Intravenous Infusion of RMP-7 Increases Ocular Uptake of Ganciclovir

Peter J. Elliot,[1] Raymond T. Bartus,[1] Jasmina B. Mackic,[2] and Berislav V. Zlokovic[2,3]

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Purpose. The ability of intravenous (i.v.) infusions of the bradykinin agonist, RMP-7, to permeabilize the blood-ocular barriers (BOB) to the antiviral agent ganciclovir was investigated in guinea-pigs.

Methods. Different i.v. dosing regimens included pre-treatment with RMP-7 (0.2 μg/kg/min for 5 min) followed by either [^{3}H]-ganciclovir (1 μCi/0.2 ml/min) alone, and/or co-infusion with RMP-7 and [^{3}H]-ganciclovir. At specific times the animals were sacrificed, their eyes removed, and the retina and lens epithelium dissected and analyzed for the amount of radioactivity.

Results. Using the ratio of tissue vs. integrated plasma radioactivity concentration, a two-fold increase in ganciclovir steady-state levels were observed in the retina as well as lens epithelium following RMP-7 pretreatment. Peak uptake effects were achieved with a 4.5 min ganciclovir infusion. Neither longer infusions of ganciclovir alone, nor co-infusions of RMP-7 and ganciclovir further enhanced the uptake effects. Kinetic analysis indicated that RMP-7 increased the rate of ganciclovir entry (K_{IN}) in studied ocular tissues, while the efflux of drug (K_{OUT}) was not affected by this treatment. Finally, ganciclovir retina:plasma ratios elevated by RMP-7 pre-treatment, remained higher than control ratios within 60 min following cessation of 4.5 min ganciclovir infusion.

Conclusions. These data offer further evidence that BOB and in particular the blood-retinal barrier can be permeabilized via bradykinin receptor stimulation. As the i.v. infusions of RMP-7 enhanced the retinal uptake of ganciclovir, it is suggested that a combination of RMP-7 and ganciclovir may provide a novel approach for treating cytomegalovirus retinis.

KEY WORDS: blood-ocular barriers; bradykinin; drug delivery; retina; guinea pig.

INTRODUCTION

The anterior and posterior compartments of the eye are not readily accessible to blood-borne agents due to the blood-ocular barriers. These barriers limit the diffusion of certain therapeutic agents from the systemic circulation into the eye, thus attenuating the potential beneficial effects. Attempts to breach these barriers and achieve therapeutic drug levels within the eye usually rely on escalating drug dosing regimens. Since the agents presently used to treat ocular infections are inherently toxic, administration of high drug doses is limited by unwanted, and sometimes life threatening side-effects (1–5). In an attempt to attain higher ocular drug levels while reducing toxic liability, long dosing protocols are often employed. However, such procedures are far from effective, require costly hospitalization and are often incompatible with the life style of the patient (2,6).

Cytomegalovirus (CMV) retinitis is one of the most common ocular infections reported in patients with a suppressed immune system, in particular those with acquired immune deficiency syndrome or those given organ transplants (1). This viral condition is currently treated with daily 1 or 2 hour intravenous infusions of either foscarnet or ganciclovir (2,6). However, even with such aggressive drug infusion protocols, CMV retinitis is rarely arrested, let alone cured, and blindness eventually occurs. In addition, both drugs used to combat CMV retinitis are limited by their dose-related side-effects which include myelosupression, renal toxicity and neutropenia (3–5). Thus, over the past decade the efforts have been focused on alternative strategies to breach the BOB and to achieve therapeutic ganciclovir levels in the retina by local intravitreal injections, liposomes and/or intraocular implants designed to maintain sustained release of ganciclovir (7). The effectiveness of the local therapy in combating the disease has been suggested by recent clinical trials (8–10), and the implants have been recently approved for use in the eye.

