RMP-7, a Bradykinin Agonist, Increases Permeability of Blood–Ocular Barriers in the Guinea Pig

Peter J. Elliott, Jasmina B. Mackic, William F. Graney, Raymond T. Bartus, and Berislav V. Zlokovic

Purpose: To determine if the bradykinin agonist, RMP-7, could increase the ocular tissue concentration of agents that normally have limited access across the blood–ocular barriers. The extracellular space marker, sucrose, and the anti-viral drug, ganciclovir, were tested.

Methods: Using the perfused eye method in guinea pigs, RMP-7 (1 μg/kg over 5 minutes) or saline were administered intraarterially into the ocular circulation before either radiolabeled sucrose or ganciclovir (0.4 to 0.6 μCi/ml per minute). At time intervals ranging from 0.25 minute to 10 minutes, perfused eyes were removed, and the radioactivity within various compartments was measured using liquid scintillography.

Results: Pretreatment with RMP-7 significantly increased uptake of both sucrose (up to 4.5-fold) and ganciclovir (up to 2-fold) into the guinea pig retina and lens. Smaller and less consistent effects were observed in other eye compartments.

Conclusions: This report demonstrates that RMP-7 enhances the permeability of blood–ocular barriers, and it provides the first pharmacologic evidence for a means to enhance the concentration of ganciclovir into the retina. Thus, these data support the concept that RMP-7 may prove to be a useful adjunct for enhanced delivery of polar molecules across the blood–ocular barriers.

METHODS. Animals. All procedures were conducted in a manner consistent with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male and female Hartley guinea pigs (Charles River, Ballardville, MA), each weighing from 250 to 300 g, were used in this study. Animals were housed in pairs in a vivarium with a 12-hour dark–12-hour light cycle maintained at 70°F ± 5°F and 70% ± 5% humidity. Food and water were available ad libitum before experimentation.
Perfusion Media, System, and Conditions. The composition of the perfusion medium consisted of 20% washed sheep red blood cells suspended in mock plasma,\textsuperscript{4,5} pH 7.35, and it was gased with 96% O\textsubscript{2} and 4% CO\textsubscript{2} and warmed to 37.6°C. The medium was pumped from a reservoir through a water bath using a Rainin (Woburn, MA) Rabbit peristaltic pump. Perfusion pressure was continually monitored by a StatMed (Hato Rey, PR) P321D pressure transducer and was recorded on a Beckman (Dayton, OH) R511 Dyonograph. Perfusion pressure was maintained slightly (10 mm Hg) above each animal’s blood pressure to eliminate any possible ingress from the systemic circulation described previously.\textsuperscript{4,5} It has been shown with isotope experiments that the perfusion conditions used in the current study, i.e., perfusion of the guinea pig head at the flow to the right common carotid artery of 4 to 5 ml/minute, ensures the perfusion arterial pressure between 80 and 100 mm Hg and the complete functional separation of the artificial from systemic circulations.\textsuperscript{4,5}

RMP-7 Infusion. RMP-7 was synthesized at Alkermes Inc. (Cambridge, MA). RMP-7 was delivered to the eye by continuous arterial infusion (0.2 ml/minute) over a 5-minute period to provide a total dose of 1 \mu g/kg (0.2 \mu g/minute over 5 minutes). This dose of RMP-7 was chosen from preliminary studies because it did not produce significant changes in physiological parameters (respiration, perfusion pressure, and heart rate), and no signs of ocular toxicity were observed (see Results). After either saline or RMP-7 dosing, one of the radiolabeled compounds was infused intraarterially at the same rate. In all experiments, the infusion was terminated by severing the right common carotid artery and decapitating the animal.

Drug Infusion and Measurement of Uptake. Immediately after RMP-7 infusion, either of the radiolabeled compounds was introduced (0.2 ml/minute) into the infusion circuit using a Harvard slow-drive syringe pump at a rate of 0.4 to 0.6 \mu Ci/ml per minute over periods ranging from 0.25 minute to 10 minutes. Each compound was isotope labeled with specific activities of 560 mCi/mmol ([\textsuperscript{14}C]-sucrose; NEN, Boston, MA) or 22 Gt/mmol ([\textsuperscript{3}H]-ganciclovir; Moravek, Brea, CA). Multiple time tracer uptake series were performed at different perfusion times, ranging from 1.5 to 4.5 minutes for sucrose and from 0.25 minute to 10 minutes for ganciclovir. Tracers were introduced into the arterial perfusion inflow at a constant rate, and the concentration of either tracer in the mock plasma of the perfusion medium, $C_{\text{plasma}}$, was constant from the start until the end of each experiment.

