Does HCV Alter the Iron Status of Infected Cells?

Jane Allan1, Sally Qiu1, Hayley Clark2, K. Hofmann1, Mark Watson2, Debbie Trinder1

1School of Medicine and Pharmacology, University of Western Australia, 2Institute for Immunology and Infectious Diseases, Murdoch University, W Australia

Introduction

Iron is tightly regulated in the body since it can promote toxic reactive oxygen species with excess iron stored as ferritin within hepatocytes. However, it is essential for many biological processes, including the production of virions and the host response to pathogens, resulting in manipulation of the availability of iron by both host and pathogen. Since HCV infection is associated with elevated serum ferritin levels and iron deposits within liver, we hypothesised that HCV replication alters iron homeostasis in hepatocytes. Two independent mediators of iron metabolism, hepcidin and IL-6, are analysed together with ferroportin, exporter of iron from cells, that is degraded upon binding of hepcidin.

HCV model: human hepatoma Huh7 cells were infected with HCV JFH1 at a moi of 0.001 - 0.01. Cultures were grown in DMEM with 10% FCS and gentamycin and supplemented with iron, where specifically stated, using ferric ammonium citrate (FAC).

HCV infection: infected cells and focus forming units (FFU) were determined on fixed cultures by indirect IHC using HCV seropositive human serum as primary antibody with detection by HRP-IgG and DAB or Dylight 488-IgG for flow cytometry.

Gene Expression: SAB PCR array for cytokines with 4 housekeeping genes (HKG). RNA was also extracted by trizol and DNase treated for quantitation of individual gene expression by Sybr green based PCR using plasmid standard curves for IL-6, hepcidin, ferroportin and HCV 5’ UTR, with β-actin, PPIA and albumin as HKG.

Ferritin Assay: ferritin was determined in cellular lysate with complete protease inhibitors by ELISA with ferritin standards (JBL). Results were normalised to protein determined by BCA assay with BSA standards.

Figure 1. Intracellular ferritin is elevated by HCV infection of Huh7 cells.

(A) DFO,iron chelator, deferoxamine; FAC (0.5 ug/ml) iron excess prior to HCV at moi 0.01, day 4 pi. (B) FAC supplemented prior to infection and assayed days 7 and 13 pi. Blue bars: uninfected, orange bars: HCV infected. Pool of 3 wells, tested in duplicate. Representative data that were verified in independent experiments.

HCV infection was found to increase ferritin levels in Huh7 cells (Fig 1A), a difference still evident when culture media was supplemented with iron (Fig 1B).

Results

Ferritin levels increase in Huh7 cells infected with HCV

Fig 2A. Infection of cells by HCV is similar in the presence of ferritin.

Analysed by flow cytometry at day 7 pi Stained with serumnegative (closed) or HCV seropositive serum (open).

Fig 2B. Spread of HCV through Huh7 cultures is unaffected by ferritin

HCV infected (75% infected) and FAC pre-treated and HCV infected (65% infected) at day 4 pi. Brown, DAB revealing HCV antigen; methyl green counterstain x 200.

Pre-treatment of cells with excess iron, using up to 20 ug/ml FAC, did not reduce HCV infection of Huh7 cells (Fig 2 A B) as shown by HCV antigen staining and also by production of infectious virus by FFU assay from these cultures. Commencing iron supplementation one day after HCV infection also did not alter virus production by Huh7 cells.

Increased IL6 gene expression induced by HCV infection

Changes in gene expression relative to uninfected cultures from 3 independent experiments (n=3 each, mean and SEM, normalised to β-actin) at approx. 10⁶ copies HCV/ul. Plasmid IL6 encoding the respective genes were used for quantitation. At lower levels of infection FPN copies were similar between infected and uninfected cultures but FPN was consistently reduced in high HCV infection.

IL-6 gene expression was elevated 8-fold in response to HCV infection of Huh7 cells based on PCR microarray for cytokines. Basal IL-6 expression was low and the result was confirmed by RT-PCR using relative quantitation with plasmid encoding the same IL6 sequence (Fig 3 and 4).

Summary

- HCV infection increases intracellular iron storage, in the form of ferritin, in hepatocytes infected in vitro.
- HCV replication and spread through hepatocytes was neither promoted nor hindered by the influx of iron.
- HCV infection modulated genes associated with regulation of iron homeostasis. IL-6 gene expression increased in response to HCV infection accompanied by a modest increase (100%) in hepcidin and a reduction (50%) in ferroportin gene expression.

Drs Y-L Ng and J Flexman are thanked for providing samples. The support of the Fremantle Hospital Medical Research Foundation is gratefully acknowledged.