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Oxidized Albumin – a Trojan Horse for p38 MAPK-Mediated Inflammation in Decompensated Cirrhosis

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Overactivation of the innate immune response amplifies hepatic injury and promotes the progression to fibrosis and cirrhosis in many liver diseases. Hepatic and systemic inflammation are triggered in part by factors released from dying hepatocytes, but recent evidence demonstrates that increased inflammation occurs in end-stage cirrhotic liver disease which typically has low levels of hepatocyte injury.⁽¹⁾ Clinical decompensation in patients with cirrhosis is associated with increased inflammation which is even greater in patients with acute-on-chronic liver failure (ACLF).⁽²⁾ These recent findings suggest the possibility that cirrhosis may be a target for anti-inflammatory drug therapy. Inflammation in cirrhosis is driven partly by the increased intestinal translocation of bacterial products (pathogen-associated molecular patterns) that activate systemic monocytes and resident liver macrophages. Factors released by dying hepatocytes (damage-associated molecular patterns) are presumably decreased in end-stage cirrhosis lacking a significant component of active liver injury however, suggesting that other monocyte/macrophage activators fuel the increased inflammation in decompensated cirrhosis. A better understanding of the mechanisms of macrophage activation in cirrhosis may facilitate the therapeutic targeting of this pathway in cirrhotic patients.

Hepatocyte-synthesized albumin is the most abundant plasma protein and performs beneficial functions including maintaining intravascular oncotic pressure and acting as a molecular carrier for different endogenous and exogenous molecules such as fatty acids, metals, hormones and drugs.⁽³⁾ A free cysteine-34 amino acid residue characterizes the reduced form of human mercaptoalbumin (HMA) and is responsible in part for its antioxidant activity.⁽³⁾ Reversible or irreversible oxidation of the cysteine-34 thiol group generates human non-mercaptoalbumin 1 (HNA1) and non-mercaptoalbumin 2 (HNA2), respectively. These oxidized forms of albumin have impaired scavenger activity for reactive oxygen and nitrogen species and accumulate in the serum of cirrhotic patients serving as a biomarker of oxidative stress in this disease.^(4,5)

In this issue of *HEPATOLOGY* Alcazar-Quilez *et al.*,⁽⁶⁾ delineate a new role for oxidized albumin as a contributor to the increased systemic inflammation in advanced liver disease. Consistent with previous studies they demonstrate that in patients with stages of chronic

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liver disease ranging from compensated (CC) and decompensated (DC) cirrhosis to ACLF, serum HNA1 and HNA2 levels increase with worsening severity of liver disease. Levels of serum proinflammatory cytokines IL-6, IL-1 β , TNF- α and IL-8 were increased in DC and ACLF patients compared to patients with CC and correlated with HNA1 and/or HNA2 levels, suggesting a direct relationship between elevated oxidized albumin and systemic inflammation. To further confirm causality, serum white blood cells from healthy donors and cirrhotic patients were treated *in vitro* with *ex vivo* oxidized HNA1 and HNA2. Cytokine production increased in the cells from both groups in response to HNA1 but not HNA2. HNA1 also upregulated levels of cyclooxygenase-2 and PGE₂-synthase and eicosanoid release. Subpopulation analysis revealed that monocytes and not neutrophils were responsive to the proinflammatory effects of HNA1. A rationale for the differential effects of HNA1 and HNA2 was not supplied except that it did not result from a difference in their levels of reactive oxygen or nitrogen species. A phospho-kinase array analysis identified increased phosphorylation and therefore activation of the mitogen-activated protein kinase (MAPK) p38 α by HNA1. The mechanistic involvement of p38 MAPK was confirmed by the ability of a p38 MAPK inhibitor, but not other MAPK inhibitors, to block the proinflammatory effect of HNA1. Although confirmatory evidence from cells with a genetic knockout/knockdown of p38 MAPK would be important, the data indicate that oxidized albumin is an activator of monocyte inflammation in cirrhosis through a p38 MAPK-mediated effect.

