HEPATITIS G VIRUS CO-INFECTION MAY AFFECT THE ELIMINATION OF HEPATITIS C VIRUS RNA FROM THE PERIPHERAL BLOOD OF HEMODIALYSIS PATIENTS

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Summary. – Hemodialysis patients are at risk for hepatitis C virus (HCV) and hepatitis G virus (HGV) infection. The aim of this study was to investigate the possible influence of HGV co-infection on HCV RNA elimination from the peripheral blood of hemodialysis patients. The study involved 144 persons, all with HCV antibodies and HCV RNA. Among 144 patients 24 (16.7%) were positive for HGV RNA. After 2.5 years of observation 80 patients (55.6%) were still HCV RNA-positive. In the latter group 18 patients were co-infected with HGV and 62 were HGV RNA-negative. During 2.5 years of the follow-up study 64 patients eliminated HCV RNA from the serum. In this group only 6 patients were HGV co-infected. None of the HGV-positive patients eliminated HGV RNA from the serum. The higher incidence of HGV co-infection in the group of patients who remained HCV RNA-positive (18/80, 22.5%), in comparison to the group of HCV antibodies-positive patients who lost HCV in the blood (6/64, 9.4%, $P < 0.0001$) suggests, that the co-infection with HGV may delay the spontaneous elimination of HCV RNA from the blood.

Key words: HCV; HGV; co-infection; HCV RNA; HGV RNA; HCV RNA elimination; hemodialysis patients

Introduction

It is thought that hepatitis viruses A-E do not account for all cases of hepatitis (Zhang *et al.*, 1997). The recently discovered hepatitis G virus (HGV) is a flavivirus with sequence homology to hepatitis C virus (HCV) (Kiyosava and Tanaka, 1999; Linen *et al.*, 1996). HGV as well as HCV have been shown to be transmitted parenterally through blood and blood products (El-Zayadi *et al.*, 1999; Wreghitt, 1999).

The pathogenic significance of HGV infection alone in the development of hepatitis is still questioned (Fan *et al.*, 1999). HGV may not be a strictly hepatotropic virus and may cause hepatitis only occasionally. In accord with this hypothesis is the lack of HGV replication in the liver in HGV-positive patients (Fan *et al.*, 1999; Kiyosava and Tanaka, 1999).

Some investigators have reported a possible association between HGV infection and liver diseases (Hollingsworth *et al.*, 1998). However, many clinical studies have failed to demonstrate any significance for HGV in the etiology of acute and chronic hepatitis (Fan *et al.*, 1999; Kiyosava and Tanaka, 1999). Despite the lack of hard evidence for pathogenic role of HGV, the incidence of two or more viral infections may have some pathological consequences. Frequent hemodialysis patients as well as intravenous drug users are at risk for HGV infection (Desassis *et al.*, 1999; Fabrizi and Martin 1999; Gartner *et al.*, 1999) and show high frequency of HGV and HCV co-infection (Basaras *et al.*, 1999; Martin *et al.*, 1999; Masuko *et al.*, 1996; Schroter *et al.*, 1999; Wang *et al.*, 1997).

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Abbreviations: HCV = hepatitis C virus, HGV = hepatitis G virus, PCR = polymerase chain reaction

According to some reports infection with HGV does not seem to have any effect on the severity of chronic hepatitis C in either immunosuppressed or immunocompetent individuals (Brandhagen *et al.*, 1999; Hayashi *et al.*, 1999; Rostaing *et al.*, 1999a,b). However, it is possible that there is a competition in replication between HCV and HGV (Radkowski *et al.*, 1998; Sauleda *et al.*, 1999). This is a likely explanation taking into account the similarity of genomic organization of HGV and HCV. Therefore in the case of co-infection with HCV and HGV, either interference or synergism may occur.

In this study we investigated the possible influence of HGV infection on incidence of HCV RNA in the blood of hemodialysis patients.

Materials and Methods

Patients. The chronic hemodialysis patients selected for this study involved 144 adults, aged 29–67 years, all with HCV RNA and HCV antibodies. All the patients were dialyzed at the same center. Among these patients 24 were positive for HGV RNA in the blood. All the patients were monitored prospectively over 2.5 years for the presence of HCV and HGV and HCV antibodies.

ELISA of HCV antibodies. A third generation ELISA (Organon Teknika) was employed. Blood samples were collected from the patients in intervals of 3–6 months during the 2.5 year follow-up study.

