Intravitreal Ganciclovir Concentration after Intravenous Administration in AIDS Patients with Cytomegalovirus Retinitis: Implications for Therapy

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To determine whether therapeutic intravitreal concentrations of ganciclovir are achieved after intravenous administration, vitreous samples were obtained intraoperatively from 23 eyes of 22 AIDS patients with retinal detachments associated with cytomegalovirus (CMV) retinitis. The mean intravitreal ganciclovir concentration of all samples was 0.93 ± 0.39 μg/mL (3.6 ± 1.5 μM). This level is near the published trough serum concentrations obtained with every-12-h intravenous dosing and well below the peak. It is significantly below the concentration of ganciclovir required to achieve 50% of viral plaque formation for many human CMV strains. Only a small decrease in vitreous drug levels was observed as a function of time after last dose. Intravenous dosing and well below the peak. It is significantly below the concentration of ganciclovir required to achieve 50% of viral plaque formation for many human CMV strains. Only a small decrease in vitreous drug levels was observed as a function of time after last dose. Intravenous administration of ganciclovir results in near-steady-state subtherapeutic intravitreal ganciclovir concentrations for many CMV isolates. This may explain the difficulty of long-term complete suppression of CMV retinitis.

The long-term control of cytomegalovirus (CMV) retinitis in patients with AIDS is difficult by using either of the two available anti-CMV drugs, ganciclovir and foscarinet. In the current setting of antiretroviral therapy as well as anti-CMV therapy, median survival of CMV retinitis patients has recently been shown to be 8.5 months for those receiving ganciclovir and 12.6 months for those receiving foscarinet [1]. Longer survival has been associated with more difficulty in continuous suppression of the retinitis over this extended period. Ganciclovir and foscarinet have both been shown to be effective in the treatment of newly diagnosed CMV retinitis [1–4]. However, the rate of reactivation of CMV retinitis during subsequent maintenance intravenous therapy has been reported in 9%–50% of patients, requiring re-induction therapy within several months of diagnosis [1, 4, 5]. Not only does reactivation occur commonly, in some eyes progressive smoldering can be very difficult to control [6]. The fact that the efficacy of anti-CMV agents is more commonly mea-
sured by their ability to prolong the interval to reactivation further emphasizes the marginal clinical efficacy of current intravenous regimens of ganciclovir and foscarnet.

The cause of clinical reactivation in patients with healed CMV retinitis has not been clearly delineated. Since both drugs are virostatic, if therapy is discontinued, essentially all patients will reactivate within 4 weeks [2, 4, 5]. Reactivation during maintenance therapy can occur due to development of viral resistance [7]. One series documented in vitro resistance early in the treatment course in 40% of the CMV strains that could be isolated from patients on continuous standard maintenance ganciclovir therapy for 3 months [7]. Other causes of reactivation include the possibility that standard intravenous therapy provides drug levels inadequate for complete suppression of viral replication in the eye. The current study evaluates intravitreal ganciclovir levels after intravenous administration in patients with variable amounts of CMV retinitis and varying degrees of retinal detachments to determine the pharmacokinetics and drug levels of ganciclovir in the vitreous, which bathes the retina.

Materials and Methods

Patients. Of 22 patients with CMV retinitis and retinal detachments requiring surgical repair, 23 eyes were included in the present study. The median age of the patients was 41 years (range, 31–62). Four were women. Fourteen were white, 2 were black, 5 were Hispanic, and 1 was Asian. History of ganciclovir administration over the 2 weeks and 3 months before surgery, extent of retinitis at the time of surgery, and duration and extent of retinal detachment were recorded in each case. Seventeen patients were on maintenance ganciclovir for the 2 weeks before surgery, and 5 were on induction therapy during that period. The mean daily ganciclovir dose for the 3 months before surgery was 6.1 ± 2.1 mg/kg. The mean extent of retinitis at time of surgery, as measured by the percentage of retina, using indirect ophthalmoscopy was 44% ± 21%. The mean extent of retinal detachment at time of surgery, as measured by the percentage of retina, was 53% ± 23%. The mean duration of retinal detachment before surgery was 3.1 ± 1.4 weeks.

Surgical technique. During pars plana vitrectomy surgery, a core, undiluted vitreous sample was obtained by manual aspiration into a syringe attached to the aspiration line with no infusion of irrigating fluid until at least 1 mL of undiluted vitreous fluid had been removed. The vitrectomy probe was then removed from the eye, and the residual vitreous in the tubing was manually aspirated into the collection syringe, aliquoted, and stored at −70°C. The surgical technique for retinal reattachment was done as described previously [8].