Preparation of Samples for Counting. Samples (30 to 40 \mu l) of aqueous humor were taken immediately after the perfusion was terminated. Eyes were then enucleated, and lenses were excised rapidly by a lateral approach, 1.5 mm posterior to the limbus. The lens subsequently was blot on filter paper to remove any adhering aqueous humor and to avoid epithelial contamination by aqueous radioactivity. The anterior epithelium of the lens was dissected from the remaining lens tissue using microforceps with the aid of a microscope. Corneas were dissected circumferentially approximately 0.5 mm anterior to the limbus. After removal of the anterior segment of the eye, the posterior vitreous body was dissected from the retinal surface. Retinas were scooped from the underlying epithelium. All tissues were blotted after dissection to minimize contamination by adjacent tissue layers or fluids. All samples were homogenized and treated with 2 ml Beckman Tissue Solubilizer 450, and 16 ml of scintillant (Beckman [Irving, CA] Ready Organic) before counting in a Beckman [Irving, CA] LS-7500 liquid scintillation spectrometer.

Calculations. The uptake values are expressed as ratios of tracer concentration in different ocular fluids and tissues relative to the plasma concentration.\textsuperscript{4,5} The following equations were used to calculate ratios:

\begin{equation}
\frac{C_{\text{aqueous}} \text{ or } C_{\text{vitreous}}}{C_{\text{plasma}}} = \frac{\text{DPM/ml (aqueous or vitreous)}}{\text{DPM/ml (plasma)}} \quad (1)
\end{equation}

\begin{equation}
\frac{C_{\text{tissue}} \text{ or } C_{\text{aqueous}} \text{ or } C_{\text{vitreous}}}{C_{\text{plasma}}} = \frac{\text{DPM/g (lens or cornea or retina)}}{\text{DPM/ml (plasma)}} \quad (2)
\end{equation}

Statistical Analysis. Results are presented as means \pm SEM and were compared by analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS. Figure 1 illustrates multiple time uptake plots (up to 4.5 minutes) of the retina and whole lens obtained during infusion of [\textsuperscript{14}C]-sucrose after vehicle or RMP-7 (1 \mu g/kg) pretreatment. The low retinal uptake of sucrose observed in vehicle-treated animals is increased 3- to 4.5-fold by RMP-7 from 1.5 to 4.5 minutes of vascular uptake (Fig. 1A). Similarly, almost negligible lenticular uptake of sucrose in control animals was significantly increased by RMP-7 after 4.5 minutes of tracer infusion (Fig. 1B). Table 1 summarizes sucrose uptake measured in other studied ocular regions. A significant increase in sucrose uptake by RMP-7 was obtained in the posterior vitreous, aqueous humor, and cornea after 4.5 minutes of vascular eye perfusion. In these regions, the ratio of tracer concentration ocular fluid to plasma and tissue to plasma indicated 3.5-fold to 6-fold increases at 4.5 minutes.
Figure 2 shows multiple time uptake plots (up to 10 minutes) of retina and lens epithelia, obtained during infusion of [3H]-ganciclovir after vehicle or RMP-7 (1 μg/kg) pretreatment. In both tissues, the uptake of ganciclovir initially was linear and was followed by a significant departure from linearity in both vehicle- and RMP-7-treated animals. In all studied time points from 1.5 to 10 minutes, the uptake of ganciclovir in retina and lens epithelia was significantly higher in the presence of RMP-7, ranging from 1.5- to 2-fold. The effects of RMP-7 on ganciclovir uptake in other studied ocular regions were not significant (Table 2).

Bradykinin is known to decrease arterial blood pressure, and this could be a confounding side effect of RMP-7. However, no differences in the arterial perfusion pressure, respiration, and arterial blood pressure were observed after infusions of RMP-7 at 1 μg/kg. At the end of each study, the eyes were inspected for any signs of ocular toxicity or obvious cellular damage. No such findings were found in any animal in either the vehicle- or the RMP-7 (1 μg/kg)-treated groups. A short safety study was performed and included higher doses of RMP-7, ranging from 3 to 10 μg/kg. We found that these higher doses, when given intracarotidally, produced increases in the respiratory rate sometimes associated with respiratory arrest and a fall in arterial blood pressure. However, none of these side effects of RMP-7 were noted with the currently used dose of 1 μg/kg.

