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Increased intestinal permeability during cytomegalovirus infection in renal transplant recipients

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Abstract Cytomegalovirus (CMV) infections in renal transplant recipients can affect the gastrointestinal tract, but significant clinical manifestations are seldom seen. We hypothesize that subclinical involvement of the gastrointestinal tract may be quite frequent during CMV infection. In order to study this, we measured intestinal permeability by calculating the urinary lactulose mannitol (LM) excretion ratio after oral administration of lactulose and mannitol (normal < 0.030) in patients with symptomatic and asymptomatic CMV infection. A total of 111 patients were enrolled in the study, 104 of whom were tested on postoperative day (POD) 10. Twenty-nine patients developed CMV infection, 12 of whom could be studied with the permeability test (median POD 40). Another nine patients without CMV infection were also studied at day 40 and served as controls. The LM ratio increased significantly during CMV infection compared to measurements before active infection (median 0.060 vs. 0.030, $P < 0.01$) and was significantly higher during the infection than in the control group (median 0.007, $P < 0.01$). No correlation could be found between the LM ratio and viral load, humoral response to the virus, or symptomatology of infection. We conclude that an increased intestinal permeability is found in a substantial number of patients with an active, albeit asymptomatic, CMV infection after renal transplantation. Pathophysiological mechanisms and clinical implications remain speculative but will be subject to further study.

Key words CMV, renal transplantation, intestinal permeability · Renal transplantation, CMV, intestinal permeability · Permeability, intestinal, CMV · Intestinal permeability, renal transplantation, CMV

Introduction

Cytomegalovirus (CMV) infection is the most frequent infectious complication after renal transplantation. Although CMV infections after renal transplantation are frequently seen, a substantial number of them are asymptomatic. When CMV infection causes disease, most patients exhibit a so-called self-limiting CMV syndrome consisting of spiking fever, arthralgia, leukocytopenia, thrombocytopenia, and elevated serum liver enzymes. Less common manifestations involve the gastrointestinal tract, the lungs, the eyes, the kidney, the heart, and the nervous system. Clinical manifestations of gastrointestinal involvement include ulcerative lesions anywhere along the gastrointestinal tract, intestinal pneumatosis [13], pancreatitis, and hepatitis.

We hypothesize that there is frequent subclinical organ involvement during CMV infection in renal transplant patients. This would be in accordance with the systemic nature of this type of infection. For example, van

Son et al. [14] have already demonstrated that when subjected to sensitive pulmonary function tests, a majority of patients with an active CMV infection have pulmonary dysfunction, even without pulmonary symptoms. Thus far, subclinical involvement of the gastrointestinal tract during CMV infection has not been studied.

The epithelium of the gastrointestinal tract has important transport functions, but the barrier function with respect to luminal molecules is at least as important. Intestinal permeability relates to the barrier function, and the permeation of marker molecules is used to measure the permeability. Most intestinal permeability tests are based on quantitation in the urine of marker molecules ingested orally. However, not only the permeability of the intestinal surface but many other factors, such as gastric emptying, dilution by secretions, and renal clearance, determine the excretion of marker molecules. These factors can be eliminated by using two markers that differ in permeability but are affected equally by all of the other factors. This principle is called differential permeability testing. The excretion ratio of these two markers has been shown to be a reliable test for intestinal permeability. This method of measuring intestinal permeability is widely accepted and used for testing mucosal dysfunction, making more invasive diagnostic procedures unnecessary [15]. In this study we evaluated the subclinical involvement of the gastrointestinal tract in renal transplant recipients with CMV infection by determining intestinal permeability with lactulose and mannitol as markers.

Materials and methods

One hundred eleven patients transplanted between 1990 and 1993 were included in the study. Sixty five were men. The mean age was 44 years (range 18–68 years). The median dialysis period pretransplantation was 32 months (range 0–144 months). There were 101 first transplantations, 9 second, and 1 third. Six transplantations were living related. Fifty-three percent of the patients were seropositive for CMV before transplantation. Patients were considered seropositive when IgG antibodies against CMV late antigen (CMV LA) were present. No CMV prophylaxis (acyclovir, gancyclovir, or anti-CMV immunoglobulins) was given. Initial immunosuppression consisted of cyclosporin A and low-dose prednisolone. Patients with a second or third transplant received induction therapy with monoclonal antibodies (OKT3), followed by triple therapy (azathioprine, cyclosporin A, and low-dose prednisolone). All patients gave informed consent before participating in the study.

A differential permeability test was performed to measure intestinal permeability. We used lactulose (342 Da, 0.52 nm) and mannitol (182 Da, 0.40 nm) as markers.

After an overnight fast of at least 6 h and with an empty urinary bladder, patients drank 100 ml of water containing 10 g lactulose and 0.5 g mannitol. The osmolality of the solution was 255 mosmol/l. No oral intake was allowed during the first 2 h after ingestion of the test solution, and subsequently milk or sugars were not allowed until 5 h after commencing the test. Urine was collected during a period of 5 h after the test solution had been ingested. The volume of the urine collected was recorded and an aliquot was refrigerated at –20 °C until the time of analysis.