High-pressure liquid chromatography analysis. A 250-μL sample of vitreous fluid and 250 μL of 0.8 M perchloric acid were mixed and centrifuged in a microcentrifuge vial. A 100-μL aliquot of each prepared sample was injected into a high-pressure liquid chromatography system (334; Beckman Instruments, Fullerton, CA) equipped with a 4.6-mm ODS Ultrasphere column (Beckman Instruments), a variable-wavelength UV detector set at 254 nm (Kratos Spectroflow, Foster City, CA), and a peak integrator (GR1A; Shimadzu, Columbia, MD). The elution buffer was 20 mM ammonium acetate and 1.2% acetic acid at a flow rate of 1.5 mL/min. The ganciclovir peak eluted at 9 min and was not interfered with by any coeluting compounds. Ganciclovir levels were quantified by comparison of sample peak areas to the peak area of normal human vitreous spiked with specific amounts of added ganciclovir.

Statistical and pharmacokinetic analyses. The relationship between time after dosing and intravitreal concentration was evaluated by linear and nonlinear regression, including determination of Pearson correlation coefficients. Further analysis was done using dose-adjusted concentrations (measured concentration/patient dose/5 mg/kg/day). Subgroup analysis was also done on patients receiving maintenance therapy only. When data between two groups was assessed, Student’s t test was used. All data were analyzed using Statistical Analysis Software version 6.03 (SAS Institute, Cary, NC).

Three patients did not provide information regarding the specific time of their most recent ganciclovir infusion, but their dosing regimen was known. The intravitreal samples obtained from these 3 patients were analyzed by high-pressure liquid chromatography for intravitreal ganciclovir concentration and by statistical analysis that did not require the time of the last dose (comparison of intravitreal ganciclovir level with the extent of retinitis and retinal detachment).

Results

Intravitreal samples were surgically obtained from 23 eyes of 22 patients with retinal detachments associated with CMV retinitis during intravenous ganciclovir therapy. The samples were obtained from each eye 2–24 h after administration of intravenous ganciclovir (mean, 12.0 ± 7.2). Three patients did not provide an estimate of the time of the last ganciclovir infusion before surgery. The mean intravitreal ganciclovir concentration for all samples was 0.96 ± 0.39 μg/mL; for the 20 samples with a recorded interval between ganciclovir administration and intravitreal sample removal, it was 1.03 ± 0.37 μg/mL (figure 1). No significant correlations were observed between time after last dose and intravitreal concentration (r = −.35, P = .13), log intravitreal concentration (r = −.37, P = .11), dose-adjusted intravitreal concentration (r = −.04, P = .85), or log intravitreal dose-adjusted concentration (r = .02, P = .92). Adjusting concentrations for dose did not improve the correlation with time after dose nor did evaluating only patients receiving maintenance levels of 5 mg/kg/day. By using nonlinear regression analysis, a best-fit curve showed only a slight and nonsignificant trend for decreasing ganciclovir concentration with time after last dosing, indicating that near-steady-state levels had been achieved (figure 1). Analysis of intravitreal concentration versus time by grouping patients into 6-h groups similarly showed a slight downward trend over time. Regression analysis indicated nonsignificant correlations (r = −.93, P = .06–.07), again indicating that at the steady-state condition in the
vitreous, little fluctuation occurred in drug concentration. This analysis suggests that the slight downward trend approaches significance, but there is only about a twofold decline in concentration over the 24-h period after an intravenous dose.

The mean concentration in patients receiving an induction level of ganciclovir of 5 mg/kg/12 h (1.15 ± 0.32 μg/mL, n = 5) was higher than that in patients receiving a maintenance level of ganciclovir of 5 mg/kg/day (1.00 ± 0.39 μg/mL, n = 17). As expected, there was a difference between these two groups in the interval between last ganciclovir administration and time of intravitreal sample removal: 5.0 ± 2.2 versus 13.7 ± 7.0 h. Analysis of ganciclovir levels between the induction and maintenance groups showed no statistically significant difference; however, the number of patients on induction therapy was small, and a concentration difference of >60% between the two groups would have been necessary to show significance (α = 0.05, β = 0.2).

Statistical analysis was also done to determine whether the measured intravitreal ganciclovir levels were dependent on the extent of retinitis. The mean extent of retinitis was 44%; the mean ganciclovir concentration was 0.93 ± 0.45 μg/mL. In the low-retinitis group (mean, 25% ± 8%), the mean ganciclovir concentration was 0.98 ± 0.33 μg/mL. There was no correlation between intravitreal ganciclovir level and extent of retinitis. The data could have detected a difference of >50% in drug levels between the high and low extents of retinitis groups (α = 0.05, β = 0.2).

A similar analysis compared the extent of retinal detachment with intravitreal ganciclovir levels. The mean extent of retinal detachment was 53%. Thirteen eyes had >53% detachment, with a mean ganciclovir level of 0.99 μg/mL. For the 10 eyes with <53% of retina detached, the mean ganciclovir level was 0.91 μg/mL. No correlation was observed between the extent of retinal detachment and intravitreal concentration. As with the prior analysis, the data could have detected a difference of >50% in drug levels between groups with high and low extents of retinal detachment (α = 0.05, β = 0.2).