RMP-7 is a peptidergic bradykinin B_2 receptor agonist with three amino acid substitutions and a reduced peptide bond between residues 8 and 9 (ArgProIIypGlyThiSerProTyr(Me) CH_2NHArg) (11–13). RMP-7 has an extended plasma half life and greater receptor B_2 selectivity than bradykinin (12,13). It has been suggested that similar to bradykinin in the venules that causes a separation of junctions between endothelial cells followed by edema which accounts for the basic mechanism by which inflammation is precipitated, the RMP-7 may also cause opening of inter-endothelial junctions (13). Recently, intracarotid infusions of RMP-7 have been shown to permeabilize the blood-brain tumor barrier (BBTB) and blood-ocular barriers (BOB) in rats (13,14) and guinea pigs (15). The current paper extends these findings by employing intravenously administered RMP-7 and offers further insight into the possible mechanisms and kinetics of enhanced ganciclovir uptake across BOB and in particular the blood-retinal barrier (BRB). As such we examined uptake of ganciclovir into the retina and lens epithelium as tissues representing the posterior and anterior compartments of the eye.

METHODS

Animals

Male and female Hartley guinea pigs (220–350 g; Charles River, Ballardville, MA) were used in this study. Animals were housed in a vivarium on a 12 h dark: 12 h light cycle maintained at 21 ± 2°C and 70 ± 5% relative humidity. Food and water were present *ad libitum* throughout the study. All procedures were conducted in a manner consistent with ARVO (Association for Research in Vision and Ophthalmology) regulations for the use of animals in research.

Surgical Procedure

Briefly, under xylazine (6 mg/kg; i.m.) and ketamine (30 mg/kg; i.m.) anesthesia, a PE10 cannula was implanted into

[1] Alkermes Inc., Cambridge, Massachusetts 02139.
[2] Department of Neurological Surgery, University of Southern California, School of Medicine, 2025 Zonal Avenue, Raulston Memorial Building 506, Los Angeles, California 90033.
[3] To whom correspondence should be addressed.

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the right jugular vein. The free end of the tubing was connected to a slow drive infusion pump (Harvard Apparatus) to deliver RMP-7 and ganciclovir, or vehicle and ganciclovir, by one of dosing protocols as described below. The right carotid artery was cannulated (PE20 tubing) for sequential arterial sampling to obtain the integrated plasma profile for radiolabeled ganciclovir. Arterial sampling was performed at a rate of 0.2 ml/min to correct for the blood volume increase. The arterial blood pressure was continuously recorded using a Statham P321D transducer. Heart rate and respiration were also monitored.

Intravenous Dosing Protocol I—Pretreatment with RMP-7

RMP-7 was infused for the first 5 min of the dosing protocol, immediately followed by an infusion of ganciclovir, the duration of which varied from 0.25 to 10 min (depending upon the group), after which time the animal was sacrificed. The dose of RMP-7 was 0.2 μg/kg/min, infused at a rate of 0.2 ml/min over 5 min. The infusion time of 5 min for RMP-7 in the present i.v. protocol was chosen based on previous intraarterial infusion experiments in which the effect of RMP-7 on opening of the BOB was seen following 5 min infusion (15). At this dose, no significant changes in blood pressure, heart rate or respiration rate were noted, and no toxic effects were observed similar to previous intracarotid studies (15). [3H]-ganciclovir (mol wt. = 255 Da) was infused at a rate of 1.0 μCi/0.2 ml/min. Using an independent group experimental design ganciclovir was infused for 0.25, 0.75, 1.5, 3, 4.5 or 10 min. Ocular tissue samples were collected at the end of these periods. Arterial profile of labelled ganciclovir was based on 10 to 12 samples collected at regular intervals between the cessation of the RMP-7 pretreatment and the end of ganciclovir infusion. The RMP-7 was obtained from Alkermes stock, while the [3H]-ganciclovir (SA = 17.5 − 22 Ci/mmol) was purchased from Moravek, Brea, CA. Both RMP-7 and ganciclovir were diluted with sterile 0.9% saline, vehicle.

