Neuroprotective Effect of Memantine in a Rabbit Model of Optic Nerve Ischemia

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The purpose of this study was to evaluate the neuroprotective effect of memantine, a N-methyl-D-aspartate antagonist, in an experimental optic nerve ischemia. Endothelin-1 (ET-1) in a dosage of 0.1 μg/day was delivered to the perineural region of the anterior optic nerve by osmotically driven minipumps for 8 weeks in 10 rabbits. In 5 rabbits, 1 mg/kg memantine was administered concurrently by intramuscular injection once a daily. Morphologic optic nerve head changes were monitored with a confocal scanning laser ophthalmoscope. Multivariate statistical analysis showed a significant change in topometric parameters (cup area, cup depth and rim volume), indicating an increase in optic nerve head cupping and a decrease of neural rim volume in the ET-1 administered eyes ($P < 0.0001$). In rabbits where memantine was given concurrently with ET-1, no significant change in topometric parameters was observed after ET-1 administration ($P = 0.78$). The current results suggest that memantine has a neuroprotective effect in optic nerve ischemia. Memantine may potentially be useful in the management of various ischemic disorders of the optic nerve, including glaucoma.

Key words: endothelin-1, memantine, optic nerve ischemia

INTRODUCTION

Glutamate and aspartate have been identified as major sources of cytotoxic effects in brain injuries and disorders that are accompanied by secondary degeneration. The excitotoxic action of glutamate is primarily mediated by an overstimulation of the N-methyl-D-aspartate (NMDA) glutamate receptor, triggering an increase in intracellular calcium and initiating a cascade of events that finally leads to apoptosis or necrosis, depending on glutamate levels. Non-NMDA receptors may also play a role in glutamate excitotoxicity.

Recent studies suggest that glutamate may be involved in the pathogenesis of various optic nerve diseases. The subcutaneous injection of glutamate in
young mice led to severe destruction of the inner retinal layers, most notably the retinal ganglion cell layer. Chronic intravitreal injection of glutamate killed 42% of the retinal ganglion cells after 3 months. More recently, elevated levels of glutamate have been demonstrated in the vitreous body of humans and monkeys with high-tension glaucoma, a rabbit model of optic nerve ischemia, and a mutant quail with a glaucoma-like disorder as well as in the aqueous humor of rats with partial crush lesion of the optic nerve. Elevation of vitreal glutamate levels was also found in a rat model of retinal ischemia and in patients with proliferative diabetic retinopathy.

Several in vitro and in vivo studies have shown that memantine, an NMDA antagonist, protects against the toxic effects of NMDA receptor agonists. With regard to the possible therapeutic applications to ophthalmic disorders, Vorwerk et al. have shown that memantine protected retinal ganglion cells from glutamate toxicity in rats where the vitreal glutamate level was elevated by serial intravitreal injection. It also had a neuroprotective effect when administered early in an animal model of retinal ischemia induced by the elevation of intraocular pressure (IOP).

In the present study, we investigated whether memantine could be neuroprotective in a rabbit model of optic nerve ischemia induced by delivering endothelin-1 (ET-1, Peptides International, Louisville, KY) to the perineural region of the anterior optic nerve by an osmotically driven minipump. Morphologic change of the optic nerve head has been demonstrated in this model by a topometric analysis with confocal scanning laser ophthalmoscope. Furthermore, over a two-fold elevation of vitreal glutamate and aspartate following ET-1 administration has been shown, indicating that excitotoxicity may be associated with the optic nerve damage in this model. We believe that another demonstration of the neuroprotective efficacy of memantine in the present study would expedite the testing and application of this drug in various ophthalmic disorders associated with NMDA receptor-mediated neurotoxicity.

