The relationship between high-density lipoprotein, bacterial lipopolysaccharide, and tumour necrosis factor-α in patients with acute decompensated heart failure

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Abstract

Introduction: We attempted to assess the relationship between serum lipoproteins and plasma concentrations of bacterial lipopolysaccharide (LPS, endotoxin), tumour necrosis factor-α (TNF-α) and soluble TNF receptor 1 and 2 (sTNF-R1 and 2) in patients with chronic heart failure (CHF). It has been shown that lipoproteins are able to act as endotoxin-binding proteins, thereby diminishing its bioactivity to induce inflammatory cytokine release.

Material and methods: Endotoxin, cytokines and lipoproteins were measured in 25 CHF patients (age 68±2 years) and 10 healthy controls (age 60±6, all mean ±SE). CHF patients were divided in oedematous (n=10) and non-oedematous (n=15) according to standard criteria. Interrelationships between endotoxin, cytokines and lipoproteins were analyzed.

Results: Oedematous patients had the highest endotoxin ($P<0.05$), TNF-α ($P<0.001$), sTNF-R1 and sTNF-R2 (both $P<0.0001$, all ANOVA), and lowest HDL ($P<0.05$, ANOVA). Amongst patients, HDL correlated with TNF-α ($r=-0.60$, $P=0.002$) and endotoxin ($r=-0.50$, $P=0.01$). The endotoxin/HDL ratio, indicating biologically active LPS, was highest in oedematous patients ($P=0.005$, ANOVA) and strongly related to TNF-α plasma concentrations ($r=0.87$, $P<0.0001$), independently of NYHA class ($P<0.01$), creatinine clearance ($P=0.05$), hepatic function, and age.

Conclusions: The degree of immune activation in oedematous CHF patients is related to endotoxaemia and inversely to serum HDL, suggesting an underlying relationship. This clinical observation supports previous experimental data and observational studies; hence further studies should explore its potential therapeutic use.

Key words: chronic heart failure, endotoxin, lipoproteins, immune activation.

Introduction

Lipoproteins have been shown to be able to bind bacterial lipopolysaccharide (LPS, endotoxin) *in vitro* [1, 2] and in animal models of experimental gram-negative sepsis [3, 4]. The binding process results in

a reduction of endotoxin-bioactivity by forming lipoprotein micelles [5, 6]. We hypothesised that lipoproteins mediate physiological actions against immune activation in patients with chronic heart failure (CHF) [7, 8].

Immune activation, illustrated by elevated plasma concentrations of tumour necrosis factor-α (TNF-α) and soluble TNF receptor 1 and 2 (sTNF-R1 and sTNF-2), is very common in patients with advanced CHF [9, 10]. We have demonstrated that LPS may contribute to systemic inflammatory responses in patients with episodes of acute decompensation of CHF who present with significant peripheral oedema [11].

Assuming a beneficial endotoxin-binding effect of lipoproteins we hypothesised that the degree of immune activation in patients with CHF is related to LPS plasma concentrations but inversely to serum levels of lipoproteins [7, 8]. The objective of the present study was to assess the relationship of serum lipoproteins and particularly of high--density lipoprotein (HDL) [5] to inflammatory cytokines in CHF patients with and without signs of acute peripheral congestion.

Material and methods

Study population

We studied 25 consecutive patients with CHF (diagnosis based on a history of congestive heart failure of at least 6 months, reduced exercise tolerance, cardiomegaly, and documented left ventricular dysfunction) in comparison to 10 healthy volunteers. The patients were receiving standard medical therapy in varying combinations. The clinical details and study characteristics are given in Tables I and II. Ten patients presented with acute decompensation of CHF with peripheral oedema but without clinical evidence of central congestion (rales or orthopnoea). Fifteen patients were clinically stable without evidence of peripheral congestion. No participant had clinical signs of acute infection, rheumatoid arthritis, or cancer. The study protocol was approved by the local Ethics Committee and all participants gave written informed consent.

Laboratory measurements

Blood samples were collected in the morning between 8 and 9 a.m. after an overnight fast and a supine rest of 20 min. An antecubital polyethylene catheter was inserted and 2 × 4 ml of venous blood were drawn into endotoxin-free tubes (Endo Tube ET®, Chromogenix AB, Mölndal, Sweden). Subsequently, 20 ml of standard venous samples (10 ml EDTA plasma, 10 ml serum) were taken for biochemical and cytokine measurements [11]. After centrifugation, Endo Tubes and plasma aliquots were stored at –80°C until analysis but serum samples for lipoprotein measurements were analysed immediately.

