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VAP-1 in NASH: A novel biomarker?

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Non-alcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) is a spectrum of disorders ranging from simple steatosis to progressive steatohepatitis characterized by necroinflammation, leukocyte infiltration, lipoapoptosis leading to fibrosis and cirrhosis. A major obstacle for diagnosing NASH is that there currently are no acceptable serum biomarkers to distinguish simple steatosis from steatohepatitis¹. In the paper entitled “Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis” (Journal of Clinical Investigation, 2015 Feb;125(2):501-20), Weston CJ, Shepherd EL, Claridge LC et al.² describe VAP-1 as an important chemoattractant for leukocytes both in soluble (sVAP-1) and receptor forms in three different animal models of steatohepatitis. Furthermore, they show that VAP-1 is directly involved in stellate cell activation and is a strong profibrogenic stimulus. Thus targeting VAP-1 may result in a decrease in leukocyte recruitment and reduction of inflammation and fibrosis.

According to current concepts, during NASH pathogenic stimuli such as oxidative stress, dysregulation of the unfolded protein response, autophagy or inflammasome activation result in the infiltration of leukocytes triggering immune responses. The key homing signals that are induced in the liver to attract these cells have not been well described. Unlike in other tissues, in the liver leukocytes are known to tether directly to sinusoidal endothelial cells (SECs) without the help of selectins, and instead use a different array of adhesion molecules including hyaluronan CD44 or vascular adhesion protein-1 (VAP-1 or AOC3)³. VAP-1, also known as semicarbazide-sensitive amine oxidase (SSAO) is a multifunctional ecto-enzyme expressed by a variety of cells⁴ catalyzes the deamination of primary amines producing aldehyde, ammonia and H₂O₂. It plays a role in lymphocyte adhesion and transmigration in chronic but not acute inflammatory liver diseases⁵. Past studies showed that VAP-1 deficient mice exhibited only mild alterations in gut mucosal immunity and did not have an increased risk for infection⁶, suggesting that targeting VAP-1 could be a feasible approach to decrease leukocyte adhesion/transmigration during chronic inflammatory injury.

As elevated VAP-1 levels were also described in obesity and diabetes⁷, it was plausible that it could have a significant role in the pathogenesis of NASH. Westin and colleagues demonstrated that in a cohort of 74 patients with histologically confirmed NASH serum sVAP-1 was significantly increased, compared to BMI and metabolic phenotype-matched control patients; and in a univariate analysis it was correlated to HOMA-IR and diabetes status. After multivariate analysis, serum sVAP-1 was independently associated with fibrosis stage. The area under the receiver-operating curve (AUROC) for sVAP-1 to predict histological NASH was 0.82 (95% CI, 0.74-0.9) with estimated sensitivity of 67.7% and specificity of 89.7%. The AUROC to predict F 2 fibrosis was 0.71 (95% CI, 0.62-0.79), and

with a cutoff of 1,018 ng/ml the sensitivity was estimated to be 45.2% and specificity 91.5%.

Interestingly, VAP-1 was present not only in endothelial cells but also in fibrotic septae, and colocalized with α SMA positive myofibroblasts. Mechanistic studies demonstrated that both *in vitro* activated hepatic stellate cells (HSC) and isolated, active myofibroblasts express VAP-1 (AOC3 mRNA), and possess amine oxidase activity that is comparable to that of VAP-1 in SECs. Activated HSC induced amine-oxidase dependent lymphocyte migration *in vitro*, and this was reduced either by removal of H_2O_2 or by inhibiting GPCR signaling.

The role of VAP-1 *in vivo* was first studied in the CCl₄ model in the total VAP-1^{-/-} (AOC3^{-/-}) mice or in wild type mice receiving anti-VAP-1 antibody. VAP-1 inhibition or deficiency has attenuated fibrosis and leukocyte infiltration. There was a marked reduction in the number of infiltrating CD8⁺, CD4⁺ T cells, and CD19⁺ B cells at earlier phases (2 weeks); whereas reduction in macrophage/monocytic cells was observed at later stages.

To examine VAP-1 in steatohepatitis experiments were conducted in the methionine choline deficient (MCD), high fat or western lifestyle model (WLM) using trans-fats and fructose. In the AOC3^{-/-} mice similar to the CCl₄ model reduced CD4⁺ and NK1.1⁺T cell populations were seen. The CD11b/F4/80 positive cells were unchanged in this dietary model but there were less CD11b⁺/Gr⁺ monocytic cells. Fibrosis was reduced both in the AOC3^{-/-} mice and those treated with the anti-VAP1 antibody. Since the MCD model does not recapitulate the metabolic features of human NASH, studies were performed in the high fat diet and WLM. Surprisingly, serum ALT levels did not decrease in the AOC3^{-/-} mice in these models, although there was a significant reduction in CD4⁺ and NK1.1⁺ cells after 9 months of steatogenic diet, and this was comparable to the data from the MCD model.