**MATERIALS AND METHODS**

**Ischemic model**

Ten male New Zealand white rabbits weighing 2.5 kg to 3.5 kg were used. All experiments conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Rabbits were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (0.5 mg/kg). ET-1 was delivered to the perineural region of the anterior optic nerve by osmotically driven minipumps (Alzet minipumps; Alza Corporation, Palo Alto, CA) that deliver a test agent at a controlled and constant flow rate (0.5 μl/hour). The minipumps were implanted in a surgically created space, superior and nasal to the right eyes. A silicone delivery tube was directed from the minipump through the upper eyelid into a surgically created superotemporal sub-Tenon’s channel and under the superior rectus muscle and was finally fixed in place using a scleral fixation suture adjacent to the optic nerve and its vascular supply. A daily dosage of ET-1 0.1 μg/day was delivered for 8 weeks in 10 rabbits. Because the capacity of the minipumps in this study allowed for only two weeks of use, a new minipump was implanted every 2 weeks. Only the reservoir was replaced, whereas the delivery tube was left undisturbed.

**Drug treatment for 10 ET-1 administered rabbits**

**Memantine treatment (n = 5).** Intramuscular memantine at 1 mg/kg every 24 hours was administered as a daily single dose from 2 days before initiating delivery of ET-1. This dosage was selected because it was previously demonstrated to be well tolerated and neuroprotective in an animal stroke model.

**Control group (n = 5).** Intramuscular saline was administered once daily from 2 days before starting ET-1.

**Morphologic monitoring of the optic nerve head during optic nerve ischemia**

Morphologic changes of the optic nerve head were monitored with the Heidelberg Retina
Tomograph (HRT, Heidelberg Engineering, Heidelberg, Germany, software version 2.01). The reliability coefficients of the optic nerve head topographic parameters obtained with the HRT ranged from 89.7% to 98.7%.24 Because rabbit eyes have a shorter axial length than human eyes, a lens providing a larger field of view was used instead of the normal objective lens of the HRT. With this lens, the maximal settings available for scan depth and field of view were used for each rabbit, providing a scan depth of 4.4 mm and a field of view of approximately 29.4 degrees. Topographic images were obtained before minipump implantation and after 8 weeks of local ET-1 administration. For scaling the topographic images, the HRT software uses Gulstrand’s model eye and the value of the corneal curvature to determine the focal length of the eye. However, Gulstrand’s model cannot be applied to the rabbit eye. Its average axial length is 17 mm and its corneal curvature is 7.5 mm. By using a corneal curvature of 7.5 mm, the calculated focal length would not be correct. Therefore, an artificial corneal radius of 4.85 mm, based on an assumed focal length of 16 mm, was used.25,26

In addition, in order to correct for magnification errors with the 29.4 x 29.4 degree objective lens, calculated volumetric parameters from the software were multiplied by 4.87, surfaces and depth measurements by 2.21, and length measurements by 1.48. Rabbits were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (0.5 mg/kg) during optic nerve head imaging, and the pupils were dilated with tropicamide 0.5%. To enhance imaging quality, the cornea was moistened with artificial tears between image acquisitions. At each session, a series of 3 topographic images were obtained from each eye by alternating both eyes during acquisition of 6 images.

The software of the HRT can be used to generate a mean topographic image from a set of individual topographic images by determining the average height measurement at each image location. A mean topographic image was generated with 3 images per eye and the region of interest was defined by a contour line on this mean topographic image. “Curved surface”27 was used to define the upper delimitation of the cup toward the vitreous. The effective area (EA) estimates the optic nerve head cup area, the volume below surface (VBS) estimates the volume of the optic nerve head cup, the volume above surface (VAS) estimates the optic nerve head rim volume, the effective mean depth (EMD) estimates the mean depth of the optic nerve head cup, the maximum depth (MaxD) estimates the mean depth of the 5% picture elements with the highest depth values within the optic nerve head cup, the third moment (TM) estimates the overall shape of the optic nerve head, and the quotient EA/Area estimates the cup:disk area ratio. For the control and memantine-treated groups, the change over time of each parameter was analyzed in a 5 (between groups: five rabbits) x 2 (between groups: minipump eye, control eye) x 2 (between groups: baseline, 8 weeks) analysis of variance design. We applied the randomized complete block design with each rabbit defined as one block in order to eliminate the influence of inter-individual difference on analyzing the difference in change over time between minipump eyes and control eyes. Because seven parameters were compared, the probability that at least one of these comparisons would be significant by chance alone was 30%. To keep this probability at 5%, only a P value lower than 0.0073 was considered significant for the evaluation of the univariate comparisons. In addition to these univariate comparisons, a multivariate approach was used. To preserve a reasonable statistical power within the study, three arbitrarily chosen topographic parameters-EA, VAS, and EMD-were analyzed. These variables provided little redundant information. The change over time of EA, VAS and EMD in the five rabbits of each group was analyzed in a 5 (between groups: five rabbits) x 2 (between groups: minipump eye, control eye) x 2 (between groups: baseline, 8 weeks) multivariate (EA, VAS and EMD) analysis of variance design with each rabbit defined as one block. The interocular difference in IOP over time was analyzed in a multivariate approach of a 2 (within subject: minipump eyes, control eyes) x 2 (within subject: baseline IOP, IOP after 8 weeks) analysis of variance design for each group. IOP was measured with a tonometer (Tono-Pen; Oculab, Glendale, CA).