Table I. Clinical characteristics of chronic heart failure patients and healthy controls

Variable	Patients (n=25)	Controls (n=10)	P-value unpaired t-test
Age [years]	68±2	60±6	0.11
Peak VO_2 [ml/kg/min]	18.6±3.0	33.2±3.4	0.03
Sodium [mmol/l]	137±0.7	139±0.7	0.07
Potassium [mmol/l]	4.3±0.1	4.5±0.1	
Albumin [g/l]	41.1±0.7	43.0±1.1	0.13
Total protein [g/l]	72.1±1.3	70.0±1.1	
Creatinine clearance [ml/min]	85.7±12.2	97.5±21.7	
NYHA class [n]	2.8±0.2		
• NYHA class II	11		
• NYHA class III	8		
• NYHA class IV	6		
Medication [n]			
• Diuretics	23		
• ACE inhibition	19		
• Digoxin	11		
• Warfarin	5		
• Oral nitrates	12		
• Amiodarone	4		
• Aspirin	12		
• Statins	7		

Values presented as mean ±SE, all P-values <0.20 are given

Table II. Cytokine concentrations and lipoprotein levels in patients with chronic heart failure and control subjects

Variable	All CHF patients (n=25)	P-value all CHF vs. controls	Controls (n=10)	P-value controls vs. non-oedematous	Non-oedematous CHF patients (n=15)	P-value non--oedematous vs. oedematous	Oedematous CHF (n=10)	P-value oedematous vs. controls
TNF-α [pg/ml]	6.6±1.5	0.07	2.1±0.2	0.07	3.1±0.3	0.0003	11.7±3.1	0.0002
sTNF-R1 [pg/ml]	1868±229	0.007	835±94	0.007	1306±167	<0.0001	2922±353	<0.0001
sTNF-R2 [pg/ml]	3155±302	0.005	1704±193	0.005	2521±320	0.0004	4344±361	<0.0001
Endotoxin [EU/ml]	0.43±0.03	0.18	0.35±0.05		0.37±0.02	0.016	0.53±0.07	0.013
CRP [mg/l]	6.5 (0.8, –0.7)	0.06	4.5 (0.3, –0.3)	0.28	5.5 (0.8, –0.7)	0.03	8.8 (1.8, –1.5)	0.005
Cholesterol [mmol/l]	5.3±0.2		5.5±0.4		5.4±0.3		5.2±0.3	
LDL [mmol/l]	2.9±0.2	0.20	3.5±0.5		2.9±0.3		2.9±0.3	
HDL [mmol/l]	1.7±0.1	0.09	1.9±0.1	0.36	1.8±0.1	0.08	1.5±0.1	0.017
Triglycerides [mmol/l]	1.2±0.1		1.1±0.1		1.1±0.1		1.3±0.2	

Values presented as mean ±SE, log-transformation of CRP resulted in asymmetric standard errors
Oedematous indicates oedematous patients with chronic heart failure, non-oedematous indicates non-oedematous patients with chronic heart failure
P-values for subgroup comparisons are provided if ANOVA P<0.05

Endotoxin measurements

Lipopolysaccharide concentrations were measured using a commercially available test kit (Limulus Amebocyte Lysate QCL-1000 test kit, BioWhittaker, Inc., Walkersville, USA). In healthy subjects the normal level of LPS in this assay is <0.50 EU/ml. Lipopolysaccharide in the sample catalyses the activation of a proenzyme in the Limulus Amebocyte Lysate. The rate of activation is determined by the concentration of LPS present. The activated enzyme catalyses the splitting of p-nitroaniline from a synthetic peptide. The p-nitroaniline released is measured photometrically at 405-410 nm after the reaction is stopped with stop reagent (sensitivity 0.03 EU/ml). The within-assay coefficient of variation at concentrations of 0.35 EU/ml and 0.82 EU/ml were 9.9 and 9.6%; between-assay coefficients of variation were 16.8 and 13.3%, respectively [8, 11].

Cytokine measurements

Systemic concentrations of TNF-α were determined by ELISA (Quantikine® HS human TNF-α, sensitivity 0.18 pg/ml, R&D Systems, Minneapolis, MN, USA). This measurement of TNF-α is not influenced by soluble TNF receptors. Soluble TNF-R1 (sensitivity 25 pg/ml) and sTNF-R2 (sensitivity 2 pg/ml) were measured by ELISA (R&D Systems, Minneapolis, MN, USA) [10].

