

Selection of hepatitis C virus resistant to ribavirin

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Introduction

Ribavirin alone has minimal clinical effect in patients with chronic HCV infections, but can strongly inhibit HCV replication *in vitro*.

The mechanism of action of ribavirin *in vitro* (alone or in combination with interferon) and *in vivo* is not completely understood.

Proposed mechanisms of action of ribavirin against HCV include

- A direct effect on the HCV RNA–dependent RNA polymerase
- Induction of misincorporation of nucleotides leading to lethal mutagenesis
- Depletion of intracellular guanosine triphosphate pools (IMPDH inhibition)
- Alteration in the cytokine balance from a Th2 profile to a Th1 profile
- Induction of some ISGs

Objective

To select ribavirin resistant mutants of HCV *in vitro* to aid the understanding of the mechanism of the ribavirin effect on HCV

Methods

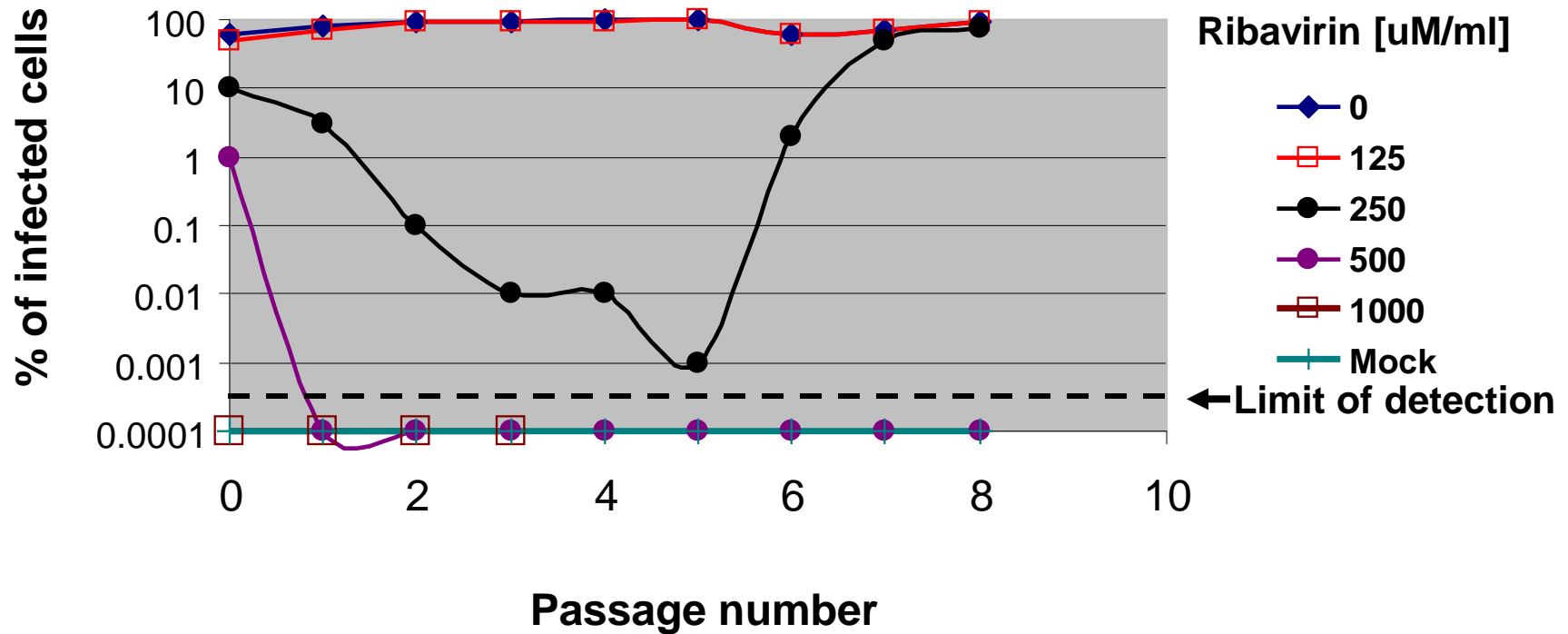
The J6/JFH1 strain of HCV was serially passed in Huh7D cells (a highly HCV replication permissive clone of Huh7 cells) in the presence of different concentrations of ribavirin.

