Intravitreal Pharmacokinetics and Retinal Concentrations of Ganciclovir and Foscarnet after Intravitreal Administration in Rabbits

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PURPOSE. To perform a detailed pharmacokinetic study and to evaluate the drug levels reached in the retina after the intravitreal administration of ganciclovir and foscarnet to rabbits.

METHODS. Retinal and vitreal levels of both drugs were measured by high-performance liquid chromatography at 1, 6, 12, 24, 36, 48, 60, and 72 hours after a single intravitreal injection of 196 µg and 800 µg of ganciclovir and 900 µg of foscarnet to three groups of 24 pigmented rabbits. A noncompartmental pharmacokinetic analysis was used.

RESULTS. Both drugs incorporated rapidly into the retina, but no equilibrium was observed between the drug levels in the vitreous humor and retina. Mean ganciclovir levels in vitreous and retina were 170.6 µg/g and 131.3 µg/g (dose of 196 µg), 755.7 µg/g and 381.6 µg/g (dose of 800 µg) at 1 hour after administration, decreasing to 0.1 µg/g, 0.6 µg/g, 0.8 µg/g, and 0.7 µg/g, respectively, by 72 hours. Mean foscarnet levels in vitreous and retina were 944 µg/g and 217.1 µg/g at 1 hour after administration, decreasing to 74 µg/g and 17.1 µg/g, respectively, by 72 hours. Whereas both doses of ganciclovir yielded retinal levels above the mean inhibitory concentration (IC50) of most human cytomegalovirus (CMV) isolates for more than 60 hours, foscarnet retinal levels were lower than the CMV IC50 before 36 hours had elapsed after administration.

CONCLUSIONS. The results suggest that the intravitreal administration of ganciclovir has a better pharmacokinetic profile than foscarnet for the treatment of retinitis caused by CMV and other herpes viruses and support the administration of intravitreal ganciclovir twice a week as a treatment for CMV retinitis. (Invest Ophthalmol Vis Sci. 2001;42:1024–1028)

The prognosis of cytomegalovirus (CMV) retinitis in patients with acquired immune deficiency syndrome (AIDS) has substantially changed in those patients who attain sufficient immunologic recovery after initiating a highly active antiretroviral treatment.1,2 However, in many patients the effectiveness of the antiretroviral treatments is either limited or transitory, and therapeutic difficulties with CMV retinitis may remain the same as in previous years in these patients.3 Intravitreal administration of drugs with activity against CMV has been frequently considered as a palliative alternative in those patients who cannot tolerate or refuse a systemic treatment. Among the available drugs, ganciclovir has probably been the most widely used. Even though comparative studies have not been performed, the efficacy of intravitreal ganciclovir could be comparable to the intravenous therapy4,5 and even more efficacious at very high doses.6 Satisfactory results have also been reported with the intravitreal administration of foscarnet.7,8 Furthermore, there are data supporting the use of intravitreal treatment for acute retinal necrosis and other forms of retinitis caused by varicella zoster virus and herpes simplex virus that may lead rapidly to blindness in the absence of an aggressive antiviral treatment.9–11 However, data regarding intraocular levels of ganciclovir and foscarnet after intravitreal administration are very scanty and are related only to vitreous humor. Dosages have been suggested on the basis of the estimated half-lives (1/2) of these drugs in vitreous humor, assuming an equilibrium between vitreous humor and the target tissue, the retina.6,7,12–14 However, the actual correlation between vitreal and retinal levels of these drugs has neither been demonstrated in humans nor in experimental models. Because the rabbit eye seems to be a good model for studying intraocular pharmacokinetics of drugs,15 we used it to perform a detailed pharmacokinetic study and to evaluate the drug levels reached in the retina after the intravitreal administration of ganciclovir and foscarnet to rabbits.