Lipoprotein measurements

HDL cholesterol was measured using a direct homogenous assay without the need for any offline pre-treatment or centrifugation steps (Synchron CX® Systems, Beckman Coulter, Inc., Fullerton, CA, USA). Quantitative determination of total cholesterol and triglycerides in serum was performed using a cholesterol and triglyceride reagent, in conjunction with SYNCHRON CX Systems CX MULTI™ Calibrator (Beckman Coulter, Inc., Fullerton, CA, USA). Low-density lipoprotein (LDL) was calculated from the following formula: LDL = total cholesterol – (HDL + 0.45 triglycerides). These measurements are routine hospital analyses. All other parameters were analysed using other routine hospital systems [12].

Statistical analysis

Results are given as mean ± standard error of the mean (SE). ANOVA with Fisher's post hoc test and Student's t-test were used to compare results. Normality of distribution was assessed using the Kolmogorov-Smirnov test. A probability value of P<0.05 was considered statistically significant. The relationship between variables was analysed by simple linear and multivariate regression analyses.

Results

Intergroup comparisons

The endotoxin, cytokine and lipoprotein data are given in Table II. Patients who presented with peripheral oedema had the highest plasma levels of endotoxin (P=0.02), TNF-α (P=0.0002), sTNF-R1 (P<0.0001), and sTNF-R2 (P<0.0001) compared to non-oedematous patients and control subjects (P-values for ANOVA). Oedematous patients with CHF had the lowest HDL serum levels (P<0.05, ANOVA). The groups did not differ significantly according to total cholesterol, LDL, and triglycerides.

An increase in C-reactive peptide (CRP), a non-specific marker of inflammation, was only observed in the oedematous patients (P<0.02, ANOVA). Creatinine clearance was worse in oedematous patients than in non-oedematous patients and healthy controls (36 ± 7 vs. 105 ±12 vs. 97 ± 22 ml/min, P=0.02, ANOVA), whereas variables indicating hepatic function did not differ from each other. The non-oedematous and oedematous patients did not differ from control subjects according to age, liver function, electrolytes, serum cortisol, and fasting glucose levels.

Relationships between endotoxin, lipoproteins, and cytokines

In the total study population, there was an inverse relationship between HDL and endotoxin (r=–0.31, P=0.08) and between HDL and TNF-α (r=–0.53, P=0.001). Age (r=0.35, P=0.04), NYHA functional class (r=0.50, P=0.02), and creatinine clearance (r=–0.43, P=0.07) were also related to TNF-α concentrations. Amongst the heart failure patients, HDL levels related significantly to concentrations of LPS (r=–0.50, P=0.01) and TNF-α (r=–0.60, P=0.0016), but not in control subjects. None of the other lipoprotein fractions was significantly related to LPS plasma concentrations. There was a trend between total cholesterol, LDL, and creatinine clearance with TNF-α (P<0.10), but no such relations were observed with LPS.

The endotoxin/HDL ratio, indicating biologically active unbound endotoxin, was increased in heart failure patients compared to controls (0.27±0.03 vs. 0.17±0.02, P=0.03) and highest in oedematous patients (ANOVA, Figure 1). In the total study population, TNF-α concentrations were related to the endotoxin/HDL ratio (r=0.87, r^2=0.75, P<0.0001, Figure 2) and NYHA class (r=0.50, P=0.002), and a trend was observed for creatinine clearance (r=–0.45, P=0.05). Although oedematous patients had somewhat higher CRP levels than non-oedematous patients and controls, a significant relationship between CRP and TNF-α was not observed (r=0.32, P=0.07). The relationship between the endotoxin/HDL ratio became stronger when analysing patients only (r=0.88, r^2=0.77, P<0.0001) and was strongest when considering oedematous patients alone (r=0.91, r^2=0.82, P=0.0003, Figure 2). There was no such relationship observed when control subjects and non-oedematous patients were analysed separately. In multivariate analysis, the endotoxin/HDL ratio was the only significant predictor of TNF-α plasma concen-trations (0.45, P=0.04), independently of NYHA class (–0.18, P=0.45) and creatinine clearance (–0.34, P=0.13). This relationship was not influenced significantly by markers of hepatic function, heart failure aetiology, or medical therapy.