Intravenous Dosing Protocol II—Continuous Infusion of RMP-7

5 min pre-infusion of RMP-7 (0.2 μg/kg/min) was followed with co-infusion of RMP-7 (0.2 μg/kg/min) and [3H]-ganciclovir (1.0 μCi/0.2 ml/min) for either 4.5 min or 10 min. Again, ocular tissue samples were collected at the end of the co-infusion period. Arterial profile for ganciclovir was based on 10 to 12 samples obtained during the study.

Intravenous Dosing Protocol III—Test of Ganciclovir Retention in Ocular Tissues

As in Protocol I, 5 min pre-infusion with RMP-7 (0.2 μg/kg/min) or vehicle was followed with infusion of [3H]-ganciclovir (1.0 μCi/0.2 ml/min) for 4.5 min. However, in this protocol, the animals were not sacrificed until 30 or 60 min following the ganciclovir infusion. The arterial profile, used to calculate integrated plasma levels for ganciclovir, was based on 10 to 12 samples obtained from the beginning of its infusion until the end of the experiment.

Tissue Preparation

At the end of each study, the eyes were rapidly removed, enucleated and the lens excised 1.5 mm posterior to the limbus. Blotting of the tissue removed any aqueous humor contamination. The anterior epithelium of the lens was subsequently dissected using microforceps. The retina was carefully separated from the underlying epithelium under the dissecting microscope and blotted to remove vitreal fluid contamination. However, some contamination of the retinal tissue by the retinal epithelium could not be excluded. Brain tissue was also taken in some experiments. Once removed, the retina, lenticular tissues and brain tissue were treated with 2.0 ml of Beckman Tissue Solubilizer 450 and 15 ml of scintillant (Beckman Ready Organic). Radioactivity within the tissue samples was determined with a Beckman LS-7500 liquid scintillation spectrometer.

Calculations

The uptake values are expressed as ratios of tracer concentrations in different ocular tissues (or in brain) relative to the integrated plasma concentration (16,17). The following equation was used to calculate the ratios:

$$C_{retina} \Big/ \int_0^T C_{PL} = \text{dpm/g (retina)} \Big/ \int_0^T \text{dpm/ml (plasma)} \quad (1)$$

$$C_{epithelium} \Big/ \int_0^T C_{PL} = \text{dpm/g (epithelium)} \Big/ \int_0^T \text{dpm/ml (plasma)} \quad (2)$$

$$C_{brain} \Big/ \int_0^T C_{PL} = \text{dpm/g (brain)} \Big/ \int_0^T \text{dpm/ml (plasma)} \quad (3)$$

When multiple-time uptake series were performed (i.e., Protocol I), the mathematical treatment, based on previously developed transport models (17–19), was used to calculate the entry or influx (K_{IN}) and exit or efflux (K_{OUT}) transfer coefficients, and the steady state or equilibrium ratio (R). Briefly, calculations were based on equation:

$$dC_{IN}/dt = K_{IN} \int_0^T C_{PL} - K_{OUT} C_{IN} \quad (4)$$

that is integrated to give

$$C_{IN} \Big/ \int_0^T C_{PL} = R(1 - e^{-K_{IN}T}) \quad (5)$$

where C_{IN} is the radioactivity (dpm) per unit mass of retina, lens epithelium and brain (g) and $\int_0^T C_{PL}$ is the radioactivity (dpm) per unit plasma (ml). R is the steady state ratio, or the ratio $C_{IN}/\int_0^T C_{PL}$ and the ratio K_{IN}/K_{OUT} at infinite time, and T is ganciclovir infusion time. Numerical values for K_{OUT} may be obtained from the slope of the plot of ln $(R - C_{IN}/\int_0^T C_{PL})$ against T, since from equation 4,

$$K_{OUT} = -\ln(R - C_{IN} \Big/ \int_0^T C_{PL})/T \quad (6)$$

The value for K_{IN} is derived by substituting the number for K_{OUT} in:

