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Serum free thiols in chronic heart failure

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ABSTRACT

Oxidative stress is a key element of the pathophysiology of heart failure (HF). As free thiols are readily oxidized by reactive oxygen and sulfur species, their circulating level may directly reflect the systemic redox status. This study addresses the role of serum free thiols in chronic HF, which is of particular interest as free thiols are amenable to therapeutic modulation and thus are a potential target for therapy.

Free thiols were measured in serum of 101 previously characterized stable chronic HF patients (93% male, age 63.7 ± 10.0 y, left ventricular ejection fraction 34.6 ± 8.2%), adjusted for total serum protein, and subsequently analysed for associations with clinical and outcome parameters.

The mean serum free thiol concentration was 3.6 ± 0.5 μM/g protein. Patients with above-average levels were younger, had better renal function, lower levels of NT-proBNP and PTH, and higher levels of cholesterol. Furthermore, above-average levels were associated with favourable disease outcome, i.e. a decreased rehospitalisation rate and increased patient survival (HR 0.27 (95% CI 0.11–0.62), P = 0.002) independent of associated clinical parameters, age and PTH. After adjustment for cholesterol or established prognostic factors in HF, eGFR and NT-proBNP the association was no longer significant, suggesting involvement of these variables in a common pathophysiological pathway.

This exploratory study demonstrates favourable associations of serum free thiols with markers of HF severity and prognosis as well as disease outcome, which should be further investigated in larger prospective studies. Restoring redox status by therapeutic modulation of free thiols may be a promising strategy to improve disease outcome in CHF.

1. Introduction

Heart failure (HF) remains a leading cause of morbidity and mortality, especially among the growing population of the elderly [1,2]. A recent systematic review reported a median prevalence of HF of 11.8% in people over 60 years of age. In contrast to HF with a preserved ejection fraction, HF with a reduced ejection fraction is more common in men than in women and affects 3.3% of the older population [2]. In general, the prognosis is poor and half of all patients diagnosed with HF die within 5 years [1].

HF is a complex clinical syndrome that results from abnormal cardiac structure and/or function [3]. These abnormalities cause failure of the heart to deliver oxygen and other nutrients at a rate that meets the body's metabolic demands [4]. In an attempt to resolve the imbalance, the body responds with adaptive mechanisms, including activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system. Paradoxically, these adaptive mechanisms are associated with increased production of reactive oxygen species (ROS) [5]. Unaccompanied by adequate upregulation of antioxidative defence mechanisms, excess ROS production leads to oxidative stress, which in turn contributes to the development of myocardial and vascular dysfunction [5]. Under oxidative stress conditions, various cellular and tissue

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components are known to become targets for oxidation through reactions in which free thiols and membrane lipids play prominent roles. Typically, a shift in redox status with reductions in reduced and increases in oxidized thiols, along with rises in the concentration of lipid oxidation products is observed [6,7]. These processes are accompanied by perturbations of cardiac physiology due to progressive changes in redox signalling at multiple levels [8].

Systemic oxidative stress can be measured as the depletion of the free thiol pool in serum [9]. In contrast to the intracellular pool, which mainly consists of low molecular weight (LMW) thiols, in serum LMW thiols have a small share and protein thiols predominate [10]. Since reduced thiols are readily oxidized by ROS and other reactive species, their level may be interpreted as a direct reflection of the overall redox status [9,11]. Once oxidized, the thiols in serum are less readily reduced compared to their intracellular counterparts, and may therefore provide a relatively stable reflection of the systemic redox status. More importantly, free thiols are active components of the antioxidant machinery, which are known to be receptive to therapeutic modulation, for example by cysteine derivatives such as N-actylcysteine (NAC) [10,12,13]. Hence, they form a potential target for therapy.

Serum free thiol depletion has been reported in patients with cardiovascular disease (CVD), including acute myocardial infarction, when compared to controls [9,14]. Also, thiol oxidation has been linked to risk factors of CVD, including aging, smoking, and obesity [15]. A large body of evidence supports the role of oxidative stress in the pathogenesis of HF. Bearing in mind the relationship between free thiols and oxidative stress, in the present study we aimed to address the role of free thiols in chronic heart failure (CHF). This is of particular interest as free thiols form a potential target for therapy [12,13].

