Emergence of Drug-Resistant Cytomegalovirus Retinitis in the Contralateral Eyes of Patients with AIDS Treated with Ganciclovir

Yumi Imai,1 Carol Shum,1 Daniel F. Martin,4 Baruch D. Kuppermann,2 W. Lawrence Drew,2 and Todd P. Margolis1
1Francis I. Proctor Foundation for Research in Ophthalmology and Department of Ophthalmology, and 2Department of Laboratory Medicine, University of California at San Francisco, San Francisco, and 3Department of Ophthalmology, University of California at Irvine School of Medicine, Irvine; 4Department of Ophthalmology, Emory University School of Medicine, Atlanta, Georgia

The purpose of the present study was to examine the emergence of ganciclovir-resistant virus in the contralateral eyes of patients who received treatment for cytomegalovirus retinitis with either a ganciclovir implant plus oral placebo, a ganciclovir implant plus oral ganciclovir, or intravenous (iv) ganciclovir. Viral DNA was amplified from vitreous specimens and was assayed for UL97 and UL54 resistance mutations. Resistant viral genotypes were found in the contralateral eyes of 0 of 28 patients treated with a ganciclovir implant plus oral placebo, in 5 of 23 patients treated with a ganciclovir implant plus oral ganciclovir, and in 1 of 6 patients treated with iv ganciclovir. All resistance mutations were in codons 591, 592, or 594 of the UL97 gene. Treatment of unilateral cytomegalovirus retinitis with systemic ganciclovir decreases the risk of development of secondary sites of infection, but, in contralateral eyes that develop retinitis, this approach to treatment is associated with a higher prevalence of drug resistance, compared with treatment with the ganciclovir implant alone (P = .023; Fisher’s exact test).

Despite recent advances in antiviral therapy, cytomegalovirus (CMV) retinitis remains a serious, vision-threatening problem for organ transplant recipients and patients with AIDS [1, 2]. The nucleoside analogue ganciclovir (9-[1,3-dihydroxy-2-propoxymethyl]-guanine) is the most commonly used antiviral agent for the treatment of CMV retinitis, with intravenous (iv), oral, and intravitreal routes of administration having been proven to be effective in the short-term management of this disease. The development of antiviral-drug resistance, however, complicates systemic long-term use of ganciclovir [3–6]. In a large prospective study of patients with AIDS who had CMV retinitis, Jabs et al. [4] reported that 11.4% of the patients who received systemic treatment with ganciclovir for 6 months, as well as 27.5% of the patients who received systemic treatment with ganciclovir for 9 months, harbored 1 drug-resistant CMV isolate in their blood or urine.

Resistance of CMV to ganciclovir is most often caused by mutations in the viral UL97 gene. This gene codes for the viral phosphotransferase that is essential for the activation of ganciclovir in CMV-infected cells [7, 8]. Point mutations at codons 460, 594, and 595 of the UL97 gene are the most common causes of resistance to ganciclovir in clinical isolates of CMV [9–11]. Resistance to ganciclovir can also be caused by mutations of the CMV UL54 gene, which codes for the viral DNA polymerase, the target of phosphorylated ganciclovir [12]. However, UL54 resistance mutations are much less common in clinical isolates of CMV than are UL97 mutations.

Studies of the development of antiviral-drug resistance in patients with AIDS and CMV retinitis have largely focused on CMV isolated from blood or urine. This has been because of the relative ease of obtaining and propagating virus from these nonocular sources.
However, patients with AIDS can be infected by multiple strains of CMV, with strains in different organ systems subjected to different evolutionary pressures [13–16]. For this reason, a few studies have focused on the development of antiviral-drug resistance in the vitreous of patients with CMV retinitis [13, 14, 17]. In a study of 204 eyes with newly diagnosed, treatment-naive CMV retinitis, Liu et al. [17] found a very low prevalence (<0.5%) of resistant viral genotypes (<0.5%) in the vitreous, a finding that established a clear baseline for the interpretation of studies of the development of intraocular antiviral-drug resistance. Liu et al. [13] also found UL97 resistance mutations in CMV DNA from 6 of 11 eyes with clinical resistance to ganciclovir, as well as different UL97 genotypes in 2 of 3 patients with bilateral retinitis. In a prospective study of 87 patients (125 eyes) with CMV retinitis that was managed with a variety of treatment protocols (median follow-up, 8.2 months), Hu et al. [18] found viral DNA with established UL97 resistance mutations in the vitreous of 5 study eyes. Most recently, Kuo et al. [14] demonstrated UL97 or UL54 resistance mutations in the viral DNA from 2 of 8 eyes with CMV retinitis that were poorly responsive to iv ganciclovir but responded well to the ganciclovir implant.

