Original Article

Heat-shock protein 90α in plasma reflects severity of fatigue in patients with Crohn's disease

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Abstract

Heat-shock proteins (HSPs) are evolutionarily conserved proteins with important cellular homeostasis functions during harmful conditions, including inflammation. Some HSPs are secreted extracellularly and act on distant cells by down-regulating inflammation and increasing cellular stress defence mechanisms. HSP90α has been postulated to signal fatigue in chronic inflammation. We investigated whether HSP90α is associated with fatigue in patients with Crohn's disease. Fifty-three patients with newly diagnosed Crohn's disease were included in a cross-sectional study. Data on demographics and disease distribution were obtained. Fatigue was measured by the fatigue visual analogue scale (fVAS). Disease activity was assessed by the Simple Endoscopic Score for Crohn's disease and Harvey Bradshaw Index. C-reactive protein, faecal calprotectin and HSP90α were also measured. The median fVAS score was 52 mm, indicating significant fatigue. HSP90α scores correlated significantly with fVAS ($r = 0.31$, $P = 0.03$). In a multivariate regression model, HSP90α was the only significant contributor to fVAS scores ($\beta = 0.31$, $P = 0.03$). When patients were dichotomised into groups with high and low HSP90α concentrations, significantly higher fVAS scores were demonstrated in the group with high HSP90α ($M = 62.4$, confidence interval 53.0–71.8 vs. 43.3, 31.6–55.0; $P = 0.01$). Thus, HSP90α may contribute to fatigue generation and/or modulation in patients with Crohn's disease.

Introduction

Fatigue can be defined as 'an overwhelming sense of tiredness, feeling of exhaustion, and a lack of energy'[1] and has been recognised as a common and substantial complaint in many chronic inflammatory conditions, including rheumatoid arthritis, primary Sjögren's syndrome, primary sclerosing cholangitis and inflammatory bowel diseases (IBDs).[2,3] Fatigue reduces quality of life, can be so severe that it leads to social withdrawal and/or inability to work and is sometimes debilitating.

The mechanisms that lead to and modulate fatigue are mostly unknown. Depressive mood, psychosocial factors and pain influence fatigue, but growing evidence indicates that inflammation and/or different cellular stress conditions trigger neuro-immune mechanisms involved in the generation of fatigue.[4,5] A prevailing hypothesis is that fatigue is part of an evolutionarily conserved protective mechanism ('sickness behaviour') activated in animals and humans during infection or inflammation in order to increase their ability to survive. Fatigue constitutes a

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substantial part of this behaviour, which also includes sleepiness, lack of thirst and appetite, depressive mood and social withdrawal.[6]

IL-1β is an important molecule in the generation of sickness behaviour, and thus fatigue, in both animals and humans.[7,8] IL-1β is produced by activated innate immune cells during inflammation and is actively transported to the brain, where it binds to specific receptors.[9] IL-1β inhibition reduces fatigue, indicating its importance in fatigue signaling.[10]

Heat-shock proteins (HSPs) are highly conserved proteins classified according to their molecular weight. They have important cell-protective functions, acting as intracellular chaperones in protein trafficking and folding.[11] Some HSPs are secreted extracellularly and may influence distant cells, such as innate immune cells and neurons.[12] Production of HSPs is triggered by different types of cellular stress, such as inflammation, pathogens, thermal stress and oxidants. HSP90 consists mainly of two isoforms: the inducible HSP90α and constitutively expressed HSP90β.[13] During inflammation and other forms of cellular stress, HSP90α is secreted extracellularly. We previously showed that a high concentration of HSP90α in the blood is associated with severe fatigue in patients with primary Sjögren's syndrome,[4] and we have postulated that peripherally produced HSP90α passes the blood–brain barrier and binds to TLR4 on microglia. This leads to intracerebral production of IL-1β, which induces fatigue.

Crohn's disease is a chronic IBD that can affect any part of the gastrointestinal tract from the oral orifice to the anus but most frequently involves the ileocolon.[14] Clinically significant fatigue is reported in 40–80% of patients with Crohn's disease and constitutes a substantial problem.[3]

The aim of the current study was to investigate whether this association between HSP90α and fatigue is evident in patients with newly diagnosed and untreated Crohn's disease.

