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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Oxidized Low-Density Lipoprotein Is a Novel Predictor of Interferon Responsiveness in Chronic Hepatitis C Infection

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SUMMARY

The unexplained association between interferon responsiveness and serum low-density lipoprotein (LDL) in chronic hepatitis C is likely due to oxidized LDL, a subfraction that blocks viral cell entry by perturbing the interaction between hepatitis C virus and its primary receptor.

BACKGROUND & AIMS: Hepatitis C virus (HCV) cell entry is mediated by several cell surface receptors, including scavenger receptor class B type I (SR-BI). Oxidized low density lipoprotein (oxLDL) inhibits the interaction between HCV and SR-BI in a noncompetitive manner. We tested whether serum oxLDL levels correlate with sustained virologic response (SVR) rates after interferon-based treatment of chronic hepatitis C.

METHODS: Baseline oxLDL was determined in 379 participants with chronic HCV genotype 1 infection from the INDIV-2 study using a commercial enzyme-linked immunosorbent assay. The mechanistic in vitro studies used full-length and subgenomic HCV genomes replicating in hepatoma cells.

RESULTS: In the multivariate analysis, oxLDL was found to be an independent predictor of SVR. Oxidized LDL did not correlate with markers of inflammation (alanine transaminase, ferritin), nor was serum oxLDL affected by exogenous interferon administration. Also, oxLDL did not alter the sensitivity of HCV replication to interferon. However, oxLDL was found to be a potent inhibitor of cell-to-cell spread of HCV between adjacent cells in vitro. It could thus reduce the rate at which new cells are infected by HCV through either the cell-free or cell-to-cell route. Finally, serum oxLDL was significantly associated with the estimated infected cell loss rate under treatment.

CONCLUSIONS: Oxidized LDL is a novel predictor of SVR after interferon-based therapy and may explain the previously observed association of LDL with SVR. Rather than being a marker of activated antiviral defenses it may improve chances of SVR by limiting spread of infection to naive cells through the cell-to-cell route. *(Cell Mol Gastroenterol Hepatol 2015;1:285–294; http://dx.doi.org/10.1016/j.jcmgh.2015.03.002)*

Keywords: Cell-to-Cell Spread; oxLDL; SVR; SR-BI.

The hepatitis C virus (HCV) is a small enveloped virus with a single-stranded, positive-sense RNA genome. It has chronically infected an estimated 160 million individuals worldwide and is a leading cause of end-stage liver disease. Pegylated interferon α (peg-IFN) in combination with ribavirin (RBV) had long been the standard treatment. Beginning in 2011 several direct-acting antiviral drugs (DAA) have been approved, and more are expected to follow.[1] Currently, both peg-IFN-containing and interferon-free treatments are available.

About half of individuals infected with HCV genotype 1 achieve sustained virologic response (SVR)—clearance of infection—after treatment with peg-IFN/RBV.[2] Numerous factors have been associated with interferon responsiveness and eventual SVR. These have been reviewed elsewhere.[3] Notably, genetic and nongenetic factors interact to determine chances of SVR.[4] Among the biochemical parameters, high low-density lipoprotein (LDL)[5–8] and low γ-glutamyltranspeptidase[9] seem to be strongly predictive of SVR. However, for these and most other factors the mechanism of how they are linked to interferon responsiveness is unclear, so our ability to predict treatment outcome in individual patients remains unsatisfactory.

There are multiple interconnections between the HCV replication cycle and host lipid metabolism: HCV particle assembly is linked to the host cell's very-low-density lipoprotein synthesis machinery, resulting in the release of

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Abbreviations used in this paper: DAA, direct-acting antiviral drug; DMEM, Dulbecco's modified Eagle medium; DTT, dithiothreitol; HCV, hepatitis C virus; HCVcc, cell culture–grown hepatitis C virus; IPS1, interferon promoter stimulator-1; ITX-5061, *N*-[5-tert-butyl-3-(methanesulfonamido)-2-methoxyphenyl]-2-[4-(2-morpholin-4-ylethoxy) naphthalen-1-yl]-2-oxoacetamide;hydrochloride; LDL, low-density lipoprotein; NLS, nuclear localization signal; oxLDL, oxidized low-density lipoprotein; PBS, phosphate-buffered saline; peg-IFN, pegylated interferon α; RBV, ribavirin; RFP, red fluorescent protein; ROC, receiver operating characteristic; SR-BI, scavenger receptor class B member I; SVR, sustained virologic response.