$$K_{IN} = RK_{OUT} \quad (7)$$

In both tissues, K_{OUT} and R values for ganciclovir were computed by fitting the experimental data to equation 4, and K_{IN}

values were calculated by equation 6. It is noteworthy that the units of concentration C_{IN} and $\int_0^T C_{PL}$ may be either mass × volume^{-1} or radioactive mass × volume^{-1}, the latter being essentially equivalent to the former. The units of the transfer constants are time^{-1}, and the constants represent the fraction of the total volume of the compartment exchanged in unit time. Thus, K_{IN} values are related to the entry rates of radioactive solutes relative to the concentration of these solutes in plasma, and K_{OUT} values are related to the exit rates of radioactive solutes from the ocular tissues (i.e., retina, lens epithelium) relative to the concentration of these solutes in ocular tissues. Advanced graphics software (Slide Write Plus) was used to compute transfer coefficients and obtain graphic plots.

Statistics

Results are presented as mean ratios of the integrated plasma concentration of the drug (±sem) and were analyzed using an ANOVA and a two-tailed Students *t*-test.

RESULTS

Protocol I—RMP-7 Pretreatment Studies

In agreement with earlier studies using intracarotid infusion, ganciclovir levels in both the retina and lens epithelium reached steady state equilibrium after 4.5 min of ganciclovir infusion, in saline treated animals. Following pretreatment with a 5 min intravenous infusion of RMP-7 (0.2 μg/kg/min), a significantly greater uptake (up to 2 fold) of ganciclovir was measured in both the retina and the lens epithelium compared to control tissues (Figure 1A/B). Furthermore, the ocular uptake of ganciclovir also reached steady state equilibrium after 4.5 min infusion of ^{3}H-ganciclovir following RMP-7 pretreatment (Figure 1 A/B). This result is reminiscent of data obtained from previous studies with intracarotid infusion of RMP-7 (15). In contrast, no effect of RMP-7 was observed on brain uptake of ganciclovir in this same set of experiments.

The increased ocular uptake that occurs with RMP-7 could be attributed to either an increase in the influx of ganciclovir or a decrease in efflux. Subsequent kinetic analysis of data using eqs. 6 and 7 revealed that the increases in the steady state tissue:blood ratios of ganciclovir were not due to a decrease in efflux but resulted from an increase in the influx of ganciclovir in each ocular tissue, as indicated by significant increases in the K_{IN} constant, and no apparent change in the K_{OUT} constant (Table I).

Protocol II—Continuous RMP-7 Infusion Studies

To test whether ganciclovir uptake into the eye could be enhanced further, RMP-7 (0.2 μg/kg/min) was infused both during a 5 min pretreatment phase as well as throughout the subsequent ganciclovir administration. Two different protocols were investigated; in one, RMP-7 and ganciclovir were co-infused for 4.5 min and in the other the two drugs were co-infused for 10 min.

Results from retinal tissue illustrate that 5 min pretreatment with RMP-7 followed by co-infusion of RMP-7 and ganciclovir did not increase the uptake of the radiolabel above those observed when RMP-7 was given only as a 5 min pretreatment followed by ganciclovir alone either for 4.5 or 10 min infusions (Table II). Interestingly, results obtained for lens epithelium indicated that the uptake of ganciclovir actually decreased with the co-infusion protocol ($p = 0.06$ and 0.02 for 4.5 and 10 min, respectively).