Materials and methods

A total of 53 patients with newly diagnosed and untreated Crohn's disease at the Unit of Gastroenterology, Stavanger University Hospital, were consecutively included in a cross-sectional study and underwent one study visit between 1 January 2013 and 31 December 2016. Descriptive data are given in Table 1. Inclusion criteria were age ≥16 yr and newly diagnosed Crohn's disease based on clinical, laboratory, endoscopic, histological and radiological criteria according to European Crohn's and Colitis Organisation (ECCO) guidelines. Exclusion criteria were a history of previous IBD or pregnancy. All patients were recruited at the time of endoscopy,

Table 1. Selected demographic and clinical characteristics of 53 patients with newly diagnosed Crohn's disease.

Variable	
Age (yr)	35 (16–78)
Males, n (%)	23 (43.4)
Disease distribution, n (%)	
Ileal	28 (53.8)
Colonic	3 (5.8)
Ileocolonic	21 (40.4)
fVAS (mm)	52 (1–100)
HSP90α (ng/ml)	17.2 (6.4–55.1)
HBI	5 (0–14)
CRP (mg/l)	9.8 (1–139)
Faecal calprotectin (mg/kg), n = 47	254 (15–4432)
SES-CD	7 (1–37)
Haemoglobin (g/dl), M (SD)	13.4 (1.6)
Ferritin (µg/l)	84 (7–1382)

Data are presented as the median (range) unless otherwise noted.
fVAS: fatigue visual analogue scale; HSP: heat-shock protein HBI: Harvey Bradshaw Index; CRP: C-reactive protein; SES-CD: Simple Endoscopic Score for Crohn's Disease.

with all study data collected within 3 d after colonoscopy.

Assessment of fatigue

The fatigue visual analogue scale (fVAS), a generic and unidimensional fatigue instrument, was used to rate the severity of fatigue. The fVAS comprises a 100 mm horizontal line with vertical anchors. The wording on the left end (0 mm) was 'no fatigue' and on the right end (100 mm) was 'fatigue as bad as it can be'. The fVAS is a frequently used and widely accepted fatigue instrument, and clinically important fatigue has been defined as an fVAS score ≥50.[3]

Assessment of disease activity

Harvey Bradshaw Index. Disease activity was rated using the Harvey Bradshaw Index (HBI). This index is based on patient symptoms and includes general well-being, abdominal pain and number of loose stools the previous day, in addition to the presence of a palpable mass in the abdomen and extra-intestinal manifestations, such as arthralgia, fistula and abscesses.[15]

Simple Endoscopic Score for Crohn's Disease. For optimal objective rating of disease activity, we used the Simple Endoscopic Score for Crohn's Disease (SES-CD) to grade intestinal inflammation.[16]

Inflammatory markers. Serum C-reactive protein (CRP) measured at the time of endoscopy (± 3d.) and faecal calprotectin (from 4 wk before until 3 d after endoscopy) were measured in all patients.

Markers of anaemia and iron deficiency

Haemoglobin and ferritin were measured in all patients at the time of endoscopy (± 3d.).

HSP90α analysis

At the time of inclusion, blood samples were drawn into EDTA tubes from all patients. These samples were kept cold on ice before centrifuging at 2400 *g* for 10 min at 4°C. Plasma was immediately separated, aliquoted and stored at −80°C until analysis.

HSP90α was measured in duplicate using a commercially available ELISA kit (Enzo Life Sciences, Farmingdale, NY). Samples were diluted 1:25 and analysed following the manufacturer's protocol. The final absorbance was read at 450 nm using a Synergy H1 plate reader (BioTek, Bad Friedrichshall, Germany). The HSP90α concentration was determined using a standard curve generated from calibrating solutions of known concentrations. According to the manufacturer, the detection range of the method is 0.0625–4 ng/mL, with a sensitivity of 50 pg/ml and no cross-reactivity with HSP90β. The calculated coefficient of variance (CV) between duplicates was < 13%, and the inter-assay CV was < 8%.

Statistical analysis

The normality of the data was tested using the Shapiro–Wilk test. Correlations were analysed by Spearman's rank correlation test. Differences between two independent groups were analysed using the independent-samples *t*-test. Univariable linear regression models were explored using fVAS scores as the dependent variable and HSP90α, SES-CD, CRP, haemoglobin, ferritin, age and sex as independent variables.

Multivariable analyses were performed first in a preliminary model including all variables used in univariable analysis. Then, we selected five independent variables (HSP90α, SES-CD, CRP, age and sex), and finally a backwards stepwise model selection was used to exclude non-significant independent variables. A significance level of 0.05 was used. As this was an exploratory study, no power-estimation was performed.

Data are available from the author upon reasonable request.

