“In Vitro Inhibition of HCV Replication”

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Abstract

The outcome of interferone plus ribavirine treatment of hepatitis C virus (HCV) genotype 4 is unfortunately poor. Development of alternative therapy for this genotype is of a paramount importance. Inhibitions of HCV gene expression in vitro by the use of antisense phosphorothioate oligodeoxynucleotides (S-ODN) against (IRES) elements were associated with favorable results. To assess S-ODN activity, previous studies utilized viral subgenomic or full cDNA fragments linked to reporter genes transfected into adhered cells or in a cell free system. In the present study we utilized HepG2 cells infected with native HCV RNA genomes in a replication competent system. S-ODN against stem loop IIId (S-ODN2, nt 252-270) and the AUG translation start site (S-ODN1, nt 325-348) of the viral polyprotein precursor were used as potential inhibitors for viral replication. Intracellular viral replication was monitored both by nested RT-PCR and real time PCR technology. These experiments indicated that intracellular replication of HCV genotype 4 was completely arrested by using either S-ODN structure (with more efficacy of S-ODN1 than S-ODN2) at concentrations as low as 1 uM after 48 h. in culture. The inhibitory effect of S-ODN appeared to be specific to HCV replication in light of the consistent levels of human glyceralehyde 3-phosphate dehydrogenase (GAPDH) gene expression throughout culture conditions and S-ODN treatments. In conclusion, the present study provides a direct evidence for the potential antiviral activity of antisense oligonucleotides on native genomic replication of HCV genotype 4, the most common type in Egypt.

Dedication

I dedicate this work
To my family:

My Kind Mom
My Great Dad
My Dearest Husband
My sweetheart Karim

I have to thank God for choosing you to be my family
I can not imagine how was my life without you
Thank you for your presence in my life
Thank you for supporting me
With kindness, patience,…….and love

Yours
Noha Gamal El Din Samy.

To My Dear Husband
Indeed I have to thank God
for choosing you to be my husband

I am appreciate & grateful for
everything you do it for me

Thanks a lot
For your
Encouragement
Assistance
Patience
Care
And
Love

To My little angle

Karím

I hope one day,
You read this thesis
& be proud that
I'm your mother

Thanks God
For the Gorgeous
& Precious Gift
My sweetheart

Karím

Who fill my life with
Happiness
Smiles
Blesses

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Contents

Chapter III Material & Methods

Chapter IV : Results

List of Figures

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LIST of ABBREVIATIONS

A	Adenine
ALT	Alanine Transferase
AML	Acute Myelogenous leukemia
Anti-HCV	HCV antibody
AS-ODN	Antisense Oligdeoxynucleotides
ATCC	American Type Culture Collection
bp	Base pair
bDNA	Branched Deoxy ribonucleic acid
C	Cytosine
$CaCl_2$	Calcium Chloride
CDC	Centers for Disease Control and Prevention
cDNA	Complementry DNA
CMV	Cytomegalovirus
CO_2	Carbon Dioxide
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dd H_2O	Double distilled water
DEPC	Diethylpyrocarbonate
dGTP	Deoxyguanosine triphosphate
DMEM	Dulbecco's Modified Eagle's Medium
DTT	Dithiothreitol
dTTP	Deoxythymidine triphosphate
dUTP	Deoxyuracil triphosphate
DNA	Deoxy ribonucleic acid
E1	Envelope protein 1
E2	Envelope protein 2
EASL	European Association for the Study of the Liver
EDTA	Ethylene diamine tetra acetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay

ETOH	Ethanol
ETR	End of therapy response
FCS	Fetal Calf Serum
FDA	Food and Drug Administration
G	Guanine
g	gram
GAPDH	Glyceraldehyde-3- Phosphate Dehydrogenase
h	hours
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCl	Hydrochloric acid
HCMV	Human cytomegalovirus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HVR1	Hypervariable region 1
IDU	Injection drug users
IFN	Interferon
IL	Interleukin
IMPDH	Inosine monophosphate dehydrogenase
IPTG	Isopropyl-β-thiogalactopyranoside
IRES	Internal ribosome entry site
KCl	Potassium Chloride
KDa	Kilo Dalton
KOAc	Potassium Acetate
KPO_4	PotassiumPhosphate
LDL	Low density lipoprotein
M	Molar
M2	Viral membrane matrix protein,
mg	Milligram
$MgCl_2$	Magnesium Chloride
µg	Microgram