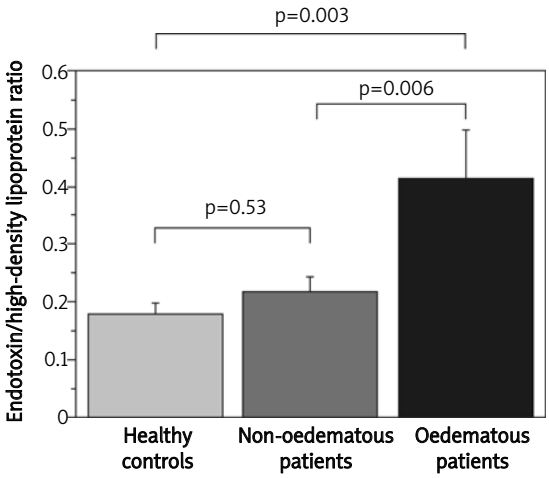

Figure 1. Bar plots illustrating the differences in the endotoxin/high-density lipoprotein ratio between controls and patients after subgrouping into non-oedematous and oedematous patients with chronic heart failure

Discussion

This study confirms that oedematous patients with CHF, compared to non-oedematous patients and healthy controls, have increased endotoxin plasma concentrations, as previously shown [11]. Markers of immune activation were also significantly elevated in oedematous patients. Serum levels of HDL related inversely to endotoxin and TNF-α, and the endotoxin/HDL ratio emerged as a powerful predictor of TNF-α plasma concentrations, particularly in oedematous decompensated CHF patients. In patients with CHF, HDL may bind LPS and prevent its biological activity. When HDL is low, heart failure patients may be more likely to develop features of inflammatory immune activation.

Bacterial translocation has been proposed to trigger the production of inflammatory cytokines [13]. We have demonstrated that LPS is increased in the plasma of oedematous patients with CHF and that endotoxaemia can be reduced by intensive treatment [11]. In oedematous patients there is clinical evidence for intestinal ischaemia, which may lead to diminished intestinal barrier function, thereby possibly enhancing bacterial translocation. In the present study we confirm our previous findings that oedematous CHF patients exhibit significantly higher plasma levels of LPS than non-oedematous patients and healthy individuals of similar age. Endotoxaemia was accompanied by significant immune activation. When bacterial LPS enters the portal circulation it is first transported to the liver [14]. Liver function, as assessed by hospital routine analyses, was not found to be abnormal in patients with oedema in the present study, or with cachexia in a previous investigation [9]. The concentrations of LPS measured in the hepatic vein

