does not exclude possibility that these elderly heart failure patients might have hypertension previously.

Cytokine activation in hypertensive heart failure

The spectrum of heart failure biomarkers continues to grow. Heart failure biomarkers can be categorized empirically as neurohormonal mediators, markers of myocytes injury and remodelling, and indicators of systemic inflammation.

BNP/NT-pro BNP is most widely studied. Strong evidence exists for use of BNP/NT-pro BNP in the diagnosis of heart failure and as a prognostic indicator as well as for improved clinical outcomes with a BNP/NT-pro BNP-guided approach to heart failure care.

Biomarkers of inflammation have emerged as potential preclinical indicators to identify individuals at risk of developing clinical heart failure. However, heart failure is heterogeneous with its diverse aetiology. IL-6 belongs to the proinflammatory cytokine family and can be expressed in myocardium under various forms of stress and is capable of inducing hypertrophy and apoptosis in cardiomyocytes.

Our study has demonstrated increased myocardial expressions of mRNA encoding for IL-6 in SHR. Our finding from the present study is in line with a recent study by Zen et al. who reported that the plasma levels of IL-6 was slightly increased in patients with idiopathic nonobstructive hypertrophic cardiomyopathy, but significantly increased in dilated-phase of hypertrophic cardiomyopathy suggesting that the proinflammatory cytokine IL-6 may play an important role in the status of hypertrophic cardiomyopathy and its progression to dilated-phase of hypertrophic cardiomyopathy [Sanz-Rosa 2005]. Moreover, IL-6 seems more involved in high blood pressure than other proinflammatory cytokines. High blood pressure levels were shown to be associated with increased IL-6 plasma levels, but not TNF-α levels, in SHR compared with WKY, implying that in SHR stimulation of inflammatory mediator IL-6 was involved [Zen 2005]. IFNγ was reduced in our study. This is in accordance with a previous study. IFNγ has been shown to have a protective role in myocardial damage [Nishio 2003].

Our clinical study has shown increased IL-6 and TNF-α in heart failure patients in the elderly. Moreover, IL-6 and TNF-α were significantly increased in those patients who did not survive

within one year. These are in line with previous findings in younger heart failure patients. Logistical analyses of totally 37 available risk factors after adjustment for imbalance showed that only IL6 is significant predictor in one-year mortality. This is the first time, to our knowledge, to show that heart failure patients in octogenarian group displayed increased proinflammatory cytokines but in a different pattern from those reported in the younger heart failure patients. By dividing patients in quartiles, there is almost 4-fold increase in one-year mortality in those with IL6 >10 compared with those < 10. We have also observed in our study that IL-10, which is often regarded as anti-inflammatory cytokine, was also increased. This is probably compensatory mechanism secondary to increased proinflammatory cytokine such as IL-6 and TNF-α.

Further analyses in subgroups with different comorbidities in CHF in the elderly showed heterogeneous pattern. For instance, in a subgroup of heart failure with atrial fibrillation there were significant increases in IL-2, I-L4, IL-6, IL-10 and TNF-α. However in subgroups such as coronary heart diseases and diabetes cytokines were less activated. This is particularly interesting and clinically relevant since heart failure in the elderly is indeed a heterogeneous group complicated with diversified aetiologies, clinical symptoms, cardiac dysfunctions, coexistence of multiple diseases and higher incidences of both morbidity and mortality. Accordingly, in subgroup CHF as described above, it does not necessarily mean that subgroup CHF only has one underlying cause. In most cases the aetiology to CHF in the elderly is multifactorial.

Our data had also shown significantly increased adiponectin levels in CHF as compared with control group for those over 70 years old. What is the underlying mechanism for increased adiponectin secretion? Is increased adiponectin level a biomarker or a pathophysiological mediator? Theoretically, progression of

CHF is closely associated with a reduction in total body weight as part of a wasting process. This decrease in body weight will result in an increase in adiponectin plasma concentrations. Recently, it has been shown that cachexia in CHF is associated with an increase in adiponectin concentration [McEntegard 2007]. In this case, increased adiponectin level in CHF is most probably compensatory as an indicator of disease severity rather than as a pathophysiological mediator. This also means that the increased adiponectin level in CHF does no longer exert its cardiovascular protective role. As suggested by Kintscher, this might imply a state of a 'functional adiponectin resistance', which occurs in CHF [Kintscher 2007]. The factors controlling adiponectin secretion are still poorly understood. It is possible that increased proinflammatory cytokines may stimulate adiponectin secretion. For example, previous studies have shown that TNF-α has been suggested to increase adiponectin secretion, although this literature is conflicting [Bruun 2003; Carey 2006; Degawa-Yamauchi 2005; Wang 2006].

Subgroup analysis has shown that heart failure with underlying non-ischemic cause hade higher level of adiponectin as those with ischemia. Hypothetically higher level of adiponectin in non-ischemic group is expected due to that adiponectin is a natural molecule that protect heart from ischemia. Tao et al showed recently that adiponectin was able to protect hearts from ischemia/reperfusion injury by inhibition of iNOS and nicotinamide adenine dinucleotide phosphate-oxidase protein expression and resultant oxidative/nitrative stress [Tao 2007]. Our study confirmed our hypothesis that adiponectin was significantly increased in CHF due to non-ischemia than those with underlying ischemia.