The novelty of these findings is that they identify albumin as a Trojan horse in cirrhosis. While maintenance of adequate serum HMA levels is important for the many beneficial functions of albumin, measures of total albumin in the clinic do not include HNA1 or HNA2 which require a more sophisticated analysis to detect. This hidden oxidized fraction of albumin can trigger an injurious proinflammatory macrophage response that promotes cirrhotic decompensation or ACLF. Whether albumin oxidation occurs exclusively systemically, or within the liver as well to activate hepatic resident macrophages, is an unanswered question. Infusion of human albumin is a common therapy in cirrhotic liver disease. An increased supply of functional HMA should benefit the patient, but oxidation of the exogenous albumin could possibly worsen the clinical condition by increasing inflammation. Therefore, the situation in cirrhosis is more complex than the simple need to replenish HMA and ideally albumin-directed therapies should remove harmful HNA1 as well. Contributing to the lack of efficacy for liver support devices could be that they fail to reduce levels of oxidized albumin, whereas plasma transfusion is more effective because it removes harmful oxidized albumin as well.⁽³⁾

MAPKs are known to be important modulators of liver pathophysiology that are up regulated by oxidative stress.⁽⁷⁾ Although studies such as the current one point to multiple mechanisms by which oxidative stress promotes liver disease, antioxidant therapies have been unsuccessful for diseases of the liver as well as other organs.⁽⁸⁾ MAPKs as the downstream effectors of oxidant stress therefore represent a potentially more efficacious target than global oxidant neutralization. The present findings are novel in that they involve the p38 MAPK pathway whereas previous studies have largely implicated the MAPKs c-Jun N-terminal kinase (JNK) and to a lesser extent extracellular signal-regulated kinase in liver pathophysiology. The finding of p38 MAPK as a regulatory element in inflammation defines this kinase as a new candidate for inhibiting systemic and possibly hepatic inflammation as

well. Unexamined in the present study is the receptor by which HNA1 activates the monocyte p38 MAPK pathway. Receptor identification would be interesting as it might identify an alternative, more selective approach to the inhibition of this proinflammatory pathway.

Phosphorylation of p38 MAPK results from activation of a cascade of upstream kinases including apoptosis signal-regulating kinase 1 (ASK1). ASK1 has been identified as a potential therapeutic target in a number of diseases because of its involvement in apoptosis, inflammation and fibrosis, and human ASK1 inhibitors have been developed.⁽⁹⁾ A randomized phase 2 trial recently suggested that the ASK1 inhibitor selonsertib may be effective in reducing fibrosis in patients with nonalcoholic steatohepatitis and stage 2 or 3 fibrosis.⁽¹⁰⁾ This benefit could have been secondary to inhibition of downstream p38 MAPK activation and/or JNK inhibition. An important question becomes whether HNA1-induced p38 MAPK activation in monocytes results from ASK1 activation. If activation is ASK1-dependent, the findings of the present study suggest that an ASK1 inhibitor already in human clinical trials for liver disease may prevent decompensation in end-stage cirrhosis. Alternatively direct p38 MAPK inhibitors could be employed as a therapy (Fig. 1). Such new molecular approaches are desperately needed for a highly prevalent disease with significant mortality and a lack of effective therapies.

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Abbreviations

ACLF	acute-on-chronic liver failure
ASK1	apoptosis signal-regulating kinase 1
CC	compensated cirrhosis
DC	decompensated cirrhosis
HMA	human mercaptoalbumin
HNA	human non-mercaptoalbumin
JNK	c-Jun N-terminal kinase
MAPK	mitogen-activated protein kinase

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FIG. 1.

The oxidized albumin form HNA1 promotes systemic inflammation and decompensation in cirrhosis. With liver cirrhosis induced by viral infections, alcohol or obesity, within the liver or systemically human mercaptoalbumin (HMA) is reversibly (HNA1) and irreversibly (HNA2) oxidized. Serum HNA1 is taken up by an unknown mechanism into circulating monocytes to phosphorylate (P) and activate p38a MAPK which induces the production and secretion into the blood of proinflammatory cytokines such as TNF- α and IL-1 β . The mechanism of p38 MAPK activation is unknown but may be through effects on ASK1 which phosphorylates downstream MAPK kinases (MKK4/7 and MKK3/6) that then phosphorylate JNK and p38 MAPK, respectively. Proinflammatory cytokines released as the result of increased macrophage activation have deleterious effects on the liver and other organs such as the kidney to promote clinical decompensation or ACLF in cirrhotic patients. The ASK1 inhibitor selonsertib or other direct p38 MAPK inhibitors may be a prevention or treatment of decompensated cirrhosis by down regulating this inflammatory pathway.