Virus replication in Huh7D cells was assessed by immunofluorescence detection of HCV antigens of infected cells and titration of recovered virus present in the supernatant.

Genomes from viruses grown in 0 and 250 uM concentrations of ribavirin were sequenced.

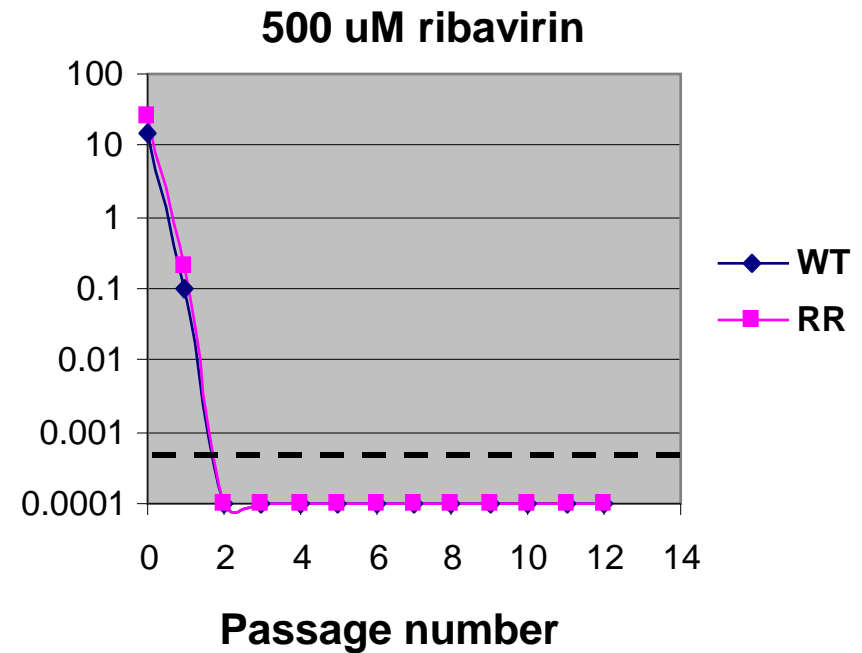
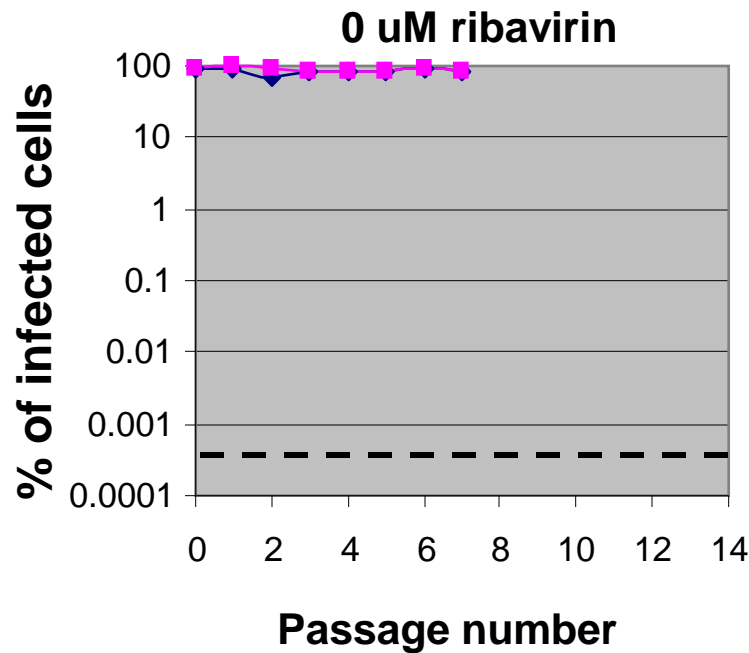
Selection of a ribavirin resistant virus