MATERIALS AND METHODS

Study Design, Drug Administration, and Sampling

Three groups of twenty-four healthy, pigmented rabbits weighting between 2.6 and 3.4 kg (mean weight: 3.03 kg) were used. The study was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Spanish Agriculture Department guidelines. After animals were anesthetized with intramuscular xylazine (12 mg/kg of body weight), each rabbit was given a single intravitreal injection of 196 or 800 µg of ganciclovir (Roche, Madrid, Spain) or 960 µg of foscarnet (Astra Pharmaceutical, Södertälje, Sweden), respectively, in a volume of 0.04 ml as previously described.1,16 These doses were chosen to simulate the usual doses in humans of 400 and 2000 µg of ganciclovir and 2400 µg of foscarnet, taking into account the differences in vitreous humor volumes between humans (~4.5 ml) and rabbits (~1.2 ml). After they were killed with intravenous pentobarbital (50 mg/kg), eyes in groups of three rabbits were enucleated at 1, 6, 12, 24, 36, 48, 60, and 72 hours after administration. After the eyes were sectioned just behind the lens, vitreous humor was obtained by dissecting it carefully from the retina (mean volume collected: 750 ± 195 µl). Afterward, the retina itself was bluntly separated from the choroid, taking care that no vitreous humor remained adherent to the retina (mean weight collected: 45 ± 10 mg). Samples were stored at −80°C until testing. Before analysis, vitreous humor and retinal tissues were sonicated at 0.5 Hz for 40 seconds (50-watt sonicator, Sonics & Materials, Danbury, CT). For
processing, each sample was transferred to a micropartition tube (Centricon 30; Amicon, Beverly, MA) and centrifuged at 1500g for 20 minutes.

**Assay Procedure**

Concentrations of ganciclovir were determined using a high-performance liquid chromatograph with an ultraviolet detector (Gilson Medical Electronics, Middleton, WI) and an analytical column (250 × 4 mm, inside diameter [ID]) with 5-μm particles (Lichospher 100 RP-18; Merck, Darmstadt, Germany). The volume injected was 20 μl, and the flow rate was 1.5 ml/min. The mobile phase consisted of a mixture of ammonium acetate (20 mM) with the addition of acetic acid 1.2% vol/vol (Merck). Quantification was based on measuring standard solutions of ganciclovir in 0.9% wt/vol NaCl solution. The detection limit was 0.1 ± 0.05 μg/ml. The intra- and interassay coefficients of variation were 4% and 5%, respectively. Mean recovery of ganciclovir from vitreous humor and retina spiked with known concentrations of ganciclovir were 95.8% ± 2.6% and 77.2% ± 6.5%, respectively.

Concentrations of foscarnet were determined according to the modified method of Petersson and Nordgren, using the same high-performance liquid chromatograph with an electrochemical detector with an analytical column (125 × 4 mm ID) with 5-μm particles (Lichospher 100 RP-18; Merck). The volume injected was 20 μl, and the flow rate was 0.7 ml/min. Quantification was based on measuring standard solutions of foscarnet in 0.9% wt/vol NaCl solution and hydrochlorothiazide as the internal standard. The detection limit was 20 μg/ml. The intra- and interassay coefficients of variation were 1% and 1.7%, respectively. Recovery of foscarnet in vitreous humor and retina, after adding known concentrations, was 82.7% and 79.8%, respectively.

**Pharmacokinetic Analysis**

The pharmacokinetic parameters were calculated using a noncompartmental method from the mean concentration of six measurements per time point. Results were expressed per gram of tissue. The terminal-phase elimination rate constant (λz) was determined by linear regression of the natural logarithms of the concentrations against time for the log-linear elimination phase as follows: λz = ln C0 − ln Cn/Δt. The terminal half-life (t1/2z) was calculated from the equation: t1/2z = 0.693/λz. The area under the concentration-time curve from time 0 to the time t (AUC(t)) was calculated by the trapezoidal rule and extrapolated to infinity according to the following equation: AUC(t) = AUC(∞) + (C/λz), in which C is the last drug concentration available, taken at time t (72 hours). The area under the first moment concentration-time curve (AUMC) was calculated as

\[
\sum_{t_{n-1}}^{t_n} \left( C_{n-1} \cdot t_{n-1} + C_n \cdot t_n \right) / 2 - C_0 \cdot (t_n - t_{n-1}) + C_0 \cdot t_x / \lambda_z + C_t / \lambda_z^2
\]

