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ORIGINAL ARTICLE

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Assay of cytomegalovirus susceptibility to ganciclovir in renal and heart transplant recipients

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Abstract Ganciclovir (GCV) prophylaxis or pre-emptive therapy significantly reduce the rate of cytomegalovirus (CMV) disease and viremia, but increase the potential for emergence of ganciclovir-resistant CMV strains. The inhibitor concentration at 50% (IC_{50}) of GCV from 156 CMV isolates from 59 renal or heart transplant recipients was calculated by means of a rapid phenotypic susceptibility assay. Twenty-seven strains were from 14 patients undergoing GCV therapy. The IC_{50} was higher in patients under the prophylaxis regimen. One CMV strain, from a heart transplant recipient, became GCV-resistant after 1 month of therapy (IC_{50} = 13.7 μmol/l). These data, together with clinical and virological markers, suggested that a switch to foscarnet was necessary, and good evolution was observed. Thus, assay of CMV susceptibility to GCV could be helpful in clinical management.

Keywords Cytomegalovirus susceptibility assay · Ganciclovir · Renal transplant recipients · Heart transplant recipients

Introduction

Cytomegalovirus (CMV) has long been recognized as the most common opportunistic pathogen in transplant recipients [12, 19, 21]. Active CMV infection occurs in 30%–75% of transplant recipients, with current mortality rates of approximately 5%. A number of effects and sequelae has been linked to CMV infection, such as CMV-related symptoms and organ dysfunction; contribution to the so-called net state of immunosuppression after organ transplantation; the clinical observation of a mutual influence between CMV infection and acute transplant rejection; a possible role of CMV in the development of chronic transplant dysfunction, such as accelerated coronary atherosclerosis after heart transplantation; and so on [3].

As more-potent immunosuppressive drugs are becoming available, there will be an inevitable tendency to intensify immunosuppression, with an inherently increased risk of opportunistic infections. This development will obviously influence our considerations and recommendations with regard to the diagnosis and therapy of CMV. The practical consequence is that reliable and efficient diagnostic tools and effective therapy will become increasingly important. Intensification of antiviral measures, especially prophylactic ones, will also be a logical consequence [3].

The goal of CMV management is to prevent or treat CMV disease with a minimum of side effects. Although prophylaxis with ganciclovir (GCV) has undoubtedly been associated with a substantial decline in CMV-associated morbidity, the optimal approach to treatment and how best to utilize GCV for prophylaxis remain controversial and unresolved [18]. Another controversial point is the potential risk of an emergence of GCV-resistant CMV strains. So, CMV susceptibility

(phenotypic or genotypic) assays would be very useful in clinical management. In this study, we performed a new phenotypic assay to test CMV susceptibility in isolates from clinical samples (urine or peripheral blood leukocytes (PBL)). The relationship between CMV susceptibility to GCV and antiviral administration was evaluated.

Patients and methods

Between May 1997 and December 2000, 59 transplant recipients were included in our study. In weekly follow-ups they were observed for at least 3 months after transplantation to detect active CMV infection or disease. Thirty-five were renal transplant recipients (24 male and 11 female; mean age 52.6 ± 13 years; range 18–60 years). GCV was administered intravenously to eight of them (22.2%) for a mean of 19.2 ± 7.2 days. For five of them, GCV was used as pre-emptive therapy over 4 weeks, and for the other three, as a prophylaxis regimen for 2 weeks. The dosage of GCV was 5 mg/kg/12 h, adjusted to renal function. Seventy-seven isolates from renal transplant recipients were studied, 12 of them were from eight patients under GCV regimen.

We also studied 79 strains from 24 heart transplant recipients (half of them male; mean age 53 ± 7.5 years; range 35–66 years); GCV was administered intravenously to six for an average of 19.8 ± 6.8 days (for one of them as prophylaxis regimen for 2 weeks, for two as CMV disease treatment over 2–3 weeks, and for the other three as prophylaxis and CMV-disease treatment for 4–5 weeks, at the same GCV dose as above and also adjusted to renal function); 15 isolates were recovered from them, and nine were from the same patient.

For urine and leukocytes recovered from PBL samples, conventional cultures of MRC-5 cells were done under standard protocol. Tests for CMV antigenemia (CMV-Ag) in PBL were also performed [1]. The susceptibility assay consisted of an early antigen reduction test. Briefly, trypsin was added to a conventional culture of MRC-5 cells with at least 30 foci of an intracellular CMV CPE. Cells were suspended in 3 ml of viral media, and seven new tubes of MRC-5 cells were infected with 300 µl of the inoculum. We also inoculated another two tubes with 4–4 dilution to check that we were working with at least 100 $TCID_{50}$. After 1 h at 37 °C and 5% CO_2 atmosphere, the inocula were removed and 1.5 ml of viral media were added to different concentrations of GCV: two tubes of 2.7 µM GCV, two more of 5.5 µM, another one of 11.1 µM, and two further tubes without GCV. After 7 days of incubation at 37 °C and 5% CO_2 (when 30 foci were present), the cells in each tube were added to trypsin, washed, and diluted in 300 µl of medium. From each tube, a shell-vial was inoculated by centrifugation at 2,000 rpm for 45 min. After 24 h at 37 °C and 5% CO_2, an immunofluorescence stain for CMV-E13 antigen (Argene-Biosoft, Varilhes, France) was added. The amount of positive cell reduction at each GCV concentration was evaluated in comparison with the control, and the inhibitor concentration at 50% (IC_{50}) was calculated. The limit of susceptibility to GCV was 11.1 µmol/l (when a GCV dosage of 5 mg/kg/12 h is administered intravenously, the peak of drug concentration reached in the plasma of the patients is 5–7 mg/l). This assay was previously compared and validated with the traditional plaque reduction assay and late antigen expression assay [10].