**RESULTS**

The IOP values at baseline and after 8 weeks are
Table 1. Intraocular pressure in rabbits implanted with endothelin-1 (ET-1)-filled osmotic minipumps for 8 weeks *

<table>
<thead>
<tr>
<th>Rabbit group</th>
<th>IOP, mmHg</th>
<th></th>
<th>8 weeks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minipump eye</td>
<td>Fellow eye</td>
<td>Minipump eye</td>
<td>Fellow eye</td>
</tr>
<tr>
<td>Control group (n=5)</td>
<td>11.0 ± 0.5</td>
<td>10.7 ± 0.7</td>
<td>10.8 ± 0.3</td>
<td>10.4 ± 0.7</td>
</tr>
<tr>
<td>Memantine group (n=5)</td>
<td>10.6 ± 0.5</td>
<td>10.2 ± 0.4</td>
<td>10.2 ± 0.8</td>
<td>10.7 ± 0.6</td>
</tr>
</tbody>
</table>

*: Values are mean ± SEM.

Table 2. Mean values of topometric parameters in the control group *

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minipump eye</th>
<th>Fellow eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>EA (mm²)</td>
<td>3.641</td>
<td>3.883†</td>
</tr>
<tr>
<td>VBS (mm³)</td>
<td>1.277</td>
<td>1.437</td>
</tr>
<tr>
<td>VAS (mm³)</td>
<td>0.072</td>
<td>0.054†</td>
</tr>
<tr>
<td>EMD (mm)</td>
<td>0.342</td>
<td>0.372</td>
</tr>
<tr>
<td>MaxD (mm)</td>
<td>0.826</td>
<td>0.840</td>
</tr>
<tr>
<td>TM</td>
<td>-0.109</td>
<td>-0.072</td>
</tr>
<tr>
<td>EAA</td>
<td>0.776</td>
<td>0.828†</td>
</tr>
</tbody>
</table>

EA: effective area, VBS: volume below surface, VAS: volume above surface, EMD: effective mean depth, MaxD: maximum depth, TM: third moment, EAA: effective area/area (cup: disk area ratio). †: The change of the three topometric variables-EA, VAS and EMD-was statistically significantly different between the eyes subjected to endothelin-1 and the contralateral eyes (multivariate analysis of variance; p < 0.0001), ‡: p < 0.0073 in univariate comparison of an analysis of variance design.

shown in Table 1. A multivariate approach showed no significant change over time in the eyes subjected to ET-1 or the fellow eyes in either the control (P = 0.45) or memantine-treated groups (P = 0.47).

The mean values of topometric parameters in the control group are shown in Table 2. Among the univariate comparisons of the seven topometric parameters of the control rabbits, EA, VAS and EA/A showed a statistically significant difference in the interocular difference in change over time (P < 0.0073). In the multivariate approach, the change of the three topometric variables- EA, EMD and VAS- was statistically significantly different between the eyes subjected to ET-1 and the fellow eyes (multivariate analysis of variance, P < 0.0001). Statistical contrast analysis disclosed a significant change of these three topographic parameters for the ET-1-administered eyes (P = 0.0051), whereas no significant change occurred in the fellow eyes (P = 0.23).

