Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Oxidative albumin damage in chronic liver failure: Relation to albumin binding capacity, liver dysfunction and survival

Karl Oettl[1,*], Ruth Birner-Gruenberger[2,3], Walter Spindelboeck[4], Hans Peter Stueger[5], Livia Dorn[6], Vanessa Stadlbauer[4], Csilla Putz-Bankuti[4], Peter Krisper[7], Ivo Graziadei[6], Wolfgang Vogel[6], Carolin Lackner[3], Rudolf E. Stauber[4]

[1]*Institute of Physiological Chemistry, Medical University of Graz, Graz, Austria;* [2]*Center for Medical Research, Core Facility Mass Spectrometry, Medical University of Graz, Graz, Austria;* [3]*Institute of Pathology, Medical University of Graz, Graz, Austria;* [4]*Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Graz, Austria;* [5]*Austrian Agency for Health and Food Safety, Department of Data, Statisics, Risk Assessment, Graz, Austria;* [6]*Division of Gastroenterology and Hepatology, Department of Internal Medicine II, Medical University of Innsbruck, Innsbruck, Austria;* [7]*Division of Nephrology and Hemodialysis, Department of Internal Medicine, Medical University of Graz, Graz, Austria*

See Editorial, pages 918–920

Background & Aims: Impaired binding function of albumin has been demonstrated in end-stage liver disease. This and other functional disturbances of albumin may be related to oxidative stress which is believed to play an important role in the pathogenesis of liver failure as well as sepsis. The aim of the present study was to relate oxidative modification of albumin to loss of albumin binding function in advanced chronic liver failure and in sepsis.

Methods: Patients with decompensated cirrhosis or sepsis and healthy controls were investigated. Three fractions of albumin were separated by chromatography according to the redox state of cysteine-34: non-oxidized human mercaptalbumin, reversibly oxidized human non-mercaptalbumin-1, and irreversibly oxidized human non-mercaptalbumin-2 (HNA2). Binding properties of albumin site II were measured using dansylsarcosine as a ligand.

Results: Both in cirrhotic and septic patients, fractions of oxidized albumin were increased and binding capacity for dansylsarcosine was decreased. Mass spectroscopy confirmed specific oxidation of cysteine-34. In cirrhotic patients, dansylsarcosine binding correlated strongly with liver function parameters and moderately with HNA2. Baseline levels of HNA2 accurately predicted 30-day and 90-day survival in cirrhotic patients and this was confirmed in an external validation cohort.

Conclusions: Our results suggest that oxidative damage impairs binding properties of albumin. In advanced liver disease, reduced binding capacity of albumin site II is mainly related to impaired liver function. The plasma level of HNA2 is closely related to survival and may represent a novel biomarker for liver failure.

Introduction

Albumin, a 66.5 kDa protein (Fig. 1), is the most abundant plasma protein, the main determinant of colloid osmotic pressure and an important carrier for endogenous and exogenous substances [1]. Among its most important functions are fatty acid transport (on several binding areas), drug binding and transport, metal chelation, and free radical scavenging which is mediated by the anti-oxidant properties of its thiol moiety at cysteine-34 (reviewed in [2]).

Albumin is the major extracellular source of reduced sulfhydryl groups, which are potent scavengers of reactive oxygen and nitrogen species [3]. Depending on the redox state, there are three major fractions of albumin: human mercaptalbumin (HMA), the non-oxidized form with a free thiol group on cysteine-34, and two different oxidized forms: (i) human non-mercaptalbumin-1 (HNA1) with cysteine, homocysteine or glutathione bound to cysteine-34 by a disulfide bond and (ii) human non-mercaptalbumin-2 (HNA2) with cysteine-34 irreversibly oxidized to sulfinic or sulfonic acid [4,5]. Oxidative stress is believed to play an important role in advanced liver failure [6] and may be reflected in oxidative modification of albumin. Decreased HMA and/or elevated HNA levels have been reported in chronic liver disease and these

Keywords: Oxidative stress; Cirrhosis; Mercaptalbumin; Non-mercaptalbumin; Dansylsarcosine.

