Diffusion of HCV through peritoneal membrane in HCV positive patients treated with continuous ambulatory peritoneal dialysis


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Abstract Purpose of the Study. We evaluated the presence of HCV in the peritoneal effluents of viraemic patients treated with continuous ambulatory peritoneal dialysis (CAPD) to evaluate the risk of transmitting the infection with this procedure.

Procedure. Fifteen of 81 CAPD patients (18.5%) had anti-HCV antibodies and eight were viraemic. At the beginning of CAPD two of the viraemic patients had ascites with a clinical picture of chronic active hepatitis and cirrhosis. Peritoneal dialysates were collected after an overnight exchange with 1.36% glucose and after a 4-h exchange with 3.86% glucose. Fluids from the overnight exchange were spun to obtain a cellular pellet and the supernatant 100-fold concentrated.

Results. No viral genome could be detected in unconcentrated samples and in cellular pellets, while HCV-RNA at low titre was detected in concentrated dialysates from the two patients with active liver disease.

Conclusions. Our findings confirm that HCV may be present in the CAPD effluent of some patients; however, the titre of virus in the effluent was extremely low, at the limit of detection of the PCR assay. Peritoneal fluids originating from patients with HCV associated severe liver disease may be a potential source of infection.

Key words: hepatitis C virus; peritoneal dialysis; polymerase chain reaction; ascites

Introduction

Hepatitis C virus (HCV) is a frequent cause of hepatitis in haemodialysis (HD) patients. The reported prevalence of HCV antibodies in HD patients ranges between 5 and 54% [1]. The increased risk for HCV infection in HD patients has been related to blood transfusion [2,3] and to the length of HD treatment [4–6]. Nosocomial spread of HCV in HD units has been suggested by several authors [7–9] and a direct demonstration that HCV can pass into dialysis fluids, albeit at low concentration, has been provided [10].

In patients treated with continuous ambulatory peritoneal dialysis (CAPD) the prevalence of HCV markers is lower than in HD, ranging from 2 to 15% [11–16]. The presence of HCV in peritoneal dialysate effluent has been investigated by few authors with conflicting results [15,17,18], and at present the prevalence of HCV-RNA positivity in peritoneal dialysate, the determinants influencing its presence, and its relevance as a potential source of infection are not well defined.

In this study we evaluated the presence of HCV in the peritoneal effluents of CAPD patients with HCV viremia in order to evaluate the risk of transmitting the infection with this procedure.

Subjects and methods

Eighty-one CAPD patients (45 males, 36 females, age range 23–85 years, median 63 years) were investigated for the presence of HCV antibodies and HCV-RNA in serum. These patients had been on CAPD for 1–145 months (median 27) performing 4–5 exchanges per day, 1.5 or 2 l each, using different double-bag disconnect systems (Baxter Y set, Bieffe L3).

Anti-HCV antibodies were detected by Elisa III (Ortho Diagnostic Systems, Raritan, NJ) and confirmed by supplemental testing with RIBA 3.0 (Ortho Diagnostic Systems, Raritan, NJ).

Viral RNA was prepared by spin chromatography (QIAamp HCV kit; Qiagen GmbH, Hilden, Germany). Qualitative detection of HCV-RNA was performed by a single-tube reverse-transcription-heminested-PCR procedure [19] with primers from the 5-untranslated region of the viral genome. Quantitation of HCV-RNA was performed using the commercial assay Amplicor HCV Monitor® (Roche Diagnostic Systems, Branchburg, NJ).

In viraemic patients the presence of HCV-RNA was investigated in the CAPD fluid. For this purpose 20 ml of fluid were obtained from the exchange bag in sterile conditions: (i) after an overnight exchange with 1.36% glucose

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solution, (ii) after a 4-h exchange with 3.86% glucose solution. The CAPD fluid obtained after an overnight exchange performed with 1.36% glucose solution was spun to obtain a cellular pellet, and an aliquot of the supernatant 100-fold concentrated using an ultrafiltration device (Centriprep 100, Amicon Division, Beverly, MA, USA). Total protein concentration was determined in the supernatant. Viral RNA was extracted from aliquots of native and concentrated fluid and from cellular pellets.

**Results**

Fifteen of 81 patients (18.5%) were found to be positive for anti-HCV antibodies. Nine were males and six were females (age range 45–81 years, median 65 years) with a mean time on dialysis of 56 ± 43 months. Twelve had been treated only with CAPD and three with haemodialysis and then with CAPD. None was a drug addict or positive for anti-HIV antibodies.