Results

In 65 strains from renal transplant recipients without GCV, the IC_{50} was 4.3 ± 1.7 µmol/l. The IC_{50} was significantly higher in 12 strains recovered from patients treated with GCV (6.08 ± 1.6 µmol/l; $P = 0.0016$, Student's t-test). These 12 samples were isolated 70 ± 48.2 days after therapy. From heart transplant recipients, 79 isolates were studied: 64 of them from naïve patients and 15 from patients under a GCV regimen, of which five were recovered during GCV therapy. The IC_{50} was 4.2 ± 1.7 µmol/l in isolates from naïve patients, and an IC_{50} of 3.9 ± 1.2 µmol/l was detected in four strains recovered during the antiviral regimen, three of them recovered on the 1st day and one on the 14th day from the beginning of therapy (resistant strain not included), but the IC_{50} rose to 5.8 ± 3.1 µmol/l in ten strains recovered after 104 ± 61 days of treatment (nine from the same patient).

In the patient with these nine samples, the first strain, recovered after 3 weeks of GCV treatment, was resistant to GCV: the IC_{50} was 13.7 µmol/l. In this case, GCV was administered over 4 weeks; clinical symptoms persisted and CMV-Ag was highly positive: 700 infected cells per 10^5 were detected. These data suggested that a switch of antiviral agent was necessary, and foscarnet was prescribed. After 20 days of treatment, the CMV-Ag became negative and clinical symptoms disappeared [16]. The next eight strains were isolated 108 ± 50.6 days (34–188 days) after treatment with GCV, and the average IC_{50} of these isolates was 5.4 ± 1.04 µmol/l, higher than the IC_{50} of strains isolated from naïve patients.

Discussion

The prophylactic application of GCV is the main focus of a growing number of studies. Prophylaxis has delayed the onset of CMV disease and viremia in transplant recipients [2, 4, 5, 6, 7, 8, 9, 14, 15, 17]. GCV-resistant CMV has, thus far, been considered rare in patients undergoing transplantation. Recent data, however, suggest that in the setting of suboptimal suppression and prolonged GCV use, GCV-resistant CMV may be emerging as a clinically relevant pathogen in transplant recipients. At one institution that employed prolonged prophylaxis with oral GCV, 10% of the patients who underwent solid-organ transplantation developed CMV disease within the 1st year of transplantation [14]. Of note, 20% of the patients with CMV disease had GCV-resistant CMV [11]. At another institution, 11 infections with GCV-resistant CMV in organ transplant recipients were documented; a majority of these patients had received prolonged oral GCV prophylaxis [14]. Moreover, virological and clinical troubles were related [11, 13, 14, 20].

To measure CMV susceptibility, we performed an assay developed in our laboratory that produces results from CMV isolates within 7–10 days [10]. The IC_{50} of

CMV strains isolated from patients treated with GCV was significantly higher than that obtained from patients without prophylaxis, in the case of renal transplant recipients. In heart transplant recipients, the results were similar: the IC_{50} was higher in samples isolated after patients had been treated with GCV than in samples from patients without therapy. In one case, we found a GCV-resistant CMV strain. This isolate was recovered 3 weeks after the patient had received GCV therapy. After this time, CMV-Ag remained high (700 positive cells per 10^5 leukocytes), and clinical symptoms of CMV disease persisted. These data, with susceptibility assay results, suggested that GCV be replaced with foscarnet. Then, good evolution was observed, with the CMV-Ag becoming negative and the clinical status improving 20 days after the switch [16]. The IC_{50} of samples isolated from patients under the therapy regimen was similar to that of samples from patients without treatment. This could be explained because the viruses were recovered too early after the beginning of therapy.

CMV is a latent virus that can replicate intermittently in immunosuppressed patients. A rising load or CMV-Ag level may imply suboptimal suppression and the potential for emergence of GCV resistance, mainly in patients with pre-emptive therapy or universal prophylaxis with GCV. Our data show that the IC_{50} increased in CMV isolates from patients under a GCV regimen and that prolonged use of GCV caused a resistant strain in a heart transplant recipient. If the CMV susceptibility to the drugs used in therapy (mainly GCV) were known, such knowledge could indicate a switch to another drug. Then, in spite of the low rate of CMV-resistant strains, in-vitro susceptibility assays could be helpful in clinical management, basically in protocols where pre-emptive therapy or prophylactic regimens with GCV are commonly used.

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