Passage of HCV in different concentrations of ribavirin



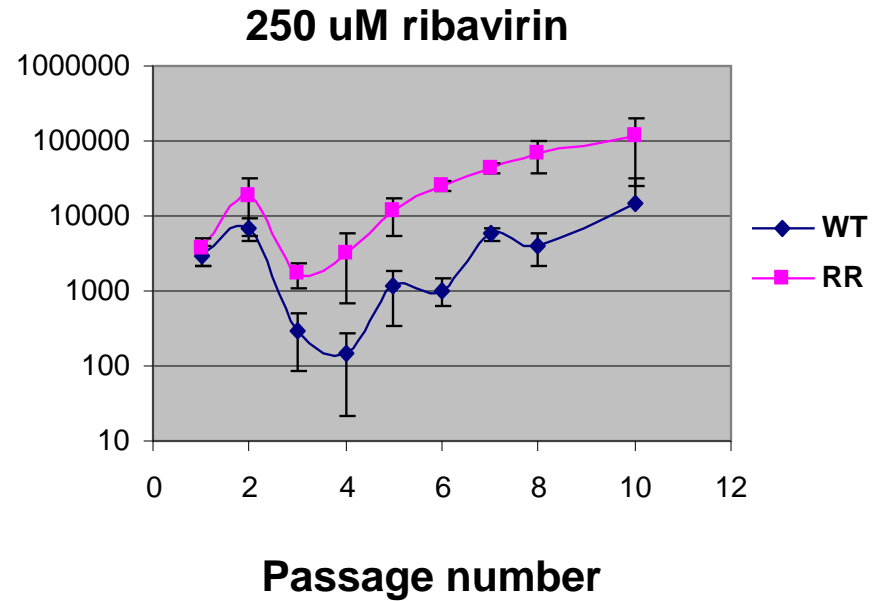
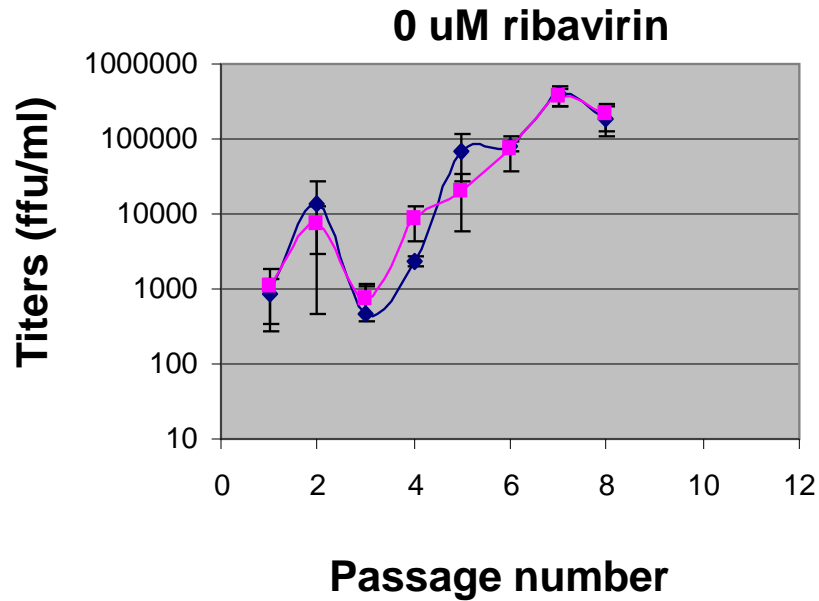
We could not select a virus resistant to 500 μ M ribavirin, even when starting with RR (250) virus

