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Plasma osteopontin in acute liver failure

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ABSTRACT

Background: Osteopontin (OPN) is a novel phosphoglycoprotein expressed in Kupffer cells that plays a pivotal role in activating natural killer cells, neutrophils and macrophages. Measuring plasma OPN levels in patients with acute liver failure (ALF) might provide insights into OPN function in the setting of massive hepatocyte injury.
Methods: OPN levels were measured using a Quantikine® ELISA assay on plasma from 105 consecutive ALF patients enrolled by the US Acute Liver Failure Study Group, as well as controls including 40 with rheumatoid arthritis (RA) and 35 healthy subjects both before, and 1 and 3 days after undergoing spine fusion (SF) surgery as a model for acute inflammation.
Results: Median plasma OPN levels across all etiologies of ALF patients were elevated 10- to 30-fold: overall median 1055 ng/mL; range: 33–19,127), when compared to healthy controls (median in pre-SF patients: 41 ng/mL; range 2.6–86.4). RA and SF post op patients had elevated OPN levels (37 ng/mL and 198 ng/mL respectively), well below those of the ALF patients. Median OPN levels were highest in acetaminophen (3603 ng/mL) and ischemia-related ALF (4102 ng/mL) as opposed to viral hepatitis (706 ng/mL), drug-induced liver injury (353 ng/mL) or autoimmune hepatitis (436 ng/mL), correlating with the degree of hepatocellular damage, as reflected by aminotransferase values (R value: 0.47 for AST, $p < 0.001$).
Conclusions: OPN levels appeared to correlate with degree of liver necrosis in ALF. Very high levels were associated with hyperacute injury and good outcomes. Whether OPN exerts a protective effect in limiting disease progression in this setting remains uncertain.

1. Introduction

Acute liver failure (ALF) results from severe hepatic injury of any kind, features coagulopathy (international normalized ratio: INR > 1.5) and varying degrees of hepatic encephalopathy (HE) [1]. On a pathophysiological level there is sterile inflammation, progression to multi-organ failure and functional immunoparesis. The progression of ALF seems to depend on the balance of pro- and anti-inflammatory responses in the liver, paralleling many features of sepsis and the systemic inflammatory response syndrome (SIRS) [2].

Many inflammatory and immune mediators are currently under investigation, including osteopontin (OPN) which was originally identified in bone [3]. OPN is composed of approximately 300 amino acids, with two distinct isoforms: a secreted and an intracellular form. At its center, OPN contains a classical binding motif, an arginine-glycine-aspartic acid (RGD) domain that is recognized by cell surface integrins. Near the RGD domain, OPN may be cleaved by proteases (thrombin and plasmin) [4]. Through RGD binding to cell surface receptors on target cells, secreted OPN can modulate cell adhesion and serve as a chemoattractant to other inflammatory mediators. OPN also acts as an autocrine and paracrine factor, playing an important role in induction and secretion

Abbreviations: ALF, acute liver failure; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APAP, acetaminophen; AST, aspartate aminotransferase; DAS-28, Disease Activity Score-28; HE, hepatic encephalopathy; INR, international normalized ratio; OPN, osteopontin; RA, rheumatoid arthritis; RGD, arginine-glycine-aspartic acid domain; SF, spinal fusion; SIRS, systemic inflammatory response syndrome; SS, spontaneous survival; USALFSG, US Acute Liver Failure Study Group; VARA, Veterans Affairs Rheumatoid Arthritis Registry; WBC, white blood cell.

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of cytokines, macrophage and neutrophil migration, and subsequent activation [5,6]. Apart from its role in bone remodeling and regulation of osteoclast activity, OPN appears to play a key role in triggering inflammation in autoimmune diseases (rheumatoid arthritis, multiple sclerosis), other chronic inflammatory states, acute inflammatory conditions (trauma) and malignancies [4,7]. Additionally, there has been interest in use of OPN as a tumor marker in patients with hepatocellular carcinoma [8,9]. While its exact functions remain unclear, intracellular OPN affects cell motility, cytoskeletal rearrangement, mitosis, signal transduction pathways downstream of innate immune receptors [10].

Elevations in serum and plasma OPN levels have been found in small studies of ALF patients [11,12]. The correlation of OPN level with eventual outcomes in ALF patients has not been well defined. Our present goals were to compare OPN levels in a large series of ALF patients, examining how etiology, disease severity and prognosis are related to OPN levels while comparing OPN levels in ALF to those observed in healthy controls, in patients with RA (chronic inflammation) with varying levels of disease activity and in spinal fusion patients pre- and post-op as an example of an acute injury/trauma model.