Ethical considerations

This study was approved by the Norwegian regional ethics committee (REK 2011/2631) and was carried out in compliance with the principles outlined in the Declaration of Helsinki. All patients provided written informed consent to participate in the study. The study was registered at ClinicalTrials.gov (NCT01551563).

Results

Baseline characteristics

The majority of patients (53.8%) had ileal disease distribution. The median (range) HBI and SES-CD were 5 (0–14) and 7 (1–37), respectively, which indicate mild to moderate disease activity at the time of diagnosis. The median (range) fVAS score was 52 mm (1–100 mm), whereas the median HSP90α concentration was 17.2 ng/ml (6.4–55.1 ng/ml). Faecal calprotectin data were missing for six subjects. Baseline data are given in Table 1.

HSP90α and fatigue

HSP90α significantly positively correlated with fVAS scores ($r = 0.31$, $P = 0.03$).

Regression models

In the univariable regression analysis, when using fVAS scores as the dependent variable, there was a modest but significant positive association with HSP90α ($r^2 = 0.09$, $P = 0.03$). In addition, CRP was significantly and positively associated with fVAS scores in the univariable models, whereas SES-CD, haemoglobin, ferritin, age and sex were not (Table 2).

In a preliminary multivariable linear regression model, all independent variables from univariable analyses were included. Assuming a target r^2 of 0.2, a power of 0.8 and a significance level of 0.05, a maximum number of five independent variables could be included in the model based on 53 cases available for calculation.[17] We chose to include HSP90α, CRP, SES-CD, age and sex. In this model, r^2 was 0.20, $P = 0.06$, and only HSP90α was a significant contributor to fVAS scores ($β = 0.35$, $P = 0.04$; Table 3). Stepwise backward model selection was then applied to exclude non-

Table 2. Univariable linear regression analysis in 53 patients with Crohn's disease using fVAS scores as the dependent variable.

	fVAS	
	β	P value
Age	0.19	0.17
Sex	0.17	0.21
CRP (mg/l)	0.29	0.04
SES-CD	0.20	0.15
Haemoglobin (g/dl)	-0.08	0.57
Ferritin (μg/l)	0.15	0.27
HSP90α (ng/ml)	0.31	0.03

fVAS: fatigue visual analogue scale; HSP: heat-shock protein HBI: Harvey Bradshaw Index; CRP: C-reactive protein; SES-CD: Simple Endoscopic Score for Crohn's Disease.

Table 3. Multivariable regression model in 53 patients with Crohn's disease using fVAS scores as the dependent variable ($r^2 = 0.20$, $P = 0.06$).

	fVAS	
	β	P value
Age	-0.22	0.11
Sex	-0.19	0.17
SES-CD	-0.07	0.69
CRP (mg/l)	0.14	0.44
HSP90α (ng/ml)	0.35	0.04

fVAS: fatigue visual analogue scale; HSP: heat-shock protein HBI: Harvey Bradshaw Index; CRP: C-reactive protein; SES-CD: Simple Endoscopic Score for Crohn's Disease.
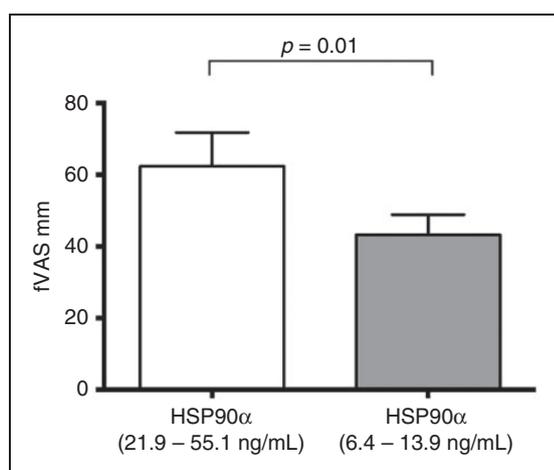
Figure 1. Fatigue visual analogue scale (fVAS) scores (mm) for the 20 patients with the highest heat-shock protein 90α (HSP90α) concentrations (ng/ml) versus the 20 patients with the lowest HSP90α concentrations. Data are presented as means with 95% confidence intervals.

significant independent variables. Ultimately, HSP90α was shown to influence fVAS the most ($\beta = 0.31$, $P = 0.03$).

When selecting the 20 patients with the highest HSP90α concentrations versus the 20 patients with the lowest HSP90α concentrations (21.9–55.1 ng/ml vs. 6.4–13.9 ng/ml), fVAS scores were significantly higher in the high HSP90α group ($M = 62.4$, confidence interval (CI) 53.0–71.8) compared to the low HSP90α group ($M = 43.3$, CI 31.6–55.0, $P = 0.01$; Figure 1).