Received 20 August 2012; received in revised form 5 June 2013; accepted 13 June 2013; available online 25 June 2013

☆ DOI of original article: http://dx.doi.org/10.1016/j.jhep.2013.08.001.

* Corresponding author. Address: Institute of Physiological Chemistry, Medical University of Graz, Harrachgasse 21, A-8010 Graz, Austria. Tel.: +43 316 380 7544; fax: +43 316 380 9610.

E-mail address: karl.oettl@medunigraz.at (K. Oettl).

Abbreviations: HMA, human mercaptalbumin; HNA1, human non-mercaptalbumin1; HNA2, human non-mercaptalbumin2; ACLF, acute-on-chronic liver failure; INR, international normalized ratio; CRP, C-reactive protein; MELD, Model for End-stage Liver Disease; HPLC, high performance liquid chromatography; DS, dansylsarcosine; ROC, receiver operating characteristics.

changes have been shown to correlate with its severity as estimated by the Child-Pugh score [7,8]. Recently, we demonstrated a 4-fold increase of the irreversibly oxidized albumin fraction, HNA2, indicating a marked alteration in the redox state of circulating albumin, in patients with advanced liver disease [9].

Albumin harbours two specific binding sites described by Sudlow: site I, which binds large heterocyclic compounds and dicarboxylic acids (such as bilirubin), and site II, which binds aromatic carboxylic compounds (such as benzodiazepines) [10]. Oxidized albumin shows altered binding capacities for several substances used to assess albumin function [11,12]. Decreased binding of dansylsarcosine – a model ligand for the benzodiazepin binding site II – was found in patients with end-stage liver disease [13,14]. The pathogenesis of this impaired binding capacity and, specifically, its relation to oxidative albumin damage remain unknown.

Several disturbances of albumin binding function are known to occur in cirrhotic patients [9,14,15]. Based on these pathophysiologic changes, albumin infusions are being used therapeutically to normalize the serum albumin level and/or restore albumin function in various conditions such as prevention and treatment of hepatorenal syndrome, prevention of postparacentesis circulatory dysfunction, and extracorporeal albumin dialysis for removal of potentially toxic substances accumulating in liver failure (reviewed in [16]). These beneficial effects are believed to be mediated at least in part by improved toxin binding and transport following infusion of fresh albumin or, in case of albumin dialysis, regeneration of toxin-laden albumin.

Acute-on-chronic liver failure (ACLF), i.e., acute exacerbation of chronic liver failure, is frequently triggered by infection and its clinical features such as end-organ failure resemble those of sepsis [17]. Since ACLF is believed to be mediated by oxidative stress [6], oxidation of albumin is likely to occur. The specific aims of the present study were (i) to assess oxidative modification of albumin in these clinical conditions, (ii) to relate these changes to albumin dysfunction, specifically the disturbance of site II specific binding, and (iii) to determine the prognostic significance of the observed changes in chronic liver failure.

Patients and methods

Patients

The study population comprised 67 consecutive cirrhotic patients hospitalized for acute decompensation at the Medical University of Graz, including 16 patients requiring ICU treatment, and 18 consecutive non-cirrhotic patients with sepsis without evidence for cirrhosis or underlying hematologic disease admitted at our ICU. In addition, 15 age- and sex-matched blood donors served as healthy controls. Patients with hepatocellular carcinoma, recent gastrointestinal bleeding (<7 days before enrolment) or liver transplantation within 90 days from enrolment were excluded. Sepsis was diagnosed according to international guidelines [18] as the presence of the systemic inflammatory response syndrome (SIRS) in response to a confirmed infectious process. Systemic inflammatory response syndrome was defined by the presence of two or more of the following criteria: temperature >38 °C or <36 °C; heart rate >90 beats/min; respiratory rate >20 per min or $PaCO_2$ <32 mmHg; white blood cells >12,000 cells/mm^3 or <4000 cells/mm^3. Infection was defined as positive cultures of blood, ascites, urine, sputum or wounds, and/or clinical findings suggestive of infections.