2. Methods

2.1. Patient population

This study is a post-hoc analysis of an open-label, blinded end point, randomized prospective trial (VitD-CHF trial) [16]. From March 2010 to November 2011 101 stable CHF patients presenting at the outpatient clinic of the University Medical Center Groningen, in Groningen, the Netherlands were included in this trial. Patients included in this trial were ≥18 years of age, had a left ventricular ejection fraction (LVEF) <45% and were treated with optimal HF medication (i.e. angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs), β-blockers, and mineralocorticoid-receptor antagonists (MRAs) when indicated). These patients were randomized to receive either 2000 IU of vitamin D_3 (vitD) daily or no extra medication for six weeks. The cohort has previously been described in more detail [16,17]. The study was conducted in accordance with the Declaration of Helsinki. The local Institutional Review Board approved the study protocol and all study subjects provided written informed consent.

2.2. Baseline characteristics

Data on participants' disease state, medical history and medication were extracted from patient records. Systolic and diastolic blood pressure and heart rate were measured according to protocol. The body mass index (BMI) was calculated by dividing body weight by height squared. Participants were instructed to collect 24-h urine the day before visiting the outpatient clinic. On the day of the visit, after an overnight fast, serum and plasma samples were obtained and routine laboratory measurements, including N-terminal pro-B-type natriuretic peptide (NT-proBNP), albumin, total protein, creatinine, urinary albumin and sodium, HbA1c, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), calcium, and parathyroid hormone (PTH) were performed. Aliquots of blood and urine samples were stored at −80 °C for future analysis. Measurements of plasma renin concentration (PRC), plasma renin activity (PRA), and aldosterone have been described before [16]. The estimated glomerular filtration rate (eGFR) was calculated using the 4-variable Modification of Diet in Renal Disease (MDRD) formula.

2.3. Detection of free thiols

Serum samples were stored at −80 °C until free thiol measurement. Thiol groups were detected as previously described, with minor modifications [18,19]. In short, 75 μl serum samples were diluted 1:4 with a 0.1 M Tris buffer (pH 8.2) and then transferred to a microplate. Using a Sunrise microplate reader (Tecan Trading AG, Männedorf, Switzerland), background absorption was measured at 412 nm with a reference filter at 630 nm. Subsequently, 10 μl 3.8 mM 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB, CAS-number 69–78–3, Sigma Aldrich Corporation, Saint Louis, MO, USA) in a 0.1 M phosphate buffer (pH 7) was added to the samples. Following 20 min of incubation at room temperature, absorption was measured again. The concentration of free thiols in the samples was determined by comparing their absorbance reading to that of an L-cysteine (CAS-number 52–90–4, Fluka Biochemika, Buchs, Switzerland) standard in the concentration range of 15.6–1000 μM in 0.1 M Tris and 10 mM EDTA (pH 8.2). Since proteins are by far the predominant source of thiols in serum, free thiol concentrations are expressed per gram of total serum protein [10].

2.4. Outcome parameter

The outcome parameter of this study is a composite of HF related rehospitalisation and all-cause mortality. The mean follow-up period was 4.6 ± 0.5 years. No patients were lost to follow-up.

2.5. Statistical analysis

Statistical analysis was performed with STATA software (version 13.0, Stata Corp, College Station, Texas, USA). Graphs were drawn in GraphPad Prism 5.0.

The distribution of all variables was examined using histograms, probability plots and the Kolmogorov–Smirnov test. Normally distributed continuous data are presented as mean ± standard deviation (SD). Skewed data are presented as median (interquartile range (IQR)) and were normalized by logarithmic transformation for analysis. Nominal data are presented as *n* (%).

Associations are shown with protein-adjusted free thiols as a continuous variable (for clinical parameters) or above and below the mean (for the outcome parameter). Differences in baseline characteristics between groups were determined using the Student's *t*-test for normally distributed continuous data, the Wilcoxon rank-sum test for skewed data and the Chi-square test for nominal data.

A Kaplan-Meier plot and log-rank test were used to test the association between free thiols per gram of protein above and below the mean and NT-proBNP with the outcome parameter. Univariable and multivariable linear regression analyses were applied to identify variables that are independently associated with free thiols per gram of protein. Subsequently, these variables were included in a Cox proportional hazard model. Additionally, the association of free thiols per gram of protein above and below the mean with the outcome parameter was adjusted for established prognostic factors in HF, eGFR and NT-proBNP in a second Cox proportional hazard model [20,21]. The discriminative power of these models