The purpose of the present study was to determine whether different drug-treatment strategies for unilateral CMV retinitis are more, or less, likely to give rise to ganciclovir-resistant viral genotypes in contralateral eyes that also develop CMV retinitis. The 3 drug-treatment strategies that were studied included a ganciclovir implant plus oral placebo, a ganciclovir implant plus oral ganciclovir, and iv ganciclovir. A multicenter clinical trial that assessed the incidence of contralateral CMV retinitis among patients receiving these 3 treatments was reported elsewhere [19].

PATIENTS, MATERIALS, AND METHODS

Patients. Vitreous specimens were obtained from all study eyes as part of a multicenter randomized clinical trial that enrolled patients with AIDS and unilateral CMV retinitis from May 1994 through July 1996 (Roche 2304). Patients in the study were randomly assigned to 1 of 3 treatment groups: ganciclovir implant plus oral placebo (122 patients), ganciclovir implant plus oral ganciclovir (123 patients), or iv ganciclovir (132 patients).

Vitreous specimens obtained from the eyes of 21 patients who had AIDS and retinal infections other than CMV retinitis (e.g., retinitis due to varicella-zoster virus, herpes simplex virus, or Toxoplasma gondii) served as negative controls. All vitreous specimens were obtained during vitreoretinal surgery scheduled for purposes other than obtaining vitreous specimens. All vitreous specimens were stored at −80°C and were incubated for 10 min at 95°C just before analysis. As described elsewhere [14], further processing of vitreous specimens, to purify CMV DNA, was not performed before polymerase chain reaction (PCR) amplification. The present study was approved by the University of California at San Francisco Committee on Human Research. All study patients provided informed consent.

UL97 genotyping. UL97 genotyping was performed as described elsewhere [14]. In brief, resistance mutations at codons 460 and 520 of the CMV UL97 gene were assayed by restriction fragment–length polymorphism (RFLP) analysis of PCR-amplified viral DNA, and resistance mutations at codons 591–607 of the UL97 gene were detected by direct DNA sequencing of PCR-amplified products. Positive results of the RFLP analysis were confirmed by DNA sequencing. Positive results of the sequence analysis were confirmed by sequencing the reverse strand of the PCR product, as well as by repeat amplification and sequencing. Amplified PCR products were purified before sequencing, by use of the QIAEX II kit (Qiagen), and double-strand DNA sequencing was performed using an automated DNA sequencer (ABI Prism 377; Applied Biosystems).

UL54 genotyping. UL54 genotyping was performed as described elsewhere [14]. In brief, resistance mutations in regions IV, C, VI, and III of the UL54 genome were detected by direct DNA sequencing of PCR-amplified products of the UL54 genome encompassing codons 379–421 (region IV), 492–539 (region C), and 765–839 (regions VI and III). RFLP analysis of additional PCR-amplified CMV DNA was used to screen for the UL54 resistance mutation at codon 987. Positive results of RFLP analysis were confirmed by DNA sequencing. Positive results of the sequence analysis were confirmed by sequencing the reverse strand of the PCR product, as well as by repeat amplification and sequencing.

RESULTS

Ganciclovir implant plus oral placebo. Of the 122 patients who received a ganciclovir implant plus oral placebo, 41 developed contralateral CMV retinitis [19]. Vitreous specimens from the eyes of 28 of the 41 patients were available for evaluation, because 28 patients chose to have the contralateral eye treated with the implant. For these 28 patients, the median treatment time was 91 days (range, 20–254 days) before vitreous biopsy of the contralateral eye was performed. All targeted regions of the CMV UL97 gene and 81% of the targeted regions of the CMV UL54 gene were successfully amplified from these eyes, for analysis. As assayed by RFLP analysis and DNA sequencing, there were no UL97 or UL54 ganciclovir resistance mutations in the amplified viral DNA from the eyes of the 28 patients (table 1).