Passage of WT and RR viruses in 0 or 500 μ M ribavirin



RR virus is more resistant to 250 μ M ribavirin than WT virus

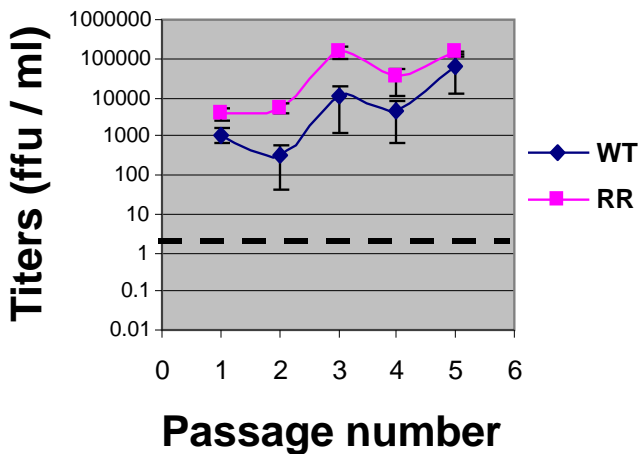
Growth of WT and RR viruses in 0 or 250 μ M ribavirin



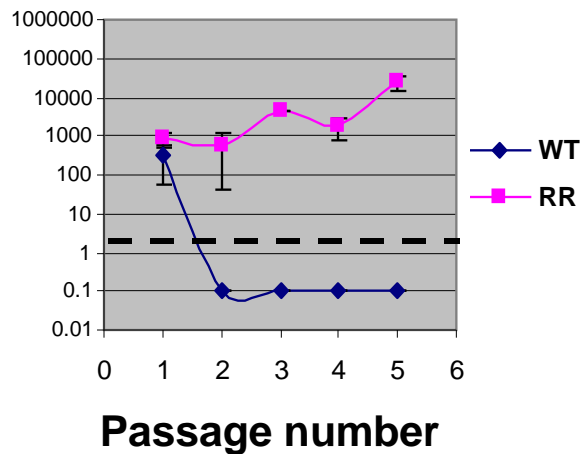
RR virus is resistant to 300 uM ribavirin