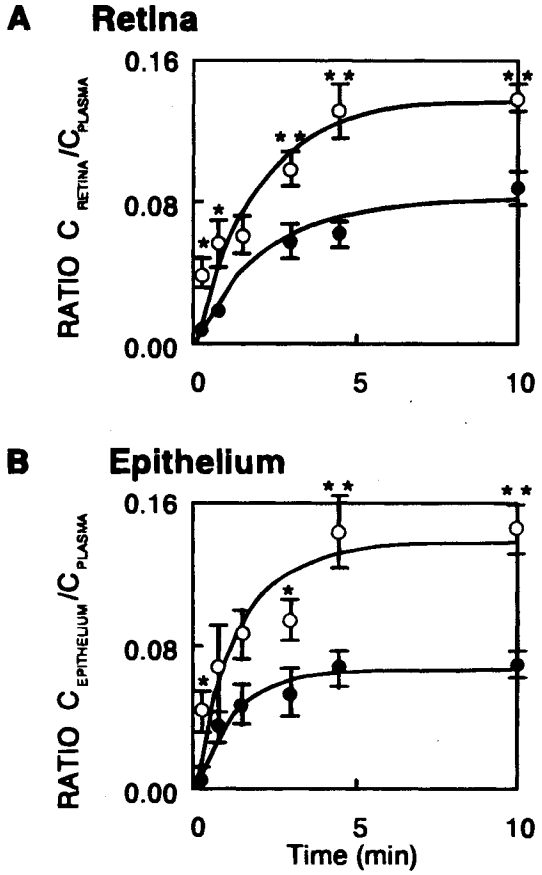

Fig. 1. Effect of i.v. pre-treatment with RMP-7 (0.2 μg/kg/min for 5 min) on the ocular uptake of [^{3}H]-ganciclovir. Ganciclovir was infused for periods of 0.25 to 10 min immediately following cessation of the 5 min RMP-7 (open circles) or vehicle (closed circles) administration. Upon completion of the ganciclovir infusion, the animals were sacrificed and the uptake of ganciclovir in the retina (A) and lens epithelium (B) determined. Data are expressed as a ratio of tissue levels:integrated plasma levels (mean ± sem) using eqs. 1 and 2; $n = 3 - 9$. * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

Table I. Influx (K_{IN}) and Efflux (K_{OUT}) Constants and Steady State Ratios (R) for ^{3}H-Ganciclovir in the Retina and Lens Epithelium in Vehicle and RMP-7 Treated Guinea Pigs

Ocular tissue	K_{IN} (min^{-1})	K_{OUT} (min^{-1})	*R*	*n*
Retina				
Vehicle	0.040 ± 0.006	0.49 ± 0.07	0.081 ± 0.004	31
RMP-7	0.071 ± 0.010[a]	0.52 ± 0.07[b]	0.137 ± 0.007[a]	32
Lens Epithelium				
Vehicle	0.053 ± 0.005	0.80 ± 0.09	0.067 ± 0.004	29
RMP-7	0.098 ± 0.015[a]	0.73 ± 0.11[b]	0.135 ± 0.008[a]	26

Note: Values are means ± sem; *n* is number of experiments. K_{OUT} and R values were computed by eq. 6, and K_{IN} values by eq. 7.
[a] $p < 0.01$ by ANOVA.
[b] Non-significant.

Table II. Plasma:Tissue Ratios for ^{3}H-Ganciclovir in the Retina and Lens Epithelium (eqs. 1 and 2) After a 5 Min i.v. Pretreatment of RMP-7 (0.2 μg/kg/min) Immediately Followed by Either i.v. ^{3}H-Ganciclovir Alone (Protocol I) or Co-Infusion of ^{3}H-Ganciclovir and RMP-7 (Protocol II) for 4.5 and 10 Min

Protocol	Retina	Lens Epithelium
I. RMP-7 pretreatment & ganciclovir for 4.5 min	0.1303 ± 0.0178 (9)	0.1419 ± 0.0253 (7)
II. RMP-7 pretreatment & co-administration of RMP-7 and ganciclovir for 4.5 min	0.1247 ± 0.0108 (10)[a]	0.0889 ± 0.0104 (7)[a]
I. RMP-7 pretreatment & ganciclovir for 10 min	0.1369 ± 0.0063 (6)	0.1426 ± 0.0157 (4)
II. RMP-7 pretreatment & co-administration of RMP-7 and ganciclovir for 10 min	0.1448 ± 0.0110 (8)[a]	0.1056 ± 0.0065 (7)[b]

Note: Mean ± sem, n is number of animals.
[a] Non-significant.
[b] $p < 0.02$, ganciclovir alone vs. co-infusion ganciclovir and RMP-7 (2-tailed tests).