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"lipoviral particles" containing both lipoprotein and viral components.[10–13] Indeed, HCV particles produced in cell culture (HCVcc) appear to be less dense and larger than expected for a member of the *Flaviviridae*. Moreover, HCVcc are heterogeneous in size, and larger particles appear to be more highly infectious.[14,15] An essential HCV receptor on hepatocytes is scavenger receptor class B type I (SR-BI),[16] physiologically the main receptor for high-density lipoprotein on hepatocytes. The LDL receptor, on the other hand, has long been proposed to also be involved in HCV cell entry,[17] but its exact role has remained controversial, and unlike SR-BI it has not been shown to be essential for productive infection.[18]

Despite these clear links between the HCV replication cycle and both very-low-density lipoprotein and high-density lipoprotein, LDL is the lipid species most consistently associated with SVR after peg-IFN/RBV as well as first-generation telaprevir-based triple therapy.[5–8,19] This is somewhat puzzling because the link between LDL and HCV biology is not obvious. We have previously identified and characterized oxidized low-density lipoprotein (oxLDL)—a naturally occurring derivative of native LDL that has undergone oxidative modifications—as a potent endogenous inhibitor of HCV cell entry.[20,21] In vivo oxLDL is detectable at low levels in human serum. Oxidized LDL binds to SR-BI, and our earlier work suggested that it perturbs the interaction between HCV and SR-BI in a noncompetitive manner. Moreover, we were able to show that endogenous oxLDL, like the in vitro generated oxLDL which is mostly used for experimentation, inhibits HCV infectivity. However, whether endogenous oxLDL has an impact on interferon responsiveness in the setting of chronic HVC infection is unknown. This study was undertaken to address this question.

Materials and Methods

Patient Cohort

We examined the samples and clinical data of 379 treatment-naive patients infected with HCV genotype 1 from the treatment arm of the INDIV-2 study. Details of the study have been described elsewhere.[22] Briefly, the patients received peg-IFN/RBV for 24–72 weeks depending on their baseline viral load and on-treatment viral kinetics. The INDIV-2 study protocol was approved by the independent ethics committees at all 20 German study centers.

Oxidized Low-Density Lipoprotein Enzyme-Linked Immunosorbent Assay

To quantify serum oxLDL, we used a commercial enzyme-linked immunosorbent assay system (cat. no. 10-1143-01; Mercodia, Uppsala, Sweden) that we had previously evaluated.[21]

Cell Lines and Reagents

Huh-7 and Huh-7.5 cells were maintained in Dulbecco's modified Eagle medium (DMEM) (cat. no. 11966-025; Life Technologies, Darmstadt, Germany) supplemented with 10% fetal calf serum (cat. no. F7524-500ML; Sigma-Aldrich, Munich, Germany), nonessential amino acids (cat. no. 11140-035; Life Technologies), L-glutamine (cat. no. 25030-024; Life Technologies), and penicillin/streptomycin (cat. no. A 2212; Biochrom, Berlin, Germany). Oxidized LDL was purchased from Intracel Resources (cat. no. RP-047; Frederick, MD) and LDL from Kalen Biomedical (cat. no. 770200-4; Montgomery Village, MD). Peg-IFN for in vitro experimentation was obtained from Roche Pharma (Basel, Switzerland).

Plasmids

For pseudo-particle production, we used the following three plasmids: (1) a vector expressing the human immunodeficiency virus (HIV) gag and pol genes (HIV gag-pol), a plasmid encoding the G-protein of the vesicular stomatitis virus, and pTRIP-tagRFP-NLS-IPS1 encoding red fluorescent protein (RFP) fused to a nuclear localization signal (NLS) and the mitochondrial anchor domain of IPS1 (interferon promoter stimulator-1).[23] The plasmids pFK_JFH1/J6/C-846.dg and pFK_i389luc_EL_JFH1/J6/C-846.dg encoding the full-length chimeric HCV genotype 2a genome Jc1 with or without a firefly luciferase reporter (Jc1 and Fluc-Jc1, respectively) have been described by Pietschmann et al.[24] Moreover, subgenomic replicons containing a luciferase reporter representing genoytpes 2a (Luc-JFH1-NS3-5B) and 1b (Fluc-Con1 NS3-5B) were used.

Production of Pseudo-particles and Transduction of Target Cells

Pseudo-particles encoding the tagRFP-NLS-IPS1 reporter were produced as previously described elsewhere.[25] Briefly, human embryonic kidney 293T (HEK293T) cells (8×10^5 cells/well) were seeded onto a six-well plate 1 day before transfection. Three plasmids expressing HIV gag-pol, tagRFP-NLS-IPS1 and the G-protein of the vesicular stomatitis virus were mixed with OptiMEM (cat. no. 31985-047; Gibco/Life Technologies, Darmstadt, Germany) and polyethylenimine (cat. no. P4707-50ML; Sigma-Aldrich) as a transfection reagent. The mixture was incubated for 20 minutes at room temperature. Seeded cells were washed once with 1x phosphate-buffered saline (PBS), and 1 mL of fresh DMEM supplemented with 3% fetal calf serum, L-glutamine and nonessential amino acids was added. After incubation, 80 μL of the transfection mixture was added per well and incubated at 37°C. The medium was changed 6 hours after transfection. The pseudo-particle–containing supernatant was harvested 48 and 72 hours after transfection and pooled. The filtered supernatant was used to transduce Huh-7.5 cells. To this end, cells were incubated for 8 hours with pseudo-particle–containing supernatant before the medium was changed. The transduction efficiency was determined by flow cytometry (Supplementary Figure 1).

Flow Cytometry

Cell populations expressing an RFP reporter were trypsinized and resuspended with DMEM complete. The cells were washed two times with 1x PBS before being resuspended in 1x PBS to a final cell number of 2×10^6 cells/mL.