**DISCUSSION.** The compartments of the eye are isolated from blood-borne agents by various blood-ocular barriers. For the first time, we have demonstrated that these barriers can be pharmacologically modified by systemic administration to elevate ocular concentrations of compounds that have restricted access to the eye. This study has shown that the bradyki-
nin B<sub>2</sub>-receptor agonist RMP-7 enhances the permeability of blood–ocular barriers to sucrose and ganciclovir, resulting in significant increases in the concentrations of ganciclovir in ocular tissues including the retina.

In the posterior compartments of the eye, RMP-7 significantly increased the retinal and posterior vitreous levels of the extracellular space marker sucrose up to 6-fold over the 4.5-minute infusion of the radiolabel tracer (Fig. 1; Table 1). The effect of RMP-7 in the retina preceded that in the vitreous. As such, the delayed effect of RMP-7 in the vitreous fluid probably reflects a lag time caused by the initial sucrose diffusion across the retinal layers. In the anterior segment of the eye, RMP-7 also significantly increased the sucrose levels in the aqueous humor, lens, and cornea (Table 1; Fig. 1). These results suggest that RMP-7 may be more effective at the fenestrated ciliary body capillaries than the iris capillaries because there was a delay between the entry of the tracer into the anterior chamber from the ciliary body to the aqueous humor (Table 1). Vehicle-treated animals had aqueous sucrose uptake values comparable to those previously reported<sup>4</sup>–<sup>5</sup>. The slower uptake of sucrose by the lens probably reflects the limited size of its extracellular space. However, the vehicle-treated animals had lentilucic sucrose uptake values similar to those previously reported<sup>4</sup>–<sup>7</sup>. The RMP-7 effect on corneal uptake of sucrose could occur by enhanced passage across the endothelial barrier, by an increase in conjunctival vascular permeability, or both. This in turn could elevate the sucrose concentration in the extracellular space of the limbus, resulting in a diffusion gradient from the limbus to the cornea.

RMP-7 also was shown to increase significantly levels of ganciclovir into the retina and lens epithelium by approximately 2-fold during the 10-minute infusion of the radiolabeled tracer. The uptake into the retina appears to be self-limiting (even in vehicle-treated animals) as demonstrated by the plateau effect occurring after 3 minutes of tracer infusion. That the uptake curve departs from linearity suggests that ganciclovir may be removed by an active efflux mechanism. Alternatively, the effect of RMP-7 pretreatment may start to wane during infusion of the drug. Moreover, identical effects of RMP-7 on ganciclovir uptake into the eye have been reported using intravenous administration of RMP-7<sup>8</sup>. Another possibility to explain the plateau effect seen with ganciclovir in the retina may result from the saturation of its transport system at the blood–retinal barrier. Because ganciclovir is a guanine nucleoside, its potential to interact with the putative nucleoside transporter in the retina<sup>1</sup> and perhaps other ocular tissues deserves to be explored in greater detail. The plateau seen in retinal uptake with ganciclovir versus the linear uptake obtained with sucrose may indicate a different mechanism of uptake of these two tracers at the blood–retinal barrier, or it simply could be caused by differences in experimental duration and the number of data points taken (Figs. 1, 2). Additional work is under way to address these questions and to determine the relationship between RMP-7, ganciclovir, and the nucleoside transporter in the eye. In the other eye compartments, no effects of RMP-
TABLE 2. Effects of Bradykinin Agonist RMP-7 (1 μg/kg) on [3H]-Ganciclovir Uptake in Ocular Fluids and Cornea in the Perfused Guinea Pig Eye