An additional cohort of 40 cirrhotic patients hospitalized for acute decompensation at the Medical University of Innsbruck, Austria, who fulfilled the same inclusion and exclusion criteria as the original cirrhosis cohort, was used as an external validation set for determining the prognostic value of HNA2.

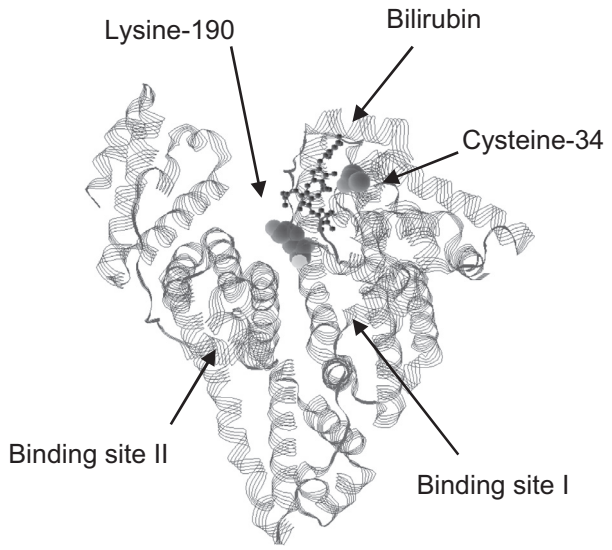

Fig. 1. Structure of human albumin. Classical binding sites I and II, bilirubin bound to a binding site (lysine-190), where it is bound covalently, and cysteine-34 are indicated by arrows. The figure was created using a PDB-file by Zunszain *et al.* [28], PDB-ID: 2VUE.

Patients were managed following local treatment protocols for liver failure and sepsis, including full intensive care support. Patients with suspected hepatorenal syndrome received an intravenous fluid challenge with albumin 1 g/kg according to current guidelines. The study protocol was approved by the Ethics Committee of the Medical University of Graz and informed consent was obtained in accordance with the Declaration of Helsinki.

Study design

Blood samples were collected within 24 h after admission, immediately centrifuged at 4 °C and plasma aliquots were stored at −70 °C until batch analysis. Bilirubin, albumin, creatinine, prothrombin time (international normalized ratio, INR), and C-reactive protein (CRP) were routinely assessed. To estimate severity of liver disease, the model for end-stage liver disease (MELD) was calculated [19,20].

Albumin analysis

Albumin was fractionated by high performance liquid chromatography to give three peaks according to cysteine-34 in the free sulfhydryl form (HMA), as a mixed disulfide (HNA1) or in a higher oxidation state (HNA2), as previously described [21]. Briefly, 20 μL of diluted plasma were injected into the HPLC system using a Shodex Asahipak ES-502N 7C anion exchange column (7.5 × 100 mm, Bartelt Labor- & Datentechnik, Graz, Austria) and 50 mM sodium acetate, 400 mM sodium sulfate, pH 4.85 as mobile phase. For elution, a gradient of 0–6% ethanol and a flow rate of 1 mL/min were used. The column was kept at 35 °C. Detection was carried out by fluorescence at 280/340 nm. Quantification was based on peak heights determined by EZ Chrom Elite chromatography software (VWR, Vienna, Austria).

Mass spectrometry

In order to further determine oxidative modification of HNA2, mass spectrometry was performed in a plasma sample of three additional patients with ACLF. Disulfide bridges of plasma proteins were reduced by incubation with 5 mM DTT and then alkylated by incubation with 10 mM iodoacetamide. Subsequently, protein was digested by adding modified trypsin (purchased from Promega, Mannheim, Germany; trypsin to plasma protein 1:50 by mass) and shaking overnight at 550 rpm and 37 °C. The peptide solution was acidified by adding 0.05% trifluoracetic acid (TFA, final concentration) and diluted in solvent A to a theoretical final total peptide concentration of 25 ng/μL. Digests (500 ng of sample) were separated by nano-HPLC (Dionex UltiMate 3000 RSLC system, Vienna, Austria)