The mean residence time (MRT) was calculated as follows: MRT = AUMC/AUC(t). The total clearance (Cl) of the drug in vitreous was calculated as follows: Cl = dose/AUC(t). The apparent volume of distribution (Vd) was calculated by the following equation: Vd = Cl/λz. Drug flow in vitreous (J) was calculated as J = Cl · Cm/A, in which Cm is the mean drug concentration in vitreous from 0 to 72 hours and A is area of vitreous, considering the eye as a complete sphere. The coefficient of diffusion for drugs in vitreous (D) was calculated as follows: D = J/(πx2), in which PC is the drug octanol/water partition coefficient determined experimentally (0.01 for ganciclovir and 0.436 for foscarnet). The mean absorption time (MAT, i.e., the mean residence time of a molecule in absorption zone from vitreous to retina) was determined as follows: MAT = MRT_vitreous − MRT_ren眼角膜. The penetration ratio was defined as the ratio of the AUC(0-72) retina to the AUC(0-72) for vitreous humor.

**RESULTS**

**Intravitreal Ganciclovir**

Figure 1 shows the mean vitreal and retinal concentration–time curves of ganciclovir after the intravitreal administration of 196 and 800 μg to the rabbits. Table 1 summarizes the values of the pharmacokinetic parameters for the two different doses of ganciclovir. Similar Cl, D, Kp, and MAT were observed for both dosages of ganciclovir. At 1 hour after administration, the retinal concentrations of ganciclovir were 151.3 and 381.6 μg/g, respective to the 196- and 800-μg doses. Thereafter, the drug levels decreased to approximately 0.7 μg/g by 72 hours, with an estimated terminal vitreal t1/2 of 7.14 and 8.66 hours.
respectively. Beyond 24 hours after administration, the levels of ganciclovir in the retina exceeded the levels in the vitreous humor. No equilibrium between the drug levels in retina and those in vitreous humor was observed throughout. By 72 hours after administration, penetration ratios from vitreous to retina, estimated as the ratios of AUCs were 89.8% and 53.6%, respectively.

Intravitreal Foscarnet

Figure 2 shows the mean vitreal and retinal concentration–time curves of foscarnet after the intravitreal administration of 960 mg to the rabbits. Table 2 summarizes the values of the pharmacokinetic parameters. The high distribution volume and the low λz estimated agree well with the high vitreal MRT; a lower MRT was observed in retina. At 1 hour after administration, the mean retinal concentration of foscarnet was 217 ± 143 µg/g. Thereafter, the levels in the retina decreased to 17.1 ± 9.5 µg/g by 72 hours. By 72 hours after administration, penetration ratio from vitreous humor to retina was 44.9%.

**DISCUSSION**

Difficulties inherent in obtaining retinal and vitreous humor samples justify that dosages and intervals of intravitreal administration of drugs with anti-CMV activity be based on acceptable results of effectiveness and toxicity and data of vitreal concentrations rather than on the knowledge of the drugs levels reached in retina.6,7,12–14 However, our results seem to contradict such a hypothesis. In this model, both drugs incorporated rapidly into the retina after intravitreal administration. Within the first 24 hours after administration, ganciclovir retinal levels are lower than those found in vitreous