Ganciclovir implant plus oral ganciclovir. Of the 123 patients who received a ganciclovir implant plus oral ganciclovir, 24 developed contralateral CMV retinitis [19]. Vitreous specimens from the eyes of 23 of the 24 patients were available for evaluation, because 23 patients chose to have the contralateral
eye treated with the implant. For these 23 patients, the median treatment time was 163 days (range, 16–373 days) before vitreous biopsy of the contralateral eye was performed. All targeted regions of the CMV UL97 gene and 78% of the targeted regions of the CMV UL54 gene were successfully amplified from these eyes, for analysis. As assayed by RFLP analysis and DNA sequencing, only 1 of the 6 patients successfully amplified from these eyes, for analysis. As assayed by RFLP analysis and DNA sequencing, 5 of these 23 eyes contained CMV DNA with UL97 resistance mutations (table 1). One eye had viral DNA with a G592 mutation, and 4 eyes had CMV DNA with the V594 resistance mutation. These mutations were confirmed by sequencing the reverse strand of the PCR product, as well as by repeat amplification and sequencing. There were no UL54 ganciclovir resistance mutations in the amplified viral DNA from these 23 eyes.

### IV Ganciclovir

Of the 132 patients who received iv ganciclovir, 17 developed contralateral CMV retinitis [19]. Vitreous specimens from the eyes of 6 of the 17 patients were available for evaluation; the remaining 11 patients continued to be treated solely with iv ganciclovir. For the 6 patients with specimens available for evaluation, the median treatment time was 184 days (range, 64–357 days) before vitreous biopsy of the contralateral eye was performed. All targeted regions of the CMV UL97 gene and 56% of the targeted regions of the CMV UL54 gene were successfully amplified from these eyes, for analysis. As assayed by RFLP analysis and DNA sequencing, only 1 of the 6 patients had an eye that contained CMV DNA with a resistance mutation (a V591 mutation of the UL97 gene) (table 1).

#### Negative controls

None of the targeted regions of CMV DNA were detected in 21 vitreous specimens obtained from patients with AIDS who had retinal infections due to agents other than CMV. This included specimens from eyes infected with varicella-zoster virus, herpes simplex virus, and *T. gondii*.

#### Statistical analyses

Using multiple-comparison analysis, we found a significant difference in the prevalence of UL97 mutations among the 3 treatment groups (*P* = .024; Fisher’s exact test). In addition, the prevalence of UL97 mutations in the contralateral eyes of patients treated with the ganciclovir implant and oral ganciclovir (5 [22%] of 23 patients) was significantly greater than the prevalence of resistance mutations in the contralateral eyes of patients treated with the ganciclovir implant plus oral placebo (0 of 28 patients) (*P* = .014; Fisher’s exact test). There were no other significant differences in the prevalence of resistance mutations among treatment groups. However, in looking at the effect of systemically administered ganciclovir on the development of resistance mutations, we found that the prevalence of resistance mutations in the contralateral eyes of all patients treated with either oral or iv ganciclovir (6 [21%] of 29 patients) was significantly greater than the prevalence of resistance mutations in the contralateral eyes of patients treated with the ganciclovir implant plus oral placebo (*P* = .023; Fisher’s exact test).

We next analyzed the data to ensure that the findings observed were not a consequence of differences in the treatment time for patients in these 3 treatment groups. Using the Kruskal-Wallis test for analysis, we found no significant difference in the treatment time for patients in these 3 treatment groups (*P* = .084).

#### DISCUSSION

Despite receiving aggressive systemic therapy with ganciclovir, many patients with AIDS who have unilateral CMV retinitis develop retinitis in the contralateral eye. Because systemic long-term use of ganciclovir leads to the emergence of antiviral-drug resistance in the blood and urine, it is tempting to attribute the development of contralateral retinitis in some of these patients to ganciclovir-resistant CMV. The purpose of the present study was to determine whether different drug-treatment strategies for unilateral CMV retinitis are more, or less, likely to give rise to ganciclovir-resistant virus in contralateral eyes that develop retinitis.