Protocol III—Test of Ganciclovir Retention in Ocular Tissues

Another issue of some importance is how long following cessation of RMP-7/ganciclovir administration were ganciclovir levels elevated (i.e., how long the permeabilizing effect of RMP-7 could be detected). To address this question, a third dosing protocol was investigated where RMP-7 (0.2 μg/kg/min) was infused for 5 min and ^{3}H-ganciclovir was infused subsequently for 4.5 min. At the end of the ganciclovir administration period, an additional 30 to 60 min period was allowed prior to sacrificing the animals.

The results showed that in both retina and lens epithelium, the ganciclovir uptake (i.e., tissue:plasma ratio) remained significantly elevated at 30 min following completion of the ganciclovir infusion compared to vehicle controls (retina tissue: $p = 0.006$; lens epithelium: $p = 0.011$, Figures 2A & 3A). These ratios were still significantly elevated at 60 min in the retina, compared to vehicle ($p = 0.02$) but had started to decrease towards vehicle values in the lens epithelium ($p = 0.08$).

While the absolute tissue levels of radiolabeled ganciclovir decreased in all conditions over time, the levels in the retina of the RMP-7 treated group when compared to vehicle treated subjects remained significantly elevated through the 60 min period following cessation of the ganciclovir infusion (Figure 2B). However, as was true for the tissue:plasma ratios, ganciclovir tissue levels in the lens epithelium were not significantly different from control levels by 60 min, in the RMP-7 treated animals (Figure 3A).

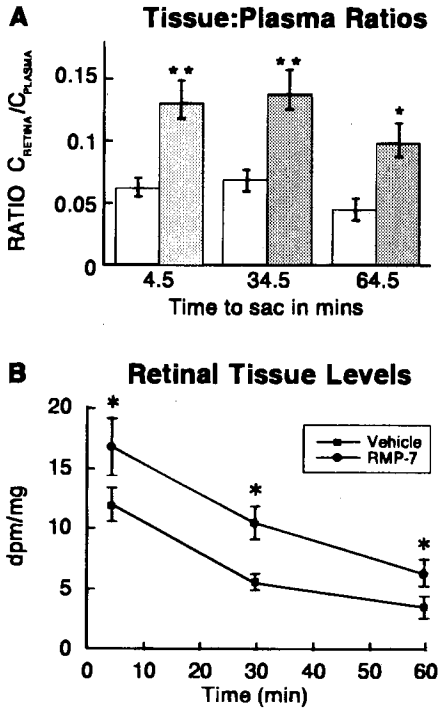

Fig. 2. Effect of intravenous infusion of RMP-7 (0.2 μg/kg/min for 5 min) on the retinal uptake of ganciclovir. Ganciclovir was infused for a period of 4.5 min immediately following cessation of the 5 min RMP-7 (solid columns) or vehicle (open columns) administration. Upon completion of the ganciclovir infusion, the animals were left for an additional 30 or 60 min before they were sacrificed. The uptake of ganciclovir in the retina was then determined and expressed as a ratio of the integrated plasma concentration of the drug (A; eq. 1). The tissue profile of ganciclovir throughout these studies is given as dpm/mg tissue (B). $n = 4 - 9$. * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

It is noteworthy that in all experiments it has been assumed that [^{3}H]-ganciclovir in plasma remains in the form of intact ganciclovir, and therefore is presented to the barriers of the eye as intact molecule. This assumption was based on previous pharmacokinetic studies with ganciclovir that have demonstrated that following systemic administration its metabolism and binding to proteins are minimal (<1%), and the drug is eliminated unchanged (unmetabolized) by renal excretion (20).