As a means of determining whether a slow equilibrium occurs between serum and vitreous ganciclovir with chronic administration, cumulative dosing for the 2-week and 3-month intervals before sample collection were analyzed. The mean daily intravenous ganciclovir dose for the 3 months before surgery was 4.7 ± 1.8 mg/kg/day and for the 2 weeks before surgery was 6.2 ± 1.7 mg/kg/day. No correlation was observed between intravitreal concentration and either 2-week (r = 0.18, P = .52) or 3-month (r = 0.16, P = .61) dosing history, although the variability in the dosing regimen was small. Similarly, no correlation was observed between log intravitreal concentration and either 2-week (r = 0.20, P = .48) or 3-month (r = 0.10, P = .77) dosing history.

Discussion

The treatment of CMV retinitis with systemic therapy is dependent on the ability of the intravenously delivered antiviral agent to penetrate the blood-retinal barrier and provide adequate therapeutic drug levels to tissue. Although an initial clinical response with resolution of retinitis is seen in 80–100% of patients receiving either ganciclovir or foscarnet, over an extended period, many patients will show clinical reactivation while on therapy [2–6]. Peak serum ganciclovir concentrations in patients with CMV retinitis receiving induction-level dosing averaged 11.5 μg/mL (range, 4.8–24.1), well above the concentration of ganciclovir necessary to achieve 50% or 90% inhibition of viral plaque formation of most clinical isolates of CMV (0.1–2.75 and 0.15–4.0 μg/mL, respectively) [9–12]. Intraocular ganciclovir levels after intravenous administration have been previously reported in 2 patients [3, 10]. However, no study has systematically evaluated intravitreal levels of ganciclovir after intravenous administration to determine whether therapeutic levels are achieved. Our data suggest that the mean intravitreal ganciclovir concentration in AIDS patients with CMV retinitis-related retinal detachment on intravenous ganciclovir therapy is 0.96 ± 0.39 μg/mL. This value is below the concentration of ganciclovir needed to achieve 50% or 90% inhibition of viral plaque formation of many initial clinical CMV isolates and is below the published trough serum concentrations obtained with every-12-h dosing (1.36 μg/mL; range, 0.11–3.50) [9–12]. In one study, 21 of 54...
initial clinical isolates required $> 1.0 \mu g/mL$ ganciclovir to achieve 50% and 2 $\mu g/mL$ (mean) to achieve 90% inhibition of viral plaque formation [9]. Significantly, the ganciclovir concentration required to achieve inhibition of viral plaque formation of clinical CMV isolates increases over prolonged periods of exposure to ganciclovir [7, 11, 12]. Thus it appears that the intravitreal levels of ganciclovir we observed are less than those required to inhibit viral replication of many initial isolates and most subsequent isolates.

The variation in intravitreal ganciclovir concentration by only a factor of two in the 24 h after intravenous administration demonstrates that the intravitreal concentration is relatively stable over time and is near steady state. This suggests that the retina is bathed for extensive periods in the relatively low levels of ganciclovir measured. Retinal tissue levels of ganciclovir are likely to be in equilibrium with those of the much larger volume of the vitreous, which in the setting of chronic intravenous drug infusion has reached a near-steady-state level of ganciclovir. The actual correlation between intravitreal and intraretinal drug levels is difficult to demonstrate because of the difficulties inherent in measuring retinal drug levels. Without the use of radiolabeled drug, we have been unable to accurately measure tissue concentrations in the retina. However, studies evaluating the ocular penetration of systemically administered drugs typically use vitreous drug levels as a measure of therapeutic efficacy [13, 14]. Because of the unique characteristics of the local ocular environment, particularly under the steady-state conditions of chronic drug infusion, the vitreous may act like a reservoir to stabilize tissue concentration of ganciclovir at low levels.

The volume of vitreous humor in the human eye is 4.5 mL. The thickness of the retina of the human eye averages 150 $\mu m$, and it covers the inner two-thirds of the eyeball [15]. Taking an average axial length of the human eye internally as 22 mm, the volume of the human retina is 0.15 mL, thus the ratio of vitreous to retinal volume is 30:1. Measurements of the rabbit eye in our laboratory indicate a fresh vitreous weight of 1.4 g and a fresh retinal wet weight of 45 mg (unpublished data). Thus, the ratio by weight (mass) of vitreous to retina is 31. The retina is a thin membrane that is bathed in the much larger vitreous. In this regard, the vitreous can be considered analogous to a culture medium containing antiviral drugs used in plaque reduction assays. We hypothesize that the relatively low vitreous levels reported here may well be an explanation of the steady-state borderline therapeutic efficacy in humans.

The results of this study suggest that current drugs and dosing for CMV retinitis result in borderline or progressively subtherapeutic levels during the course of chronic therapy. We hypothesize that this may be an explanation for the reactivation during therapy that is so frequently seen. Future drug development for CMV retinitis should take into consideration the vitreous levels of drug, particularly if they reach a steady state in the presence of large fluctuations in serum levels.

References