Growth of WT and RR viruses in 200, 300, or 400 uM ribavirin

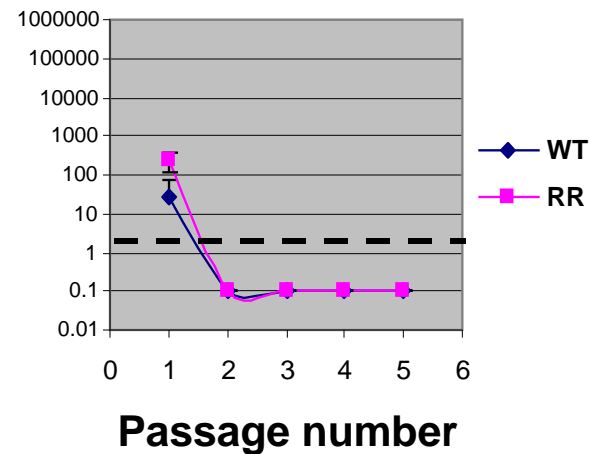
200 uM ribavirin



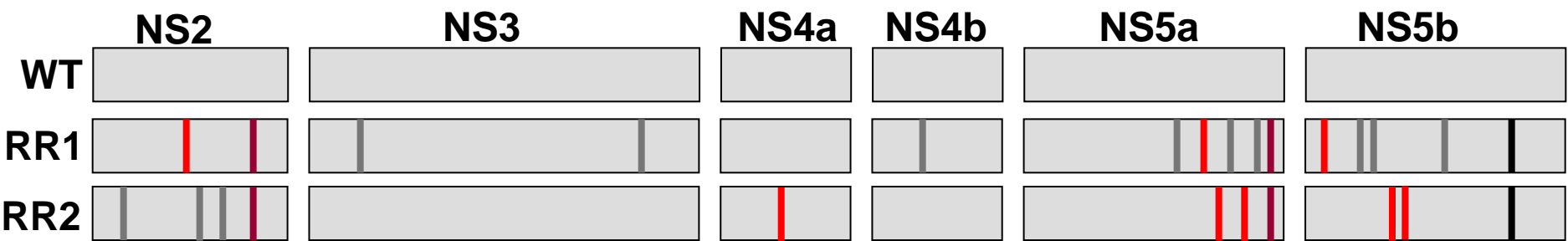
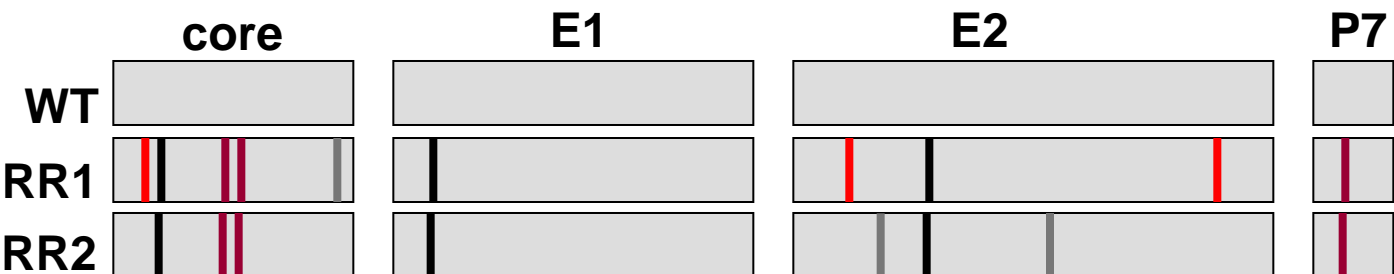
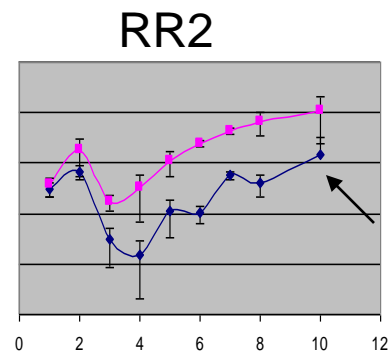
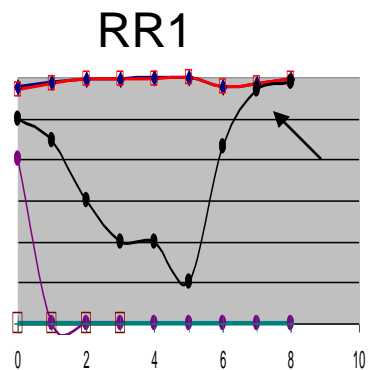
300 uM ribavirin



400 uM ribavirin



Sequence of coding regions from two RR viruses



Non-synonymous mutation

Synonymous mutation

Summary of Results

125 μ M ribavirin did not have a dramatic effect on HCV replication; 500 μ M of ribavirin lead to viral extinction.

250 μ M of ribavirin rapidly reduced virus replication

Viral resurgence reached the level of the wild type virus grown without ribavirin at passage 7.

RR virus was more resistant to 250 and 300 μ M ribavirin than WT virus, but was still completely inhibited by 400 μ M ribavirin, and showed no difference in replication relative to WT virus when grown in the absence of ribavirin.

RR virus accumulated multiple synonymous and non-synonymous mutations scattered throughout the genome.

Conclusions

It is possible to select mutants of HCV that can replicate in the presence of ribavirin to levels similar to wild type virus grown without ribavirin.

Analysis of the mutations responsible for the ribavirin resistance may aid in understanding the mechanism of action of ribavirin.

Acknowledgments

Dr. Charlie Rice, Rockefeller University (J6/JFH1 strain)