CIVACIR, HEPATITIS C IMMUNE GLOBULIN (HCiG), POTENTLY NEUTRALIZES INFECTION OF HEPATITIS C VIRUS TRANSPLANT ESCAPE VARIANTS

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Introduction

- Hepatitis C virus (HCV)-induced end-stage liver disease is the major indication of liver transplantation.
- Re-infection of liver graft is common.
- Safety and efficacy of new direct-acting antivirals (DAAs) for prevention of liver graft infection remains to be determined.
- Biotest Pharmaceuticals CIVACIR, a human hepatitis C antibody enriched immune globulin product (HCiG), has been shown to efficiently prevent liver graft infection in a phase III RCT (Terrault et al. EASL 2011).

Aim of the study

- We aimed to study the molecular mechanism of action of CIVACIR/HCiG against patient derived HCV escape variants.

Methods

- Inhibition of CIVACIR/HCiG-mediated HCV infection was studied using 22 viral variants isolated from patients before and after liver transplantation and state-of-the-art HCV cell culture models (Felt-Kremer et al. J Exp Med 2010, Fofana et al. Gastroenterology 2012; Felmlee, Fauvelle et al. EASL 2014).
- HCV pseudoparticles (HCVpp) and cell-culture-derived HCV (HCVcc) expressing patient-derived viral envelope glycoproteins from transplant escape variants were used.

CIVACIR/HCiG potently and dose-dependently neutralizes escape and non-escape variants

Figure 1. CIVACIR/HCiG potently and dose-dependently neutralizes HCVpp derived from escape and non-escape HCV variants isolated from patients before and after liver transplant. HCVpp expressing E1E2 envelope glycoproteins from escape (blue) and non-escape (black) variants isolated from 6 patients before and after liver transplantation respectively were produced and tested for neutralization against serially diluted CIVACIR/HCiG (1250 – 1.22 μg/ml). HCVpp were incubated with CIVACIR/HCiG or control IgG preparation at 37°C for 1 hour and subsequently inoculated on Huh7.5.1 hepatoma cells. The level of infection was determined after 72 hours by measuring luciferase activity expressed as relative light unit (RLU). The level of HCVpp entry is shown as percentage of the control.

Civacir/HCiG equally neutralizes escape and non-escape variants at low nano-molar concentration

Figure 2. The IC50 values (μg/ml) of CIVACIR/HCiG against 22 HCV pseudoparticles expressing E1E2 envelope glycoproteins of viral isolates from different patient derived escape (blue) and non-escape (black) variants. The IC50 values and correlation coefficients were calculated by nonlinear three parameter least squares analysis using GraphPad prism software.

Civacir/HCiG neutralizes HCVcc expressing the envelope of a highly infectious escape variant (HCV P1VL)

Figure 4. Civacir/HCiG robustly neutralizes HCVpp JFH-P1VL and JFH-P1VLsc chimera. (A) JFH-based HCV chimeras expressing structural proteins of patient isolates P1VL (genotype 1b) and P1VLsc were produced as described before (Fofana et al. Gastroenterology 2012). Replacing phenylalanine at position 447 with leucine endows phenotype of non-escape variant i.e. less infectious and increased susceptibility to neutralization with antibody preparations; akin to isolate P1VL. HCVcc was incubated with Civacir/HCiG or control IgG preparation at 37°C for 1 hour and subsequently inoculated on Huh7.5.1 hepatoma cells. Infection was read after 72 hours by measuring luciferase activity expressed as relative light unit (RLU). HCVcc infectivity was measured by determining the tissue culture infective dose 50% (TCD50) (B) IC50 values of CIVACIR/HCiG against HCVpp JFH-P1VL and JFH-P1VLsc chimeras. The IC50 values were calculated by nonlinear three parameter least squares analysis. JFH1 stands for highly infectious HCV strain isolated from a Japanese patient with fulminant hepatitis.

Conclusions

- The host antibody response is ineffective in countering the continuously evolving HCV quasispecies, leading to persistent chronic infection or escape and re-infection of liver graft post-transplantation. (B) Civacir, human hepatitis C antibody enriched immune globulin product (HCiG), is a polyclonal antibody preparation derived from human plasma enriched with HCV antibodies collected from several donors. The resulting high antibody diversity and synergy between anti-HCV antibodies targeting different epitopes is translated into effective neutralization of transplant escape variants resistant to autologous antibodies.
- These results uncover the mechanism of action of Civacir/HCiG, explain its clinical efficacy for prevention of HCV liver graft infection and indicates its potential for use against different genotypes.

Civacir/HCiG neutralizes HCVcc expressing the envelope of escape variants in primary human hepatocytes

Figure 3. Civacir/HCiG neutralizes HCVpp entry of escape and non-escape variants in primary human hepatocytes. (A) HCVpp derived from representative escape (blue line) and non-escape (black line) variants from liver transplant patients were incubated with different concentrations of Civacir/HCiG or control IgG preparation at 37°C for 1 hour and subsequently inoculated on primary human hepatocytes (PHH). The level of infection was determined after 72 hours by measuring luciferase activity expressed as relative light unit (RLU). The level of HCVpp entry is shown as percentage of the control. (B) IC50 values of Civacir/HCiG against HCVpp derived from patient variants P1VL, P1VLsc, P1VLsc, and P1VL. The IC50 values (μg/ml) were calculated by nonlinear three parameter least squares analysis.

CIVACIR/HCiG equally neutralizes escape and non-escape variants at low nano-molar concentration

Patient no. | Viral variant | IC50 (μg/ml) | Correlation coefficient
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1 | P1VA | 1.7 | 0.87 |
2 | P1V | 0.3 | 0.78 |
3 | P1VK | 6.7 | 0.91 |
4 | P1VL | 28.7 | 0.89 |
5 | P2VA | 22.0 | 0.98 |
6 | P2V | 36.6 | 0.95 |
7 | P2VI | 3.3 | 1.00 |
8 | P2V | 85.8 | 0.88 |
9 | P2V | 34.9 | 0.94 |
10 | P3VA | 37.9 | 0.97 |
11 | P3V | 66.1 | 0.92 |
12 | P3V | 10.4 | 0.97 |
13 | P4V | 53.4 | 0.94 |
14 | P4V | 5.7 | 0.72 |
15 | P5V | 34.9 | 0.95 |
16 | P5V | 6.5 | 0.99 |
17 | P6V | 38.8 | 0.94 |
18 | P6V | 2.7 | 0.92 |
19 | P6V | 8.6 | 0.78 |
20 | P6V | 12.4 | 0.96 |