**Table 1. Intraocular Pharmacokinetic Parameters of Ganciclovir after Intravitreal Administration**

<table>
<thead>
<tr>
<th></th>
<th>196 µg</th>
<th>Retina</th>
<th>800 µg</th>
<th>Retina</th>
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<tr>
<td>Vd</td>
<td>1.02 ml</td>
<td>NC</td>
<td>1.27 ml</td>
<td>NC</td>
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<tr>
<td>λz</td>
<td>0.097 h⁻¹</td>
<td>0.059 h⁻¹</td>
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<td>MRT</td>
<td>9.24 h</td>
<td>11.21 h</td>
<td>7.78 h</td>
<td>9.86 h</td>
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<tr>
<td>Terminal t₁/₂</td>
<td>7.14 h</td>
<td>11.7 h</td>
<td>8.66 h</td>
<td>8.66 h</td>
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<tr>
<td>CI</td>
<td>0.099 ml/h</td>
<td>NC</td>
<td>0.102 ml/h</td>
<td>NC</td>
</tr>
<tr>
<td>AUC₀₋₇2</td>
<td>1947.8 µg · h/g</td>
<td>1750.8 µg · h/g</td>
<td>7852.3 µg · h/g</td>
<td>4214.2 µg · h/g</td>
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<tr>
<td>AUC₁₋₇2</td>
<td>1948.9 µg · h/g</td>
<td>1760.9 µg · h/g</td>
<td>7761.4 µg · h/g</td>
<td>4223 µg · h/g</td>
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<tr>
<td>λm</td>
<td>24.27 µg/ml</td>
<td>—</td>
<td>111.66 µg/ml</td>
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<tr>
<td>J</td>
<td>0.61 µg/cm² · h</td>
<td>—</td>
<td>2.89 µg/cm² · h</td>
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<tr>
<td>D</td>
<td>1.88 × 10⁻³ cm²/h</td>
<td>—</td>
<td>2.11 × 10⁻³ cm²/h</td>
<td>—</td>
</tr>
<tr>
<td>Kp</td>
<td>3.35 × 10⁻⁵ cm/h</td>
<td>—</td>
<td>3.76 × 10⁻⁵ cm/h</td>
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<tr>
<td>AUMC</td>
<td>18.24 mg · h²/ml</td>
<td>19.75 mg · h²/g</td>
<td>61.13 mg · h²/ml</td>
<td>41.63 mg · h²/g</td>
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<tr>
<td>MAT</td>
<td>2 h</td>
<td>Penetration ratio retina/vitreous = 89.8%</td>
<td>2.08 h</td>
<td>Penetration ratio retina/vitreous = 53.6%</td>
</tr>
</tbody>
</table>

NC, not calculable (dose unknown for retina).
humor, as would be expected from the passive diffusion that takes place from an avascular milieu with a hydrogel structure. After 24 hours after administration, ganciclovir retinal concentrations remained higher than those for vitreous humor, probably in part because of its longer intracellular MRT compared with the retinal MRT. Similar vitreal AUC, Cmax, and D were observed for ganciclovir, regardless of the administered dose, suggesting that the passage from vitreous to retina is not affected by the dosage. With both doses of ganciclovir, similar retinal levels were found at 72 hours after administration, suggesting that a larger elimination through pathways other than the retina occurs with the highest dose.

By contrast, foscarnet retinal concentrations remained lower than those for vitreous humor during all the experimental time. Both the very low vitreal AUC, Cmax, and D and MAT were observed for ganciclovir, regardless of the administered dose, suggesting that the passage from vitreous to retina is not affected by the dosage. With both doses of ganciclovir, similar retinal levels were found at 72 hours after administration, suggesting that a larger elimination through pathways other than the retina occurs with the highest dose.

From a therapeutic viewpoint, there are also differences between both drugs worthy of mention. Retinal ganciclovir levels remain above the IC50 for human CMV (0.58–1 μg/ml; range: <0.1–2.75) and other susceptible herpes viruses for more than 60 hours and more than 48 hours for those CMV strains turned resistant to ganciclovir after oral or intravenous administration.23–26

On the contrary, retinal foscarnet levels after 36 hours after administration were lower than the IC50 for most human CMV isolates. This could be an important factor implicated in the progression of CMV retinitis during the intravitreal maintenance treatment when only one weekly injection is administered.

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References


