

The effect of iron on hepatitis C virus replication

Hayley Clark

School of Biological Sciences and Biotechnology
Faculty of Sustainability, Environmental and Life Sciences
Murdoch University

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Supervisors: **Assistant Professor Jane Allan & Professor Debbie Trinder**
School of Medicine & Pharmacology, Faculty of Medicine Dentistry &
Health Services, University of Western Australia

Associate Professor RJ Mead
School of Biological Science and Biotechnology, Faculty of
Sustainability, Environmental and Life Sciences, Murdoch University

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.

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Hayley Clark

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.

Table of Contents

Declaration.....	ii
Abstract.....	iii
Table of Contents.....	v
Acknowledgements.....	xi
List of Figures and Tables.....	xii
Abbreviations.....	xv

Chapter 1: Introduction.....2

1.1 Introduction to Hepatitis C Virus (HCV).....2

1.1.1 Epidemiology of HCV.....2

1.1.2 Acute and chronic HCV infection.....4

1.1.2.1 Acute HCV infection.....4

1.1.2.2 Chronic HCV infection.....4

1.1.3 Diagnosis.....6

1.1.4 Treatments.....7

1.1.4.1 Standard treatments.....7

1.1.4.2 Developing treatments.....7

1.1.4.3	Alternative treatments.....	8
1.2	Virology of HCV.....	9
1.2.1	HCV Genome structure and proteins.....	9
1.2.2	Viral life cycle.....	9
1.3	Immune response to HCV infection.....	13
1.3.1	Innate immunity.....	13
1.3.2	Adaptive immunity.....	14
1.3.3	HCV evasion of immune system.....	15
1.4	HCV models.....	17
1.4.1	<i>In vivo</i>	17
1.4.2	<i>In vitro</i>	17
1.5	Iron Metabolism.....	19
1.5.1	Role of iron in humans.....	19
1.5.2	Acquisition and distribution of iron.....	19
1.5.3	Utilisation and recycling of iron.....	21
1.5.4	Iron transport and storage.....	21
1.6	Iron homeostasis and regulation.....	24
1.6.1	Systemic iron homeostasis.....	24

1.6.2	Cellular iron homeostasis.....	25
1.7	Iron and infection.....	26
1.8	HCV and Iron.....	29
1.9	Project aims.....	32
Chapter 2: Materials and Methods.....		36
Section: Materials.....		36
2.1	Cell culture.....	36
2.2	RNA analysis.....	36
2.3	DNA Analysis.....	37
2.4	Protein analysis.....	37
2.5	Immunohistochemistry.....	38
2.6	Solutions and buffers.....	39
Section: Methods.....		44
2.7	Cell culture.....	44
2.7.1	Passage of Huh7 cells.....	44
2.7.2	Viable cell count.....	45
2.7.3	Cryopreservation of Huh7 cells.....	45
2.7.4	Thawing cryopreserved Huh7 cells.....	45

2.7.5	Treatment of Huh7 cells with iron donor compounds.....	45
2.8	HCV.....	46
2.8.1	Virus stock.....	46
2.8.2	Lysis of cultures for determination of HCV titre.....	47
2.8.3	Detection of HCV.....	47
2.9	RNA studies.....	47
2.9.1	RNA extraction and DNase treatment.....	47
2.9.2	RNA quantification	48
2.9.3	Reverse transcription	48
2.9.4	Real time polymerase chain reaction (RT-PCR).....	49
2.9.4.1	Standard curves for RT-PCR.....	50
2.9.4.2	Molecular detection of mycoplasma by RT-PCR.....	51
2.10	Plasmid DNA.....	51
2.10.1	Restriction enzyme digest.....	51
2.11	Protein studies.....	54
2.11.1	Protein extraction.....	54
2.11.2	Bicinchoninic acid (BCA) protein assay.....	54
2.11.3	Western blot.....	54

2.12	Statistical analysis.....	56
Chapter 3: Results.....		58
3.1	Introduction	58
3.2	Optimisation of the iron content in culture media to allow detection of ferritin in Huh7 cells.....	58
3.2.1	Testing two iron donor compounds, FAC and holo-transferrin.	58
3.2.2	Optimising the concentration of FAC	62
3.2.3	Summary	63
3.3	Aim 1: Does iron influence HCV replication.....	65
3.3.1	HCV infection of Huh7 cells in presence of excess iron provided by FAC	65
3.3.1.1	Summary.....	70
3.3.2	Titration of copies of HCV RNA per infectious unit.....	73
3.3.3	Infection of Huh7 cells by HCV in the presence of excess iron in the form of hemin, holo-transferrin and Fe-PIH.....	76
3.3.3.1	Summary	87
3.4	Aim 2: Does HCV infection alter cellular iron status	89
3.4.1	Optimisation of iron regulator and transporter genes for RT-PCR quantification.....	89

