The effect of iron on hepatitis C virus replication

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November 2011

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Declaration

This thesis was submitted as part of the requirements for the degree of Bachelor of Science in Molecular Biology with Honours from Murdoch University.

I declare that the work within this thesis is solely my own and is not the work of any other person unless otherwise stated.

Word count including in-text citations:

19099 words

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Abstract

Hepatitis C virus (HCV) is a small RNA encoded virus that causes acute and chronic infections of the liver and is associated with fibrosis, cirrhosis, hepatocellular carcinoma and end-stage liver disease. The infection is linked with elevated serum ferritin levels and iron deposits within the liver which are associated with an increased risk of progression of HCV disease. It is hypothesised firstly that the presence of excess iron affects HCV replication and secondly that iron homeostasis is altered by HCV replication. The main aim of this study was to investigate the influence of iron on HCV replication by analysing the spread of infection and the titre of infectious HCV produced in the presence of excess iron. The second aim was to determine if HCV infection itself alters the level of iron stored as ferritin in liver cells.

HCV Japanese fulminant hepatitis (JFH-1) infection of the human hepatoma cell line, (Huh7), was used as the model for this study. The culture conditions were first optimised to allow detection of ferritin by western blot. Huh7 cells were then treated with iron provided by 5, 10 or 20 μg/ml ferric ammonium citrate (FAC) prior to HCV infection (0.001 MOI). No evidence of inhibition of HCV replication was found on day 7 post infection (PI) by analysis of the level of viral protein detected by western blot, the proportion of infected cells determined by immunohistochemistry and flow cytometry or the titre of infectious HCV produced. A comparison of iron donors was then undertaken and conditions were changed to add iron donors 1 day after HCV. Viral RNA levels were not significantly reduced on treatment with 20 μg/ml FAC and 25 μM holo-transferrin and assays for viral protein and production of infectious virus again showed no evidence of inhibition. Fe-PIH, a lipophilic iron donor, had the greatest effect, reducing both the proportion of infected cells and the titre of infectious virus produced. When the reduction

in the number of cells present in cultures treated with Fe-PIH was taken into account, the decrease in virus titre was explained.

HCV infection was found to alter iron storage in Huh7 cells. A significantly lower level of ferritin was detected ($p < 0.05$) by western blot in HCV infected compared to uninfected Huh7 cells whereas in other situations highly infected cells were found to have increased iron storage. While it had been intended to analyse iron metabolism by examining gene expression during iron deficiency and iron storage, the conditions which give rise to these outcomes will need to be elucidated first.

This study has shown that iron has the potential to inhibit HCV replication although *in vitro* it depends on the type and concentration of iron donor used and the time during infection that excess iron is present in the cell. This study has confirmed that HCV infection alters the iron status of the liver cell. The outcome of these interactions between iron and HCV is expected to influence the course of HCV infection and the development of liver disease.

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Acknowledgements

A special thanks goes to Dr Jane Allan and Dr Debbie Trinder for all the guidance, help and supervision over the year. I would like to thank Kirsten for her friendship and support over the short time she was here, for without that it would have been struggle fighting for equipment and reagents while learning new techniques. I would like to acknowledge Dr Silvia Lee and Irma Larma for all their assistance and expertise on IHC and flow cytometry. Without Prof. Richardson, the experiment comparing iron donor Fe-PIH would not have been possible. My thanks goes to all those who helped and supported me at Fremantle hospital and made my time there enjoyable; Leona, Roheeth, Des, Kristine, Angela, Gwen, Rina, Stephan, Borat, Janina, Yoke-Leng, Francis, Anita, Caryn and Ross.

I would like to acknowledge my family for all the support, encouragement and motivation they have given me over the year.

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Abbreviations

ALT	alanine aminotransferase
APS	ammonium persulphate
BCA	bicinchoninic acid
BCP	1-bromo-3-chloropropane
BME	β-mercaptoethanol
BMP6	bone morphogenetic protein 6
BSA	bovine serum albumin
DAB	3,3'-diaminobenzidine peroxidise
DFO	desferrioaxamine mesylate
DMEM	Dulbecco's modified eagle medium
DMSO	dimethyl sulphoxide
DMT	divalent metal transporter
dNTPs	deoxyribonucleotide triphosphates
DTT	dithiothreitol
eIF3	translation initiation factor 3
ER	endoplasmic reticulum
FAC	ferric ammonium citrate
FBS	foetal bovine serum
ffu	focus forming unit
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HFE	haemochromatosis
HJV	hemojuvelin

IFA	immunofluorescence assay
IHC	immunohistochemistry
IRE	iron responsive element
IRF	interferon regulatory factor
IRPs	iron regulatory proteins
JFH	Japanese fulminant hepatitis
MHC	major histocompatibility complex
MOI	multiplicity of infection
NF-κB	nuclear factor κB
PAMP	pathogen-associated molecular pattern
PBS	phosphate buffered saline
PI	post infection
PIH	pyridoxal isonicotinoyl hydrazone
PPIA	peptidylprolyl isomerase A
PRRs	pattern recognition receptors
RT	room temperature
RT-PCR	real time polymerase chain reaction
SDS	sodium dodecyl sulphate
SIH	salicylaldehyde isonicotinoyl hydrazone
TEMED	N,N,N',N'-Tetramethylethylenediamine
TFR1	transferrin receptor 1
TFR2	transferrin receptor 2
UPR	unfolding protein response
UTR	untranslated regions
ZIP14	Zrt- and Irt-like proteins 14