

Hepatitis C Virus (HCV) is Highly Sensitive to MB/light Treatment of Plasma as Shown by a Novel *in vitro* HCV Infection System

U. Gravemann^o, M. Friesland^{*}, J. Doerrbecker^{*}, T. Pietschmann^{*}, E. Steinmann^{*}, A. Seltam^o

^o German Red Cross Blood Service NSTOB, Springe, Germany * TWINCORE, Center for Experimental and Clinical Infection Research GmbH

Background

Photodynamic treatment using methylene blue (MB) and visible light is a well established method for virus inactivation of human plasma (figures 1 and 2). It has been shown to inactivate a broad range of different DNA and RNA viruses. Due to the absence of a cell culture system for propagation of the hepatitis C virus (HCV), the inactivation efficacy against HCV had to be extrapolated from studies with the bovine viral diarrhea virus (BVDV). Although HCV is known since 1989 only recently a HCV infection system allowing the propagation of infectious HCV in cell culture has been developed (figure 3). Aim of the current investigation was the determination of the inactivation capacity of the THERAFLEX MB-Plasma system by the novel *in vitro* HCV infection system.



Fig 1. MacoTronic B2

Methods

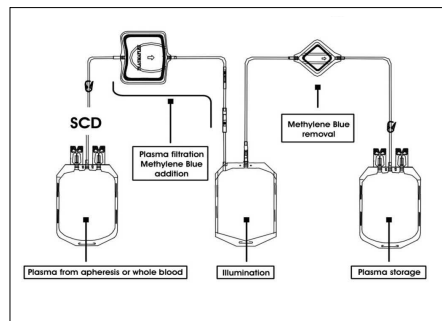


Fig 2. Theraflex bag system for MB/Light treatment

HCV Jc1 virus was generated by electroporation of 10 µg RNA into Huh7.5 cells. Cell culture supernatants were harvested 48, 72 and 96 h post transfection. Leukodepleted plasma was prepared from whole blood using standard blood banking technology. Plasma (n=3, 285 mL) was spiked with cell-culture grown HCV (30 mL). MB/light treatment was done using the MacoPharma Theraflex MB-Plasma bag system (figure 3) and the MacoTronic B2 illumination device (figure 1). Plasma (n=3; 285 mL) was spiked with cell-culture grown HCV (30 mL). Samples were taken at different steps of the process (table 1). In a second experiment the plasma was re-spiked after illumination with 40 J/cm² with an additional 30 mL of virus suspension to increase the sensitivity of the assay and to test for robustness of the inactivation process. Samples were taken at different steps of the process (table 2). Infectivity was determined by immunohistochemistry-based endpoint titration and large volume plating on Huh7.5 cells. The virus titer was determined at a dilution that was neither cytotoxic nor did interfere with the viral titer. The titer which causes a positive result in 50% of all infected cultures (TCID₅₀) was determined according to the method of Spearman and Kaerber.

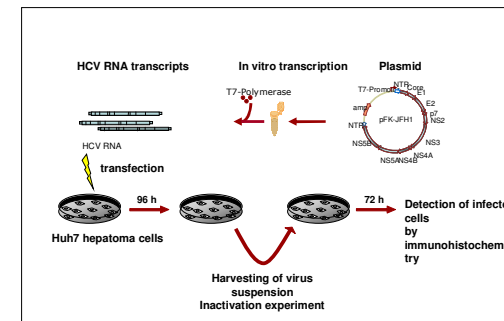


Fig 3. HCV infection system

Results

In a first experiment it was shown, that HCV in plasma was efficiently inactivated by the MB/light treatment below the limit of detection already with a light dose of 10 J/cm² (figure 4). After 40 J/cm² no infectivity was detectable by large volume plating. A log reduction factor of ≥ 3.83 was achieved (table 1). To further evaluate the inactivation capacity of the system a respiking experiment was done (figure 5). Again HCV in plasma was efficiently inactivated by the MB/light treatment below the limit of detection already with a light dose of 20 J/cm². After the second spike again treatment with a light dose of 20 J/cm² was sufficient for an inactivation of HCV below the limit of detection. A cumulative log reduction factor of ≥ 8.9 was achieved (table 2).

Conclusion

MB/light treatment of plasma using the THERAFLEX MB-plasma system (MacoPharma) efficiently inactivates HCV with a cumulative reduction factor ≥ 8.9 log steps. The procedure therefore has the potential to significantly reduce transfusion transmitted HCV infections.

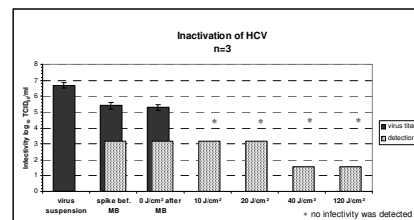


Fig 4. Inactivation kinetics of HCV by MB/light treatment

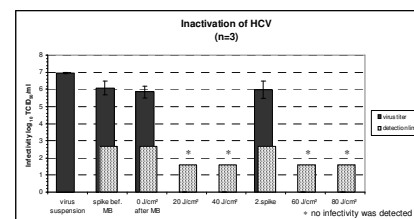


Fig 5. Respiking experiment: Inactivation kinetics of HCV

Tab 1. HCV reduction factors

Sample	log ₁₀ TCID ₅₀ ± SD	log ₁₀ reduction factor
spike before MB	5.41 ± 0.22	
0 J/cm ² after MB	5.29 ± 0.16	0.12
10 J/cm ²	< 3.15	≥ 2.26
20 J/cm ²	≤ 3.15	≥ 2.26
40 J/cm ²	≤ 1.58	≥ 3.83
120 J/cm ²	≤ 1.58	≥ 3.83

Tab 2. Respiking experiment: cumulative HCV reduction factors

Sample	log ₁₀ TCID ₅₀ ± SD	cumulative log ₁₀ reduction factor
spike before MB	6.09 ± 0.39	
0 J/cm ² after MB	5.85 ± 0.34	0.24
20 J/cm ²	≤ 1.58	≥ 4.51
40 J/cm ²	≤ 1.58	≥ 4.51
2. spike	5.97 ± 0.50	≥ 4.51
20 J/cm ²	≤ 1.58	≥ 8.90
40 J/cm ²	≤ 1.58	≥ 8.90