The lactulose and mannitol concentrations in the urine were measured by gas liquid chromatography, as described by Laker [9] and Laker and Mount [10], respectively, with minor modifications. Briefly, the samples were mixed with internal standard solution (a-methyl glucose), washed, and dried. Pyridine/hydroxysil (Chromopack, Middelburg, The Netherlands) was added and the specimens were heated at 60 °C for 2 h. Samples were analyzed in a Packard 428 chromatograph (Packard-Becker, Delft, The Netherlands) on a 200-cm column of 3 % OV-1 (Chromopack, Middelburg, The Netherlands), operated at 190 °C for 7 min and at 250 °C for 6 min; the temperature was elevated from 190 ° to 250 ° in 12 min. Mannitol, 1 mmol/l, and lactulose, 1 mmol/l, were used as standard solutions (Janssen Pharmaceutica, Belgium).

The lactulose mannitol excretion ratio (LM ratio) was calculated by dividing the urinary lactulose excretion by the urinary mannitol excretion, expressed as percentages of the ingested doses. Normal values are below 0.030 and are independent of renal function. Especially in our patients, who had a broad range of glomerular filtration rates, it was necessary to be very sure about the independence of renal function, as reviewed in the literature [4, 15]. For this reason, we measured the LM ratio in 10 non-transplanted patients without gastrointestinal diseases from the outpatient renal clinic and with glomerular filtration rates below 25 ml/min; these ratios were in the normal range.

Diagnosis of active CMV infection was made using the CMV antigenemia assay, as described by van der Bij et al. [1, 2] and reviewed by Chou [3] and by Ljungman and Griffiths [11] during the Fourth International CMV Workshop (Paris, 1993). In short, peripheral blood leukocytes were isolated, cytocentrifuged, and incubated with a mixture of monoclonal antibodies directed against a 65–66 kD CMV antigen, followed by immunoperoxidase staining. The number of antigen-positive cells per 50,000 leukocytes was counted in triplicate. The antigenemia assay was performed at least once weekly starting on postoperative day (POD) 12. In all patients the antigenemia was followed by either seroconversion (primary infection) or a significant rise (reactivation) in CMV IgG antibodies. IgM and IgG CMV antibodies were measured quantitatively by ELISA using late stage CMV-infected fibroblasts as antigens [5].

Intestinal permeability was measured with the LM test at POD 10 to avoid possible bias of the postoperative state. Those values are referred to as baseline values. During active CMV infection, diagnosed by a positive antigenemia assay, the intestinal permeability was measured again (median POD 40). In five patients we were able to perform more than one permeability test during CMV infection, and in the analysis we used the result of the test with the highest LM ratio. Since we measured the LM ratios during CMV infection on median POD 40, we also measured LM ratios on median POD 40 in a control group of renal transplant recipients without CMV.

Statistical analysis was performed using the Wilcoxon signed rank test and the Mann-Whitney U-test. Differences in creatinine clearances were evaluated with Student's *t*-test.

Results

We performed 104 lactulose mannitol (LM) tests on POD 10 (baseline values), 18 during active CMV infection, and 9 on POD 40 without CMV infection. Twenty-

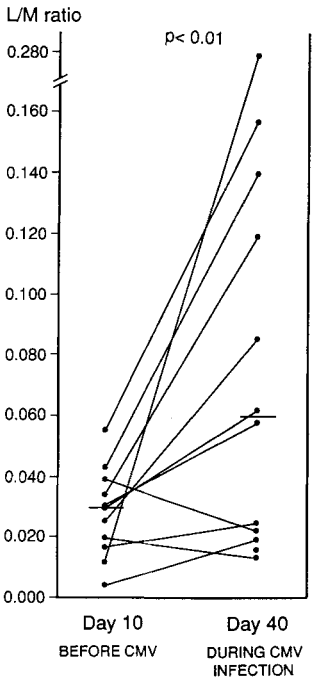

Fig. 1 Individual (•) and median (–) lactulose mannitol (LM) excretion ratios in renal transplant recipients before [postoperative day (POD) 10] and during active CMV infection (median POD 40)

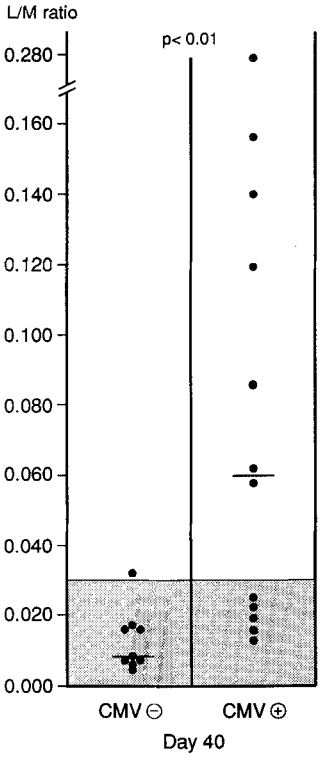

Fig. 2 Individual (•) and median (–) lactulose mannitol (LM) excretion ratios in renal transplant recipients during CMV infection (median POD 40) and in a control group without CMV infection (POD 40)

nine patients (29 %) contracted an active CMV infection, 14 secondary infections versus 15 primary ones. In 12 of them, the intestinal permeability was tested at least once during infection (7 secondary versus 5 primary infections). In this group five patients were asymptomatic and four had fever as the only symptom, while three had fever as well as leukocytopenia and thrombocytopenia. Ten of these 12 patients were hospitalized. The other 17 patients with CMV infection refused to undergo a LM test. Of these 17 patients, 15 were hospitalized. They did not differ clinically from the group that was tested. In none of the 29 patients with an active CMV infection was clinical gastrointestinal symptomatology apparent.