Discussion

In this study, we found a significant association between higher plasma HSP90α levels and more severe fatigue in patients with newly diagnosed Crohn's disease. These results were consistent in both correlation and regression analyses. In addition, HSP90α was the strongest influencing factor for fatigue when correcting for multiple independent variables. Patients with the highest HSP90α scores also demonstrated significantly more fatigue in independent group analyses.

HSPs are generally thought to be essential cellular defence proteins, which are produced in conditions that constitute a threat to cellular life, such as inflammation, toxic compounds, heat and oxidants.[11] HSPs downregulate innate immune reactions and inflammation and reduce tissue damage.[18] Some previous animal studies indicate that HSP90 may have a dual role and increase inflammation in some conditions, while being protective and down-regulating inflammation in other conditions.[19–21]

Fatigue has previously been an overlooked and ignored phenomenon in IBD but is increasingly recognised as one of the major problems for many patients. Clinically significant fatigue affects roughly 50% of patients at the time of diagnosis.[3] Although the pathophysiological mechanisms and pathways that generate fatigue are only beginning to be revealed, the strong and evolutionarily conserved 'sickness behaviour' response appears essential in understanding fatigue in both animals and humans.[7,22] Sickness behaviour is a genetically based unconscious and temporary behaviour evoked during states of infection and cellular damage to increase survival of the individual.[22] The behaviour becomes persistent in states of chronic inflammation or chronic cellular stress. Crohn's disease is a chronic inflammatory condition and therefore triggers this behavioural response in which fatigue is a major element.

IL-1β has been associated with fatigue in both preclinical and clinical studies, supporting the innate immune system being fundamentally involved in the development of sickness behaviour.[8,10] Sickness behaviour is a well-known cerebral phenomenon.[23] A leading hypothesis is that inflammatory conditions in peripheral tissues trigger the release of signal molecules that are transported into the brain, passing the blood–brain barrier and binding to specific cellular receptors.[7] This can include pro-inflammatory cytokines, such as IL-1β, but also other molecules that bind to TLR4 on microglia and lead to intracerebral production of IL-1β, consequently leading to the generation of sickness behaviour, including fatigue.[24]

A recent study from our group demonstrated that fatigue is associated with high levels of HSP90α in primary Sjögren's syndrome.[4] In the current study, we demonstrated similar findings in Crohn's disease. Our findings fit with the hypothesis that HSP90α is transported into the brain, although the exact mechanism is currently not known. Intracerebrally, HSP90α may act as a signal molecule for fatigue, possibly through the TLR4–IL-1β pathway.

Our findings also support the role of HSP90α as a fundamentally protective molecule not only for cellular life, but also by inducing the protective behaviour of the individual. As such, HSP90α is a 'super-defence' protein, providing protection for the organism on both the micro- and the macro-biological level by increasing its possibility of surviving.

A generic, validated fatigue instrument, the fVAS, was used in the current study, allowing comparisons with other diseases. We previously demonstrated that HSP90α may be a biomarker of Crohn's disease activity.[25] Thus, we included selected objective markers of disease activity and inflammation in our multivariable analysis. However, HSP90α was still the strongest contributor to fatigue.

We also included newly diagnosed and untreated patients in the current study, thus avoiding possible interference from medical treatment, such as corticosteroids, on fatigue severity.[26]

This study has limitations. We did not analyse HSP90α concentrations in the intestinal mucosa, the target tissue for inflammation in Crohn's disease. We do not have any follow-up data demonstrating whether the association between HSP90α and fVAS persists over time. All patients were newly diagnosed with active disease, so we could not assess if HSP90α were associated with the presence/absence of disease activity. In addition, we did not take into account some other factors that may contribute to fatigue, such as sleep quality, depression and anxiety.[26]

In conclusion, high HSP90α plasma levels are associated with more severe fatigue in Crohn's disease, indicating that this protein may be a modulator of fatigue, possibly acting through intracerebral signalling pathways. HSP90α inhibitors should be tested in chronic inflammatory/IBD animal models as a novel treatment to attenuate sickness behaviour and fatigue.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: T.G. has served as a speaker for Ferring Pharmaceuticals, Takeda, and advisory board member for Takeda, Janssen-Cilag and Tillotts Pharma. No other conflicts of interest were declared by the authors.

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