HCV viraemia was found in only eight patients with a serological result for HCV. The titre of viraemia was <10^5/ml in three patients, >10^5 in one patient and >10^6 in four patients. Elevation of serum ALT was seen in five of 15 (33%) patients with anti-HCV antibodies, all of them viraemic. Two viraemic patients had ascites with a clinical and biochemical picture of liver cirrhosis at the beginning of CAPD; one of them underwent liver biopsy, which showed a histological picture of chronic active hepatitis and cirrhosis. The presence of HCV-RNA in peritoneal fluids was investigated in patients with HCV viraemia. In none of the native samples obtained with a 4-h or an overnight exchange could HCV-RNA be detected by PCR, while HCV-RNA was detected on two concentrated peritoneal effluents after an overnight exchange. Both of these patients had viraemia levels in the range >10^6/ml and a clinical picture of cirrhosis. The protein concentration in their CAPD fluid (0.58 and 1.30 g/l), however, was not significantly different from that of the whole group (0.32–1.34 g/l, mean 0.79 ± 0.31). HCV-RNA titre in 100-fold concentrated effluents was <10^5/ml, at the limit of detection of the assay, while PCR on cellular pellet was negative in all patients. At light-microscopy examination cellular pellets consisted mainly of monocytes, granulocytes, and mesothelial cells with lymphocytes not exceeding 10%.

**Discussion**

The risk of HCV infection among uraemic patients is only partially related to blood-transfusions, and apparent modes of viral transmission account for a relevant proportion of cases. The highest prevalence of HCV infection is found in patients treated by HD but CAPD patients are also exposed to an increased risk of infection. Since HCV may be found in several organic fluids including ascites [20,21], it is possible to hypothesize that HCV-infected peritoneal fluid could represent a potential infectious risk in the CAPD environment.

The presence of HCV in the peritoneal effluent of CAPD has been the object of a few studies reporting partially conflicting results. In five viraemic CAPD patients reported by Caramelo et al. [18] HCV could not be detected in peritoneal fluid. In two other small series HCV was detected in the peritoneal fluid of four of nine viraemic CAPD patients by Gladziwa et al. [15] and in four of five by Krautzig et al. [17]. In the latter report HCV was determined in each patient on three samples obtained on different days and only the fluid from one patient was found positive on each of the three occasions.

Our data confirm that HCV is present in the CAPD effluent of some patients. The titre of virus in the effluent, however, was extremely low and a positive signal was detected only on the concentrated fluids of two viraemic patients. Taking into account the 100-fold concentration we can estimate that the titre of HCV in the peritoneal effluent of these patients was at least four orders of magnitude less than in the blood. Since all viraemic patients had HCV titres <10^5/ml, the concentration of viral particles in the peritoneal fluid should be in the order of 1–10 virions/ml, exceeding the sensitivity limits of most PCR tests currently employed, and raising the possibility of random sampling errors in PCR due to the extreme dilution of the target sequence. The very low titres of HCV in peritoneal fluids could account for the sporadic detection of the virus even by the most sensitive PCR procedures, and may explain conflicting results on this issue.

The two patients in whom HCV could be detected in the peritoneal fluid were the only two in our series with a clinical picture of HCV-associated chronic active hepatitis with cirrhosis and ascites when they started CAPD treatment. Since HCV-RNA can be virtually always found in the ascitic fluid of patients with HCV-related cirrhosis [21] (and our unpublished data), this suggests that the severity of liver disease and the presence of ascites may be important determinants for the infection of CAPD fluid. Both patients had viraemia at the upper limit of the range observed in our patients and hypertransaminasemia. The persistence of ascites in these two patients during CAPD could not be indirectly demonstrated by an increased protein concentration in their CAPD effluents. The expected increase in convective flux and capillary vasodilatation determined by a 4-h exchange with a high glucose concentration were apparently unrelated to the passage of HCV in the CAPD fluid. Since HCV is commonly found in a variable proportion of blood lymphocytes [22], we also tested cellular pellets obtained from the centrifugation of effluents. Lymphocytes represented a low proportion of the cell population, and none of the cellular samples was positive for HCV.

Although the virus is detected in the peritoneal fluid of only a minority of HCV-positive CAPD patients, we would recommend that infection control measures should be routinely implemented, and that closed systems should be used, in order to minimize the risk of transmission of hepatitis C through CAPD. The presence of hypertransaminasemia in HCV-positive...
patients, the level of HCV viraemia, and clinical evidence of portal hypertension and ascites can all be useful, even if indirect, indicators of an increased risk of infection of peritoneal fluids.

References


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