Compared to baseline values, the LM ratio increased during active CMV infection in 9 out of the 12 patients that could be studied. For these 12 patients a significant increase ($P < 0.01$) was observed, from a median of 0.030 (range 0.004–0.056) to 0.060 (range 0.013–0.279; Fig. 1). In addition, the LM ratio during active CMV infection was significantly ($P < 0.01$) higher than in the control patients measured at POD 40 (median 0.007, range 0.004–0.032; Fig. 2). No correlation could be found between the LM ratio and clinical signs of infection, the height of antigenemia or humoral immune response. Control patients had significantly ($P < 0.05$) better renal function than CMV patients on POD 10 [mean creatinine clearance (CrCl) on POD 10: 61 vs 40 ml/min]. During CMV infection there was a tendency towards a lower CrCl in the CMV group than in the control group on POD 40 (mean CrCl 49 vs 64 ml/min), but this did not reach statistical significance. The tendency towards a slightly higher CrCl in patients without CMV infection was most likely caused by the more frequent rejection episodes in the CMV group. The mean CrCl on POD 10 compared to that on POD 40 did not differ significantly in the two groups (40 vs 49 ml/min in the CMV group and 61 vs 64 ml/min in the control group).

Discussion

Our data appear to support involvement of the gastrointestinal tract during active CMV infection in renal transplant patients in the absence of gastrointestinal symptoms. This may indicate that the majority of patients with active CMV have subclinical enteropathy as evidenced by an increased permeability of the intestinal barrier. Previous studies have shown that about a quarter of patients with reactivation of CMV after liver transplantation have evidence of CMV in their upper gastrointestinal mucosa [12]. This study suggests that the impact of CMV infection on bowel mucosa may be much more extensive than those previous studies showed.

The LM test was used because it is simple and considered not to be stressful for patients. The LM differential permeability test has been shown to provide useful clinical information on small intestinal pathology as, for ex-

ample, in inflammatory bowel disease or celiac disease [15]. Nevertheless, there is still confusion about the permeation pathways of lactulose and mannitol. Lactulose is a disaccharide that probably diffuses between the epithelial cells of the intestinal barrier (paracellularly) through a relatively small population of large pores formed by tight junctions and extrusion zones. Mannitol is a smaller molecule and diffuses between, but also across, epithelial cells (transcellularly) through a larger population of small pores. This explains why mannitol permeation is more than 30 times higher than that of lactulose. Intestinal disease makes the barrier leakier and less selective, thus favoring lactulose and increasing the LM ratio.

Although intestinal participation in CMV infection is in accordance with the systemic nature of this type of infection, the reason for the increased intestinal permeability remains speculative and requires further studies. Aside from the cytopathic effects of the virus itself on the intestinal mucosa, it has been suggested that a more widespread infection of endothelial cells by the CMV might play a pivotal role in the pathophysiology of CMV infection, possibly explaining the protean symptomatology of the infection [7, 8]. The virus is latent in tissue cells of the transplanted organ or of the recipient. The virus may be reactivated as a result of immunosuppression in the transplanted patient. According to our hypothesis, the virus most likely spreads from cell to cell until, at some point during this process endothelial cells become infected. In the following phase, mononuclear and polymorphonuclear leukocytes adhere to the infected endothelium and ingest the virus and the pp65 matrix protein, which has been proven to be the viral protein detected in the CMV antigenemia test [6]. Finally, the infected endothelial cells detach and can be seen in the blood as circulating, cytomegalic endothelial cells [7, 8]. Endothelial damage, causing microvascular obstruction or plugging of these large, circulating CMV-infected endothelial cells, may damage the integrity of the intestinal epithelial cells by hypoxemia and may explain the increased intestinal permeability in CMV infection. A variant on this hypothesis could be that the enterocytes themselves become infected by the virus, causing cellular damage and loss of barrier function.

Clinical implications of the increased permeability can only be speculated. One important implication could be increased permeation of antigens during CMV infection or translocation of bacteria that could influence the delicate immune balance in transplant recipients and be the cause of opportunistic infections. Another implication could be an altered absorption of drugs.

In conclusion, an increased intestinal permeability indicating intestinal epithelial damage has been found in a substantial number of patients with active CMV infection after renal transplantation. While this finding does not, in our opinion, provide any useful, marker of CMV infection, it could provide some insight into the pathophysiology of the protean manifestations of CMV infection after transplantation. Further studies need to be performed to clarify the pathophysiological mechanisms and implications of this phenomenon.

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