PCR for detection of HCV RNA and HGV RNA was performed by use of PCR kits for HCV (Roche) and HGV (Boehringer). In the PCR for HCV RNA, the primers KY78 and KY80 yielded a product of 244 nucleotides within the highly conserved 5'-untranslated region of HCV genome (Linen *et al.*, 1996; Young *et al.*, 1993). The PCR for HCV RNA consisted of one cycle at 95°C for 5 mins, 35 cycles of denaturation at 95°C for 2 mins, annealing at 65°C for 2 mins and elongation at 72°C for 3 mins, and one cycle of final extension at 72°C for 7 mins. The PCR for HGV RNA consisted of one cycle at 94°C for 5 mins, 35 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min and elongation at 72°C for 2 mins, and one cycle of final extension at 72°C for 5 mins.. Normal serum, water and positive and negative samples, available in the abovementioned kits, were used as controls in each PCR. The Boehringer HGV test uses the primers define a sequence of 373 nucleotides within the highly conserved 5'-untranslated region of HGV genome (Linen *et al.*, 1996).

Agarose gel electrophoresis. PCR products were subjected to 2.2% agarose (Serva) gel electrophoresis and stained with ethidium bromide (Merck) (1 µl 1% solution of ethidium bromide/100 ml agarose gel). A DNA size marker, 50 bp DNA Ladder (MBI FERMENTAS GeneRuler™) was employed.

Results and Discussion

All the 144 patients, selected for the follow-up study, were initially positive for HCV RNA and HCV antibodies.

Table 1. Effect of HGV co-infection on the elimination of HCV RNA from the peripheral blood of hemodialysis patients

Time of evaluation during the follow-up study	HCV+			HCV–		
0 (at the beginning)	HGV+	24/144	(16.7%)	HGV+	0	
	HGV–	120/144	(83.3%)	HGV–	0	
After 2.5 years	HGV+	18/80	(22.5%)	HGV+	6/64	(9.4%)
	HGV–	62/80	(77.5%)	HGV–	58/64	(90.6%)

Primarily, we aimed at following the rate of spontaneous HCV RNA elimination in hemodialysis patients. At the same time we examined the presence of HGV RNA and the influence of HGV co-infection on the elimination of HCV RNA in these patients. Among 144 patients 24 (16.7%) were positive for HGV RNA. After 2.5 years of the follow-up study 80 patients (55.6%) were still HCV RNA-positive. In this group, 18 patients (22.5%) were co-infected with HGV and 62 (77.5%) were HGV RNA-negative. During 2.5 years of the follow-up study 64 patients eliminated HCV RNA from the blood. In this group, only 6 patients (9.4%) were HGV-co-infected. None of the HGV-positive patients eliminated HGV RNA from the blood. The obtained data are summarized in Table 1.

According to the published data the co-infection with HGV has been observed in about 15% of patients with chronic hepatitis C (Desassis *et al.*, 1999; El-Zayadi *et al.*, 1999; Martin *et al.*, 1999; Masuko *et al.*, 1996). In our study, 16.7% of the hemodialysis patients were HGV co-infected, so it can been concluded that HGV infection is frequently seen in hemodialysis patients.

On the basis of the obtained results it may be postulated that HGV infection is more persistent than HCV infection, as measured by the presence of HGV RNA and HCV RNA in the blood. There are some reports indicating a long, up to 16 years persisting HGV infection in hemodialysis patients (Masuko *et al.*, 1996; Rostaing *et al.*, 1999b).

A key question is whether the HGV co-infection alters the level of HCV, clinical course and treatment response in chronic hepatitis C. There are some reports dealing with biochemical and virological effects of HGV infection on chronic hepatitis C (Brandhagen *et al.*, 1999; El-Zayadi *et al.*, 1999). Hayashi *et al.* (1999) have concluded that HGV infection does not increase the severity of hepatitis C as evaluated by biochemical and serological parameters (Hayashi *et al.*, 1999). Other reports have revealed that HCV/HGV co-infection does not affect the biochemical and virological profile of chronic hepatitis C and that it has no effect on efficacy of interferon therapy (Rostaing *et al.*, 1999a,b). Recently, a histopathological evaluation has shown that the dual HGV and HCV infection in early chronic phases

of the disease can affect the development and relevance of inflammatory and fibrotic changes (Brandhagen *et al.*, 1999).

It is not easy to explain contradictory results, most of which do not support the etiological role of HGV. It is also difficult to consider the supportive role of HGV on the course of HCV infection. As HCV RNA is eliminated more rapidly from the blood as compared to HGV, the question arises whether the persistent HGV infection may affect the course of HCV infection. Much higher incidence of HGV co-infection in the group of patients who remained HCV RNA-positive (18/80, 22.5%) in comparison to the group of HCV antibodies-positive patients who lost HCV from the blood (6/64, 9.4%, $P < 0.0001$) suggests that the co-infection with HGV may delay the spontaneous elimination of HCV RNA from the blood.

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