3.4.2	Early and late HCV infection of Huh7 cells maintained in baseline media	90
3.4.3	Summary of Aim 2.....	95
Chapter 4: Discussion.....		97
References.....		106

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List of Figures and Tables

Figure 1.1: Global prevalence of HCV and the distribution of HCV genotypes.....	2
Figure 1.2: Progression of HCV infection.....	5
Figure 1.3: HCV Genome.....	10
Figure 1.4: HCV viral life cycle.....	12
Figure 1.5: Pattern recognition receptors and their role in innate and adaptive immune responses.....	14
Figure 1.6: Evasion of the immune system by HCV.....	16
Figure 1.7: Genomic structure of full length, subgenomic and chimeric HCV replicons.....	18
Figure 1.8: Absorption of iron by duodenal enterocytes.....	20
Figure 1.9: Distribution of iron in vertebrates.....	20
Figure 1.10: Recycling of iron by macrophages.....	22
Figure 1.11: Iron uptake by hepatocytes.....	23
Figure 1.12: Regulation of cellular iron homeostasis via IRP.....	26
Figure 1.13: Interleukin-6 induces the transcription of hepcidin through the activation and binding of STAT3 to the hepcidin promoter.....	28
Figure 1.14: Relationship between iron, HCV, oxidative stress and ER stress.....	30
Figure 2.1: Mycoplasma detection	52
Figure 2.2: <i>NotI</i> digested pGEM-T containing Albumin, PPIA and TFR1 inserts.....	53
Figure 2.3: Layout for SDS-PAGE gel to nitrocellulose membrane transfer.....	55
Figure 3.1: Ferritin detection by western blot in Huh7 cells.....	60
Figure 3.2: Quantification of ferritin levels in uninfected Huh7 cells.....	61
Figure 3.3: Viable cell count from a pool of 4 replicate wells of Huh7 cells per group 7 days after treatment with FAC at 0.1, 1, 5 and 10 µg/ml.....	63

Figure 3.4: (a) Ferritin detection by western blot in Huh7 cells. (b) Quantification of ferritin expression in Huh7 cells.....	64
Figure 3.5: Ferritin protein detection by western blot. Cultures are treated with baseline (1), 5, 10, 20 µg/ml FAC.....	66
Figure 3.6: HCV core detection by western blot.....	68
Figure 3.7: Viable cell count from 15 pooled Huh7 wells on day 7 PI.....	69
Figure 3.8: Progression of HCV infection as determined by IFA for HCV antigen in baseline cultures.....	69
Figure 3.9: Flow analysis of HCV infected Huh7 cells.....	71
Figure 3.10: Titres of infectious HCV collected from culture supernatant, 0.001 MOI, day 7 PI.....	72
Figure 3.11: RT-PCR analysis for third run.....	74
Figure 3.12: Protein detection by western blot of Huh7 cells treated with iron donors FAC, holo-transferrin and hemin.....	78
Figure 3.13: Protein detection by western blot of Huh7 cells treated with PIH, Fe-PIH and hemin.....	79
Figure 3.14: Analysis of ferritin and HCV core protein expression in Huh7 cells, 0.01 MOI, day 4 PI.....	80
Figure 3.15: Viable cell count on day 4 PI.....	82
Figure 3.16: Extent of HCV infection determined by IHC.....	84
Figure 3.17: Experimental design for mRNA analysis for each culture.....	85
Figure 3.18: Analysis of HCV mRNA levels in infected Huh7 cells, 0.01 MOI, day 4 PI.....	86
Figure 3.19: Titres of infectious HCV collect from culture supernatant, 0.01 MOI, day 4 PI.....	88

Figure 3.20: Viable cell of Huh7 cells at day 7 PI, 0.001 MOI.....	91
Figure 3.21: Extent of HCV infection in Huh7 cultures by IFA.....	91
Figure 3.22: Titres of infectious HCV collected from culture supernatant, 0.001 MOI, day 7 and 13 PI.....	93
Figure 3.23: Analysis of HCV RNA levels in infected and uninfected Huh7 cells, 0.001MOI, week 2 PI.....	93
Figure 3.24: Ferritin and HCV detection by western blot.....	94
Figure 4.1: Titres of infectious HCV collected from culture supernatant, 0.001 MOI, day 7 PI.....	99

List of Tables

Table 1.1: Functions of HCV proteins.....	11
Table 1.2: Iron proteins involved in host defence.....	28
Table 2.1: PCR master mix.....	49
Table 2.2: PCR cycling parameters.....	49
Table 2.3: Primers for RT-PCR.....	50
Table 3.1: Calculation of RNA per ffu of HCV from RT-PCR standard curve.....	75

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 peroxidase
DFO	desferrioxamine 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- κ B	nuclear factor κ B
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