The results of the present study suggest that, in contralateral eyes that develop CMV retinitis, systemic treatment of unilateral retinitis with ganciclovir is associated with a higher incidence of drug-resistant virus, compared with treatment with the ganciclovir implant alone. Thus, although systemic use of

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**Table 1. Summary of cytomegalovirus (CMV) resistance mutations in the contralateral eyes of patients in each of 3 treatment groups.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients enrolled in the study</th>
<th>With contralateral CMV retinitis</th>
<th>No. of contralateral eyes with resistance mutations found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganciclovir implant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plus oral placebo</td>
<td>122</td>
<td>41</td>
<td>28</td>
</tr>
<tr>
<td>Plus oral ganciclovir</td>
<td>123</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Intravenous ganciclovir</td>
<td>132</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A594V&lt;sup&gt;d&lt;/sup&gt; and C592G&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A591V</td>
</tr>
</tbody>
</table>

<sup>a</sup> Vitreous specimens from all eyes were available for analysis.

<sup>b</sup> All are in the UL97 gene.

<sup>c</sup> In 4 eyes.

<sup>d</sup> In 1 eye.
drug in the management of unilateral retinitis is associated with a lower incidence of the development of secondary sites of CMV infection [19], it is also associated with emergence of drug-resistant virus in the contralateral eye. Furthermore, among patients who received systemic treatment with ganciclovir and developed contralateral retinitis, we were unable to demonstrate a significant difference in the incidence of resistant virus among patients randomized to receive oral therapy, compared with that among patients randomized to receive iv therapy; however, the limited size of the study groups may have made any difference difficult to detect.

In the present study, we have demonstrated the emergence of drug-resistant virus as a cause of contralateral retinitis in \( \sim 20\% \) of patients randomized to receive treatment with systemic ganciclovir (combined median treatment time, 163 days). To our knowledge, this is the first published study to determine the prevalence of resistant virus in an infected end organ of a patient receiving prolonged drug therapy. Previous studies have focused on the emergence of resistant virus in blood, urine, or semen. For example, Drew et al. [3] found an estimated frequency of resistance of 6.5% after a median ganciclovir treatment time of 165 days, and Jabs et al. [4] found resistant virus in 11.4% of cultures by 180 days of treatment. Although the higher rate of resistance that we found in the present study might reflect real differences in the development of resistance in eyes with retinitis, compared with the detection of resistant virus shed into blood, urine, or semen, it might also be explained by differences in study design—in particular, the use, in the present study, of molecular methods to directly assay for resistant viral genotypes; the approach used in the present study is a more sensitive approach than the viral cultures performed by Drew et al. [3] and Jabs et al. [4]. Additional studies of the incidence of viral resistance in end-organ tissue will be needed to verify that higher rates of resistance may be occurring in diseased end organs in which the levels of ganciclovir that are achieved are lower than those found in blood or urine.

In the present study, an important finding that deserves further discussion is that we were unable to demonstrate a significant difference in the incidence of resistant virus among patients randomized to receive a ganciclovir implant plus oral therapy, compared with that among patients randomized to receive iv ganciclovir. This may have been because of the relatively high dose of oral ganciclovir (1.5 g 3 times daily) used in the present study, a dose that was just as effective as iv therapy in preventing the incidence of new cases of CMV disease [19]. Furthermore, the data from the present study are consistent with those from a previous study [3], which suggest that patients treated with oral ganciclovir may not have an increased risk for the emergence of resistant virus in blood, urine, or semen, compared with patients treated with iv ganciclovir. An alternative explanation for our finding is that the present study may have lacked the power to detect differences in the emergence of resistance in these 2 treatment groups. Although we obtained vitreous specimens from 23 of 24 patients who developed contralateral retinitis despite having received treatment with a ganciclovir implant plus oral ganciclovir, we obtained vitreous specimens from only 6 of 17 patients who developed contralateral retinitis despite having received treatment with iv ganciclovir. For the remaining 11 patients, a clinical decision was made to treat the contralateral eye with iv therapy only. Because of this decision, vitreous specimens from these eyes were not available for study. In the present study, the results pertaining to patients treated with iv ganciclovir may have been skewed by case-selection bias.