DISCUSSION

The current results demonstrate that intravenous infusions of RMP-7 increase the uptake of ganciclovir into the retina and the lens epithelium by two fold. In guinea-pigs the vascular supply of the retina is limited to the central fovea while the rest of the retina is mainly avascular (21). Thus it is possible that the effects of RMP-7 on the BRB in the present model are primarily controlled by the tight-junctioned retinal epithelium as this cellular layer is mainly responsible for the presence of the BRB. However, the effects of RMP-7 on the vascular tight-junctioned endothelium in the restricted foveal area could not be ruled out based on the present data. The increased uptake of ganciclovir by the lens epithelium may be associated with an increase in the permeability of the blood-aqueous barrier. Although in the present study we did not examine whether

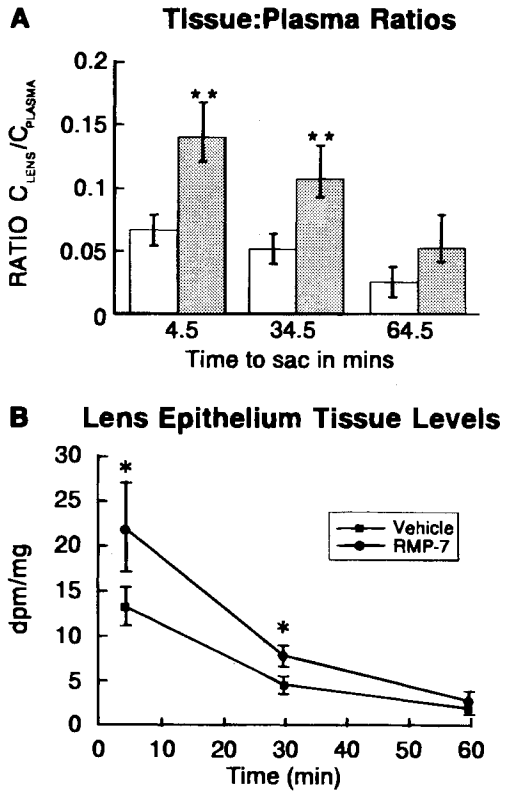

Fig. 3. Effect of intravenous infusion of RMP-7 (0.2 μg/kg/min for 5 min) on the lens epithelium uptake of ganciclovir. Ganciclovir was infused for a period of 4.5 min immediately following cessation of the 5 min RMP-7 (solid columns) or vehicle (open columns) administration. Upon completion of the ganciclovir infusion, the animals were left for an additional 30 or 60 min before they were sacrificed. The uptake of ganciclovir in the lens epithelium was then determined and expressed as a ratio of the integrated plasma concentration of the drug (A; eq. 2). The tissue profile of ganciclovir throughout these studies is given as dpm/mg tissue (B). $n = 4 - 9$. ** represents $p < 0.01$.

RMP-7 exerts an effect on blood-to-aqueous transport of ganciclovir during 10 min, our earlier study with intraarterial delivery of ganciclovir failed to demonstrate consistent RMP-7 effects at this ocular site over shorter exposure times (15).

Similar responses in the retina and lens epithelium have been reported after intraarterial infusions of RMP-7 in the eye (15). Other studies have demonstrated that RMP-7 permeabilizes the BBTB in different brain tumor models, and this effect with most dosing paradigms was obtained in isolation of significant effects on the blood-brain barrier (BBB) (13,14,23). In accordance to these data (13,14), no effects on the BBB were observed with either of the present dosing regimens with RMP-7. However, higher levels of circulating RMP-7 may be required for more generalized vascular central nervous system (CNS) effects, as recently suggested (13). Very sensitive quantitative electron microscopy has recently demonstrated that RMP-7 may increase the cerebrovascular permeability to lanthanum in mice by opening the endothelial tight junctions, but failed to provide evidence for enhanced transcellular transport (22). As suggested by a study on isolated brain microvessels, the effects of RMP-7 are mediated via activation of bradykinin B2 receptors and are associated with increased phosphatidylinositol turnover and elevated intracellular calcium levels (11,13). It is possible that RMP-7 permeabilizing effects in the eye occur through similar mechanisms that involve separation of junctions between endothelial and/or epithelial cells of the BRB.