We have also shown increased adiponectin in those died within 1 year. When univariate analysis was applied,

increased adiponectin was a prognostic indicator for CHF. However when multivariate analysis was performed, increased adiponectin was no longer significant. This may be due to limited sample size.

The heterogeneous phenotype of CHF particular in the very elderly makes clinical judgement difficult and unreliable. To find a suitable marker for this group will no doubt facilitate clinical practice. Proinflammatory cytokine IL6 is one of useful markers, adiponectin is another. In many cases it is complimentary to BNP/ NTpro BNP. It is believed that combination of different biomarkers such as BNP/NTpro BNP, inflammatory cytokines and adiponectin may have additional values.

Why TNF-α antagonist does not work in CHF?

It is possible that suppression of one cytokine, such as TNF-α, might be substituted by increases in other proinflammatory cytokines known to be elevated in CHF (e.g., IL1, IL6) since the immune system is redundant. Etanercept is a highly selective TNF-α inhibitor. It is logical to postulate, after demonstration of lack of benefit, that the highly selective nature of this compound may also be a disadvantage. Moreover, different cytokines may be dominating in different stages of the disease and be dependent on different causes.

It has previously been shown that TNF-α induces cardiodepressive effects and causes hypotension [Prabhu 2004]. Similarly, chronic exposure to IL-6 decreases contractile and sarcoplasmic reticular in adult rat ventricular myocytes [Yu 2005]. Recently, Bellahcene showed that TNF-α activates p38-MAPK, a stress-responsive kinase implicated in contractile depression and cardiac injury, and this activation likely contributes to the early cardiodepressant action of TNF-α [Bellahcene 2006]. In line with above observations we have demonstrated that TNF-α antagonist was able to induce positive inotropic effect and increase blood pressure. Therefore, TNF-α antagonist may work in short term in

acute heart failure because of positive inotropic effect. However, consequence of above positive inotropic effect will no doubt lead to gradually decreased heart function in the setting of chronic heart failure in the long term. Hitherto it is known that positive inotropic agents are effective as acute treatment e.g. pulmonary edema but so far have not shown any long-term benefit on mortality [Swedberg 2005].

Our results have clearly demonstrated that chronic treatment with TNFα antagonist was able to not only upregulate IL6 but also exert positive inotropic effect in hypertensive rats displaying early stage of heart failure, which may explain why etanercept does not work in chronic heart failure in the long term despite inflammation is indeed involved. In this type of heart failure dominated proinflammatory cytokine is IL-6 rather than TNF-α. We have observed reduction in heart weight and body weight ratio in WKY rats after chronic treatment with etanercept, which is speculated to be due to significantly increased inflammation as shown by significantly increased IL-6 level up to almost 10 fold.

Limitations

Because of limited budget and high cost of cytokine analyses only very limited number of patients were included. Because of limited sample size the interpretation of our results should be cautious particularly for subgroup analyses. No serum level of cytokine was measured in rats due to limited amount of blood sample. No gene expression of cytokine was determined in human myocardial tissues because no heart biopsy was available in the elderly heart failure patients.

Summary of results

SHR developed cardiac hypertrophy complicated with diastolic heart dysfunction with increased expression of brain natriuretic peptide, down-regulation of beta adrenergic receptors and simultaneous up-regulation of IL-6, which indicates active proinflammatory process as, at least partly, underlying mechanism during the early stage when cardiac hypertrophy associated with diastolic dysfunction occurs. Chronic treatment with etanercept in SHR resulted in favorable cardiac remodeling, but however had a positive inotropic effect and was associated with an upregulation of IL-6. These findings indicate that chronic treatment with TNF-α antagonists is not a rational and effective treatment strategy and may aggravate heart failure in the long term.

Heart failure in the elderly constitutes the majority of heart failure population and is often associated with multiple diseases. In the octogenarian group with heart failure, there are significant increases of proinflammatory cytokines which are associated with mortality and IL6 is the only cytokine which predicts one year mortality. The cytokine activation is more pronounced in subgroup of patients with heart failure concomitant with atrial fibrillation. Plasma adiponectin levels were increased in heart failure patients in the very elderly particularly with underlying non-ischemic origin. Adiponectin levels appear to be associated with increased mortality.

The “story” about inflammation is definite alive and needs to be further studied. It is obvious that these failed clinical trials [Anker 2002; Chung 2003; Mann 2004] were carried out too early before we really understand how inflammation is involved and regulated in heart failure. Therefore there is urgent need of a new strategy for anti-inflammatory therapy targeting the total cytokine network instead of a single cytokine.

Conclusions

In hypertensive heart failure, both clinically and experimentally, there are increased cytokine activations. In the elderly heart failure there is different cytokine profile as compared with that in the younger and increased IL-6 is related to increased one-year mortality. Moreover, it appears that it is not enough to suppress one single cytokine because cytokine network is redundant. However more fundamental studies are needed to understand this complex cytokine network before an appropriate anti-inflammatory therapy emerges.

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