In a well-designed prospective study, Jabs et al. [4] followed patients to assess shedding of resistant virus in the blood or urine, and they found that 11% of patients shed virus by 6 months of ganciclovir therapy and that these patients have a 9-fold greater risk of developing contralateral retinitis. Taken together, these data suggest that \( \sim 50\% \) of cases in which contralateral retinitis develops by 6 months are associated with resistant CMV in the blood or urine. However, in the present study, only \( \sim 20\% \) of cases, for which the median duration of ganciclovir exposure was 5.5 months, were the result of drug-resistant virus, a finding that is significantly less than the finding of 50% extrapolated from the study by Jabs et al. [4]. It would thus appear that finding resistant virus in the blood or urine may be a risk factor for the development of contralateral retinitis but not necessarily for the development of contralateral retinitis caused by a drug-resistant virus. This clearly deserves to be a subject of further direct study.

One limitation of the present study was that we were only able to amplify \( \sim 77\% \) of the targeted regions of the CMV UL54 gene from the vitreous samples. The reasons for this limitation include the inherent difficulty in amplifying CMV DNA from the vitreous specimens of patients undergoing treatment with systemic antivirals [20], although 100% of the targeted regions of the CMV UL97 gene were successfully amplified. This limitation is assumed to be caused by the different sensitivities of the PCR conditions designed for several targets. Despite this limitation, we believe that our chances of having missed UL54 resistance mutations in these vitreous samples was low, because resistance to ganciclovir is much less commonly caused by mutations in the UL54 gene than by mutations in the UL97 gene [10, 11] and because, among the 77% of targeted regions of the UL54 gene that we were successful in amplifying, we found no resistance mutations. A second limitation of the present study was our failure to screen for all UL97 and UL54 mutations that are known to confer resistance to ganciclovir, as established by marker transfer. Given the limited volume of the vitreous specimens that were available to us for analysis, we specifically chose not to assay for the relatively rare UL97 Q520
(CAC→CAA) and UL54 981–982 deletion resistance mutations. Given the overall size of our study population, we believe that it is unlikely that screening for these additional known resistance mutations would have altered the outcome of our study.

An important limitation of the present study was the relatively small number of vitreous specimens that were available for analysis, especially specimens from patients who received treatment with iv ganciclovir, and this needs to be taken into account when drawing conclusions from the data. Nonetheless, it is important to point out that, to our knowledge, this is the largest study, to date, of the development of drug-resistant CMV in the eyes of patients with AIDS, and it is the only study, to date, to assess differences in the development of drug resistance in the eyes of patients randomly assigned to different treatment protocols. Finally, it is highly unlikely that a larger study will be performed to address these issues in the near future, because the present study required enrollment of 377 patients to identify 68 patients who developed retinitis in the contralateral eye. Vitreous specimens from 55 (81%) of these 68 eyes were available for analysis.

In conclusion, although the use of oral or iv ganciclovir for patients with AIDS and unilateral CMV retinitis reduces the likelihood of these patients developing secondary sites of infection, including contralateral retinitis [19], systemic long-term prophylactic use of ganciclovir is associated with an increased risk that secondary sites of CMV infection will be populated with a drug-resistant genotype. In this study, ~20% of the patients who developed contralateral retinitis despite receiving systemic prophylaxis with ganciclovir had retinitis caused by drug-resistant virus. This is in sharp contrast to the absence of ganciclovir-resistant virus in the contralateral eyes of patients whose initial disease was treated with local therapy alone (i.e., a ganciclovir implant plus oral placebo). These results were not unexpected, but, to our knowledge, the present study is the first placebo-controlled clinical trial to demonstrate that prolonged systemic treatment with ganciclovir is associated with an increased risk for the development of new foci of CMV disease due to drug-resistant virus, not just for drug-resistant virus shed in the blood, urine, or semen. Future studies are needed to determine whether the drug-resistant virus that emerges in these secondary foci of infection is more difficult to control clinically, even with the use of the ganciclovir implant, than is infection with wild-type CMV.

References