The effect of RMP-7 on ganciclovir uptake reached steady state equilibrium after 4.5 min from the initiation of ganciclovir infusion, which agrees with results reported previously following intracarotid infusion of RMP-7 in this model (15). In an attempt to further enhance the elevated ganciclovir levels in the eye, the dosing protocol was modified (Protocol II). In both the previous intracarotid study (15) and the current intravenous study, RMP-7 was administered as a pretreatment infusion for 5 min (Protocol I). Due to its peptidergic nature, the plasma half life of RMP-7 is relatively short (12). Moreover, previous studies have demonstrated rapid restoration of vascular barriers when plasma levels of RMP-7 or bradykinin decrease (13,24). Therefore, rapidly declining blood levels following cessation of RMP-7 could account for the decrease in permeability observed at the later time points. Hence, it was proposed that continual infusion of RMP-7 might further enhance ganciclovir uptake by maintaining RMP-7 plasma levels through the ganciclovir administration. However, continual RMP-7 infusion did not increase further the uptake of ganciclovir in either the retina or lens epithelium above those observed following the 5 min RMP-7 pretreatment. In fact, uptake in lens epithelium was actually reduced. As such, the equilibrium reached after cessation of RMP-7 administration (Protocol I) is unlikely to be related to declining RMP-7 blood levels.

The data from the lens epithelium are intriguing in that they could be interpreted as evidence for receptor desensitization. Desensitization with continuous bradykinin receptor stimulation by either bradykinin or RMP-7 have been reported within several bradykinin receptor systems (13,25–27). Our observations that continuous RMP-7 infusions decreased uptake of ganciclovir in the lens, compared to those achieved with RMP-7 pretreatment alone, is consistent with this concept. As such, desensitization of bradykinin receptors in ocular tissues may be induced relatively rapidly (i.e., within 5–10 min), especially in lens epithelium since the longer RMP-7 infusions reduced uptake in the epithelium, while uptake in the retina was not different from the pre-treatment control. In fact, it is possible that the equilibrium reached after pretreatment with RMP-7 (Protocol I) may in part reflect latent desensitization of bradykinin receptors in the ocular barriers. Clearly, additional research exploring B2 desensitization phenomena in different vascular/ocular tissues is required.

Although ganciclovir is currently used to treat patients with CMV retinitis, for systemic administration daily 1 hour i.v. infusions are necessary to reach and maintain minimal therapeutic ocular levels (2,6). Unfortunately, higher and potentially more efficacious dosing protocols for ganciclovir are limited by systemic toxicity (3–4). The current report illustrates that both retinal and lens epithelium ganciclovir tissue:plasma ratios remained elevated above control 34.5 min after cessation of RMP-7 dosing. At 64.5 min after RMP-7 pretreatment, retinal but not lenticular tissue:plasma ratios were still elevated. The reason for the difference between these two ocular tissues is presently unclear. However, the fact that RMP-7 may still significantly elevate by about 2-fold the retinal to plasma ratios of ganciclovir even 1 hour after cessation of ganciclovir i.v. infu-

sion strongly suggests the effectiveness of RMP-7 at the level of BRB.

In conclusion, this paper demonstrates that intravenous infusion of RMP-7 can permeabilize the BOB, and in particular the BRB, thereby increasing the uptake of ganciclovir into the eye. This novel approach to drug delivery, across relatively impermeable membranes, offers hope that RMP-7 may ultimately be developed as a useful adjunct to be given with other poorly penetrating therapeutic agents used to treat eye and in particular retinal infections.

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