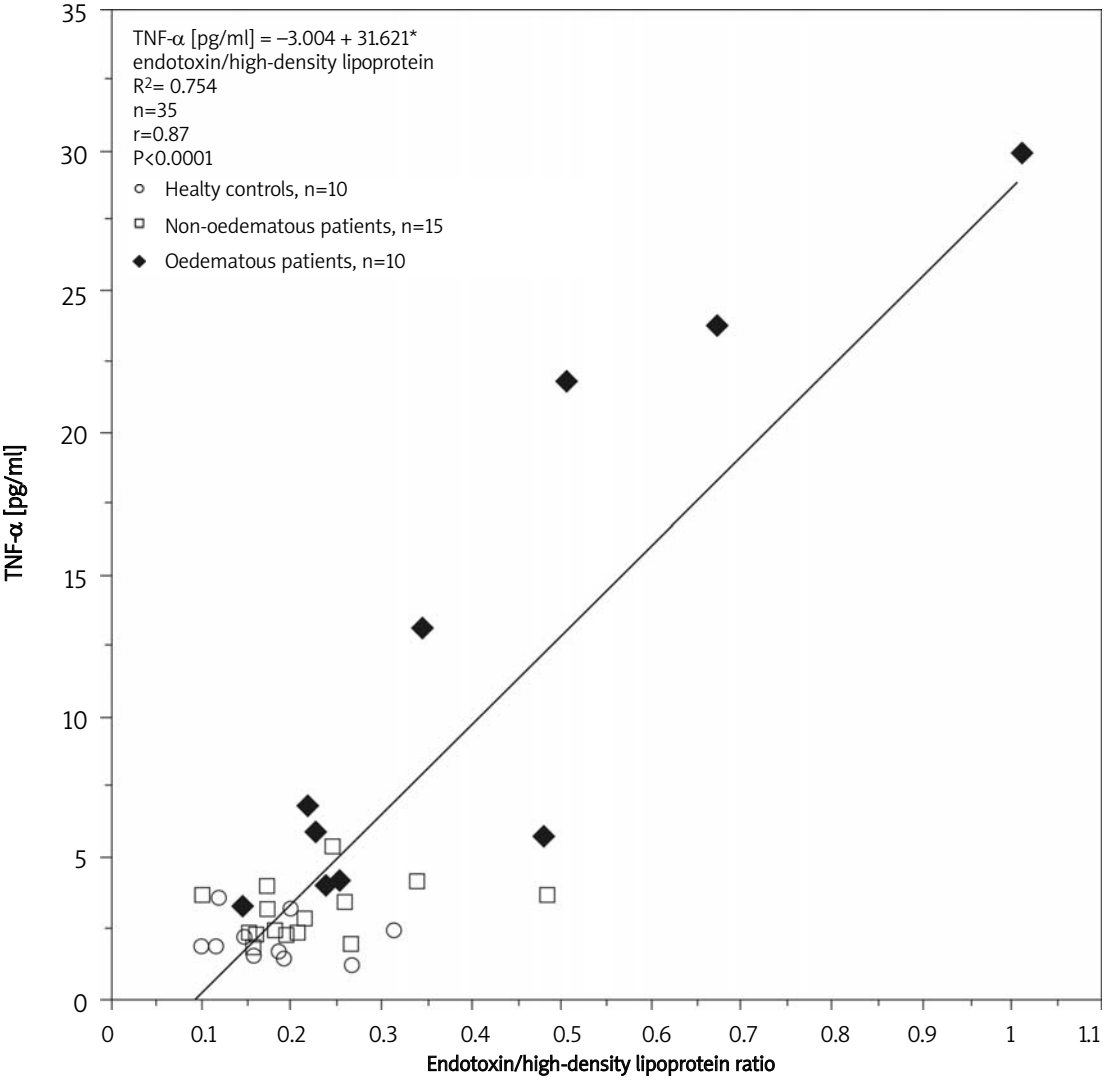

Figure 2. Univariate correlation plot presenting the relationship between the endotoxin/high-density lipoprotein ratio and TNF-α plasma concentrations in patients with chronic heart failure and in controls

were significantly higher than in the left ventricle in acutely decompensated patients with CHF [15]. Therefore, systemic concentrations of LPS may reflect intestinal LPS. Once endotoxin enters the circulation, both in patients and healthy individuals, it is not only the absolute amount, but also the biological activity that may be important [16, 17].

Regulation of lipopolysaccharide bioactivity

The degree of endotoxin bioactivity may vary and thus interactions of LPS with immune competent cells can be modified. Steroid hormones may alter cellular endotoxin sensitivity that has been raised in some patients with CHF [18, 19]. This may explain why the cortisol/dehydroepiandrosterone ratio is closely correlated to cytokine concentrations in CHF patients [20]. The most widely known and studied humoral factor to interact with LPS and modify its bioactivity is lipopolysaccharide-binding protein (LBP) [21]. LBP binds circulating LPS and the LPS-LBP complex can then bind to membrane CD14 receptors, triggering the production of inflammatory cytokines [22]. Biochemically, LBP is a member of the lipid transfer protein family. It has not been shown to be altered in oedematous heart failure patients [11], which could be interpreted as a lack in the protective physiological response to inflammatory stimuli such as bacterial LPS. Likewise, HDL could be suggested to be another regulating factor diminishing LPS bioactivity [7, 8]. In oedematous patients, HDL was lower in our study than in stable patients and healthy controls. The endotoxin/HDL ratio has been used to indicate biologically active LPS in the plasma of patients with CHF. This ratio was very closely related to TNF-α concentrations in oedematous patients. Further *in vitro* experiments and clinical studies need to explore the potential role of HDL to modify inflammatory cytokine

production in CHF patients. If this holds true for HDL and/or other lipoproteins [23], then the therapeutic use of lipoproteins (such as reconstituted HDL [24]) may be warranted, particularly in the setting of acute decompensated CHF and cardiogenic shock [25]. However, also in stable patients with CHF lipoproteins may carry prognostic power, which has been demonstrated for total cholesterol [9, 12].

In the present study a significant relationship was observed only between HDL and LPS and TNF-α, respectively, but not with LDL [9, 23]. In comparison to other clinical conditions such as sepsis [26], the concentrations of LPS in CHF are rather low, which could result in a less strong impact on lipoprotein levels. To date it is not clear whether a certain lipoprotein fraction carries a different buffer capacity. Although we used established hospital lipoprotein analyses, differen-tiation between cholesterol-rich and triglyceride-rich lipoproteins by ultracentrifugation might be more accurate to relate each lipid moiety to cytokine concentrations separately.

In conlusion, the degree of immune activation in oedematous patients with CHF is related to LPS plasma concentrations and inversely to the serum level of circulating HDL, suggesting an underlying relationship. Our findings provide clinical evidence for previously published experimental data suggesting that HDL may bind to LPS, thereby reducing its bioactivity and the induction of cytokine production. We did not find such a relationship for the other lipoproteins measured; however, the possible interplay needs further clinical and experimental research.

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