2. Materials and methods

2.1. Patients and specimens

The US Acute Liver Failure Study Group (ALFSG) was established in 1998 as a consortium of liver centers interested in better defining the causes and outcomes of acute liver failure. To date, more than 2500 subjects have been enrolled prospectively at 23 tertiary centers within the US, all of which have liver transplantation programs. All enrolled subjects met standard criteria for acute liver failure: presence of coagulopathy (prothrombin time > 15 s or INR $\geqslant$ 1.5) and any degree of hepatic encephalopathy (HE), occurring within 26 weeks of the onset of first symptoms in a patient without previous underlying liver disease [1,13]. Since the subjects were encephalopathic by definition, written informed consent was obtained from their legal next of kin. Detailed demographic, clinical, laboratory and outcome data as well as daily sera for 7 days were collected prospectively. All centers were in compliance with their local institutional review board requirements. A Certificate of Confidentiality was obtained from the National Institutes for Mental Health for the entire study.

To obtain a representative selection of ALF patients of all etiologies we studied plasma samples from 105 patients enrolled consecutively into the US Acute Liver Failure Study between 2006 and 2008, obtained on study day 1 and stored at −80 °C for 1–3 years prior to testing. Causes for ALF in this group included acetaminophen toxicity (APAP); ischemia (Shock); idiosyncratic drug-induced liver injury (DILI); autoimmune hepatitis (AIH); viral hepatitis (A or B); indeterminate (IND – cases in which a specific cause could not be determined); and other (a miscellaneous group including heat stroke, cancer, Budd Chiari syndrome). Among the 12 AIH patients studied, none had received immunosuppression (usually prednisone in moderate to high doses) for longer than two weeks at the time of admission to study.

To serve as a healthy control group and as an example of an acute inflammatory state, we obtained plasma samples from patients prior to posterior lumbar spinal fusion and on two occasions following the procedure. The 35 subjects studied prior to and after lumbar fusion (Rigshospitalet, Copenhagen, DK) all had symptomatic degenerative disc disease with chronic low back pain but were considered to be in good health otherwise, and without any autoimmune diseases. These patients would presumably mimic the findings observed in trauma patients or other similar acute inflammatory settings. Each patient's sample prior to surgery provided a control for subsequent plasma samples collected on day 1 and 3 after the operation. All patients underwent posterior lumbar un-instrumented fusion without decompression. Fusion was done from L4 to S1. Samples were obtained at 24 h prior to surgery and at 24 and 72 h after surgery and stored at −80 °C for approximately up to 10 years prior to testing; no patient was receiving corticosteroids prior to surgery. As an example of a chronic inflammatory state (rheumatoid arthritis: RA), we obtained 40 RA plasma samples from the longitudinal Veterans Affairs Rheumatoid Arthritis Registry, a database recruiting RA patients at 10 VA hospital sites in the US (VARA) [14]. From VARA, 20 patients were chosen with high disease activity (Disease Activity Score-28 (DAS-28) average 6.90) and 20 were selected with low disease activity (average DAS-28 0.97). All RA subjects were receiving either NSAIDs or immune modulating therapy at the time of the blood draw. Blood samples were obtained and the plasma samples were stored at −80 °C for 2–4 years prior to testing. In both high and low DAS score groups, 60% were receiving immunosuppressive therapy and 65% NSAIDs; all were receiving one or both forms of treatment. We did not see any difference in levels between those with only NSAIDs and those with immunosuppressive or combined treatment.

2.2. ELISA

The plasma OPN levels were determined for all samples using the Quantikine® Osteopontin ELISA assay (R&D Systems Inc. Minneapolis, MN). The lower detection limit for plasma samples with this assay was 0.011 ng/mL. We utilized plasma samples for these analyses since serum levels of OPN are recognized to be considerably lower than plasma due to cleavage of OPN by thrombin [15,16].

2.3. Statistical methods

Statistical analyses were performed using IBM® SPSS® Statistics 17.0 (SPSS Inc, Chicago, IL USA). Non-parametric analysis was used for comparing continuous variables between groups (Kruskal–Wallis with post hoc testing (Dunn method) for multiple groups and Mann–Whitney for 2 groups). Logistic regression analysis was performed to identify independent factors associated with severe hepatic encephalopathy and spontaneous survival.

3. Results

3.1. Demographic and clinical features

Detailed clinical and demographic data for the 105 ALF patients are shown in Table 1. APAP overdose patients were around 10 years younger than patients with other etiologies as has been previously observed. APAP overdose patients and those with ischemic liver injury had significantly higher alanine aminotransferase (ALT) levels, lower bilirubin levels and higher creatinine levels compared to the remainder of ALF patients.

3.2. Plasma osteopontin levels in ALF patients compared to controls

Significantly higher plasma OPN levels were observed in the overall ALF patient group (Fig. 1, median: 1055 ng/mL; Range: 33–19,127) when compared to healthy controls (SF pre-surgery group: median 41 ng/mL; range 3–86, $p < 0.001$), or chronic inflammatory controls: (RA group median: 37 ng/mL; range 4–136, $p < 0.001$) or the acute post-surgery group (median 198 ng/mL; range 58–459, $p < 0.001$).