The authors next postulated that VAP-1 SSAO activity was required for the development of steatohepatitis and generated a mouse model where VAP-1 was rendered catalytically inactive. When these mice were placed on the MCD diet similar reductions in CD4⁺ and NK1.1CD3⁺ cells were seen as in the AOC3^{-/-} mice suggesting that intact SSAO activity is required for leukocyte homing and progression of steatohepatitis. This is of particular importance, as the direct signals for leukocyte recruitment have not previously been established. The reduction of inflammatory infiltrate was in parallel with decreasing fibrogenic markers.

As VAP-1 expression was induced in fibrotic stroma, the next question was whether it had direct profibrogenic effects. Short-term treatment with recombinant VAP-1 did not induce HSC activation or proliferation however; it enhanced cell spreading and wound closure. LX2 cells, a human activated HSC cell line when transfected with the catalytically active form of VAP-1, exhibited a significant induction of COL1a1 and LOXL2. The fact that VAP-1 antibody (that does not bind to the active site) *in vivo* also blocked fibrosis suggests that VAP-1 has both enzyme-dependent and independent profibrogenic effects that would need to be addressed in future studies.

Taken together, the authors have demonstrated complex proinflammatory and profibrogenic effects of VAP-1 during steatohepatitis involving either the recruitment of leukocyte

populations and/or a direct effect on HSC activation. Moreover, serum sVAP-1 levels correlated to histological findings in progressive NASH in a well-characterized cohort of patients. Therefore sVAP-1 either alone or combined with other potential markers of fibrosis could become a biomarker of disease progression in NASH.

An important question to address in the future is the mechanism of sVAP-1 cleavage. Dysregulated insulin responses are thought to be linked to VAP-1 shedding however, the main cellular sources (SECs vs. stromal cells or myofibroblasts) or the mechanisms of cleavage in the liver are not known. Previously, TNF α was shown to regulate VAP-1 ectodomain shedding by MMPs in adipose tissue⁸, thus it is plausible that rising serum sVAP-1 reflects increasing leukocytic/monocytic infiltration, TNF α activity, and sVAP-1 cleavage leading to a vicious cycle.

Interestingly, steatosis was also reduced by the deletion of AOC3 or its inhibition by antibodies. As in these studies no cell-specific approaches were used there could be multiple explanations, e.g. the role of adipose tissue-derived adipokines could be considered. The concept that VAP-1 plays a role in HSC activation is novel. As alternative splicing is described to result in multiple VAP-1 transcripts with some encoding proteins, it would be important to know which form was responsible for the observed profibrogenic effects in NASH.

In previous studies, VAP-1 induction was also observed in the fibrotic stroma of biopsy samples with primary biliary cirrhosis, primary sclerosing cholangitis, and alcoholic liver disease. This means that VAP-1 may have inherent profibrogenic roles independent of the metabolic phenotype.

Further studies are needed to clarify the role of VAP-1 in stellate cells, or whether its decreased expression/shedding could be correlated to fibrosis reversal.

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References

1. Angulo P, Machado MV, Diehl AM. Fibrosis in nonalcoholic Fatty liver disease: mechanisms and clinical implications. *Semin Liver Dis.* 2015; 35:132–45. [PubMed: 25974899]
2. Weston CJ, Shepherd EL, Claridge LC, et al. Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. *J Clin Invest.* 2015; 125:501–20. [PubMed: 25562318]
3. Bonder CS, Norman MU, Swain MG, et al. Rules of recruitment for Th1 and Th2 lymphocytes in inflamed liver: a role for alpha-4 integrin and vascular adhesion protein-1. *Immunity.* 2005; 23:153–63. [PubMed: 16111634]
4. Weston CJ, Adams DH. Hepatic consequences of vascular adhesion protein-1 expression. *J Neural Transm.* 2011; 118:1055–64. [PubMed: 21512782]
5. Kurkijarvi R, Yegutkin GG, Gunson BK, et al. Circulating soluble vascular adhesion protein 1 accounts for the increased serum monoamine oxidase activity in chronic liver disease. *Gastroenterology.* 2000; 119:1096–103. [PubMed: 11040196]
6. Koskinen K, Nevalainen S, Karikoski M, et al. VAP-1-deficient mice display defects in mucosal immunity and antimicrobial responses: implications for antiadhesive applications. *J Immunol.* 2007; 179:6160–8. [PubMed: 17947691]

7. Salmi M, Stolen C, Jousilahti P, et al. Insulin-regulated increase of soluble vascular adhesion protein-1 in diabetes. *Am J Pathol.* 2002; 161:2255–62. [PubMed: 12466139]
8. Abella A, Garcia-Vicente S, Viguerie N, et al. Adipocytes release a soluble form of VAP-1/SSAO by a metalloprotease-dependent process and in a regulated manner. *Diabetologia.* 2004; 47:429–38. [PubMed: 14968297]

Figure 1.

Proposed actions of VAP-1 in steatohepatitis. 1. In HSC there is a VAP-1-mediated induction of fibrogenic transcripts that is SSAO dependent. 2. Cleaved sVAP-1 in the serum correlates to fibrogenic activity in the liver of NASH patients. 3. VAP-1 expressed in SECs or active HSC enhance the recruitment of CD4⁺, CD8⁺, and NK1.1⁺CD3⁺ cells likely involving SSAO activity/H₂O₂ production.