<table>
<thead>
<tr>
<th>Compartment</th>
<th>1.5 Minutes</th>
<th>3 Minutes</th>
<th>4.5 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous humor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP-7</td>
<td>0.46 ± 0.10 (3)</td>
<td>0.51 ± 0.24 (6)</td>
<td>0.55 ± 0.11 (11)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.22 ± 0.03 (2)</td>
<td>0.36 ± 0.07 (6)</td>
<td>0.41 ± 0.10 (6)</td>
</tr>
<tr>
<td>Posterior vitreous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP-7</td>
<td>0.02 ± 0.00 (3)</td>
<td>0.04 ± 0.03 (5)</td>
<td>0.11 ± 0.04 (11)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.03 ± 0.02 (2)</td>
<td>0.04 ± 0.02 (5)</td>
<td>0.11 ± 0.02 (6)</td>
</tr>
<tr>
<td>Cornea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP-7</td>
<td>1.16 ± 0.53 (3)</td>
<td>2.98 ± 0.36 (6)</td>
<td>2.92 ± 0.49 (9)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.76 (1)</td>
<td>2.32 ± 0.68 (4)</td>
<td>2.41 ± 0.15 (5)</td>
</tr>
</tbody>
</table>

Data are ratios X 10^6 of ganciclovir plasma concentrations expressed as mean ± SEM (n); n is number of animals.

7 were observed in the posterior vitreous, cornea, or aqueous humor.

The effect of RMP-7 in the retina is thought to result from the opening of tight junctions at the blood–retinal barrier, similar to its effects on the blood–brain and blood–tumor barriers reported previously.23 Such effects are mediated by the activation of B2 bradykinin receptors and probably are associated with increased intracellular calcium levels.1 It is noteworthy that the blood–retinal barrier in humans is localized both at the level of retinal capillaries (i.e., tight-junctioned microvascular endothelium) and the pigmented epithelium (i.e., tight-junctioned epithelial cells), whereas in the guinea pig, retinal blood vessels are minute and are restricted to the direct neighborhood of the optic disc,10 so that barrier primarily is represented by the pigmented epithelium. Therefore, the observed actions of RMP-7 on retina in the current model are likely to reflect its effects on the epithelial rather than the endothelial component of the blood–retinal barrier. If the action of RMP-7 decreases with time after the cessation of its infusion, a further increase in ganciclovir uptake would not be expected. Studies in which the pretreatment infusion time of RMP-7 is increased and in which coinfusion of RMP-7 occurs with ganciclovir have been started to investigate this issue.

Although ganciclovir is currently used to treat patients with cytomegalovirus retinitis, daily 1-hour intravenous infusion protocols are required to reach minimal therapeutic levels in the eye.11 Higher, potentially more efficacious dosing is limited by ganciclovir systemic toxicity.11 Should RMP-7 and ganciclovir elicit comparable results in patients with cytomegalovirus retinitis to those reported in our animal studies, then this drug combination has the potential to enhance the efficacy of ganciclovir therapy. Moreover, similar drug combinations of RMP-7 and other therapeutics may have a significant impact on other eye disorders. As such, RMP-7 could be a useful adjunct for the enhanced delivery of many agents to the eye.

Key Words

blood–ocular barriers, bradykinin, ganciclovir, permeability, RMP-7

References

Patients with strabismus have congenital or acquired misalignment of the ocular axes. If the alignment is stable, we can assume that the elastic forces across the involved extraocular muscles are at equilibrium. These elastic forces consist of the elasticity of orbital tissue other than muscle, the elasticity of the extraocular muscles to passive stretch, and the elasticity associated with muscle contraction. Strabismus surgery alters this equilibrium by changing the rotational position of the globe and by changing the resting tension of the operated muscles. Recession of a horizontal rectus muscle, for example, results in a profound drop in the resting tension of that muscle at the new insertion site. 

Studies in limb muscle have shown that long-term changes in resting tension result in changes in muscle fiber morphometry. Decreased tension causes muscle fiber atrophy; increased tension results in compensatory hypertrophy. Our previous studies in extraocular muscle indicated that a similar phenomenon occurs after strabismus surgery. Recession procedures cause atrophy of both the recessed muscle and its antagonist, whereas resection causes hypertrophy of the agonist–antagonist pair. These findings suggest that the change in resting tension caused by the procedure affects both the operated muscle and its antagonist(s). Changes in extraocular muscle morphometry are transient. Fiber diameters returned to normal within 2 to 3 months in recession and resection preparations. 

The purpose of the current study was to determine whether the atrophy observed after recession of extraocular muscles results in any changes in developed tension in the antagonist, using the cat as a model.

**METHODS.** Eighteen cats were premedicated with intramuscular atropine, 0.05 mg/kg, and a mixture of ketamine, 20 mg/kg, and xylazine, 1 mg/kg. After endotracheal intubation, anesthesia was continued with isoflurane. The animals were ventilated continuously, and core body temperature was monitored.