In the memantine-treated rabbits, no significant change over time in the eyes subjected to ET-1 or the fellow eyes was shown in either the univariate (P > 0.0073 for each parameter) or multivariate approach (P = 0.78) (Table 3).

**DISCUSSION**

Memantine is known to have anti-Parkinsonian and anti-epileptic properties and has been used clinically for Parkinson’s disease and dementia for many years in the United States and Europe. Several experiments have shown that memantine is neuroprotective at low micromolar concentrations, unlike other NMDA antagonists, memantine
Table 3. Mean values of topometric parameters in the memantine-treated group*

<table>
<thead>
<tr>
<th></th>
<th>Minipump eye</th>
<th></th>
<th>Fellow eye</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 Weeks</td>
<td>Baseline</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>EA (mm²)</td>
<td>3.364</td>
<td>3.246</td>
<td>3.094</td>
<td>3.020</td>
</tr>
<tr>
<td>VBS (mm²)</td>
<td>1.356</td>
<td>1.172</td>
<td>1.041</td>
<td>1.016</td>
</tr>
<tr>
<td>VAS (mm³)</td>
<td>0.068</td>
<td>0.072</td>
<td>0.068</td>
<td>0.067</td>
</tr>
<tr>
<td>EMD (mm)</td>
<td>0.397</td>
<td>0.367</td>
<td>0.313</td>
<td>0.316</td>
</tr>
<tr>
<td>MaxD (mm)</td>
<td>0.880</td>
<td>0.828</td>
<td>0.674</td>
<td>0.658</td>
</tr>
<tr>
<td>TM</td>
<td>-0.072</td>
<td>-0.136</td>
<td>-0.076</td>
<td>-0.089</td>
</tr>
<tr>
<td>EAA</td>
<td>0.771</td>
<td>0.744</td>
<td>0.743</td>
<td>0.724</td>
</tr>
</tbody>
</table>

EA: effective area, VBS: volume below surface, VAS: volume above surface, EMD: effective mean depth, MaxD: maximum depth, TM: third moment, EAA: effective area/area (cup: disk area ratio), *: The change of the three topometric variables-EA, VAS and EMD-was not statistically significantly different between the eyes subjected to endothelin-1 and the contralateral eyes (multivariate analysis of variance; p = 0.78).

does not impair normal synaptic transmission of NMDA receptor which plays a crucial physiological role in various forms of synaptic plasticity such as those involved in learning and memory.34,35

In the present study, the morphological analysis of the optic nerve head with HRT demonstrated that memantine blocked neuronal rim loss in an animal model of optic nerve ischemia induced by ET-1 administration. HRT is a quantitative method to analyze the topography of the optic nerve head. It seemed reasonable to use this method in this study because previous experiments using the HRT have shown comparable reproducibility of the topometric data obtained in rabbits as compared to humans.24 This is important because good reproducibility is essential to establish a useful statistical power in determining small changes.

In the ET-1 administered model, optic nerve damage has been documented by a topometric analysis using a confocal scanning laser ophthalmoscope.22 More recently, we demonstrated an elevation of glutamate and aspartate in the vitreous body of this model.10 Although the source of vitreal glutamate and aspartate remains unknown, it has been suggested that these amino acids are released from the dying retinal ganglion cells. The glutamate and aspartate thereby released, in turn, may lead to further neuronal injury. The possible association of excitotoxicity with the previously proven optic nerve damage following ET-1 administration suggests that this model is useful in screening neuroprotective agents.

Although it is widely accepted that elevated IOP is an important factor in glaucoma, microcirculatory compromise of the anterior optic nerve has been invoked as a potential causal factor or contributor to glaucoma. Many microvascular diseases, including diabetes mellitus, systemic hypertension, peripheral vascular disease and disorders associated with vasospastic tendencies are associated with glaucomatous optic neuropathy.36-39 Furthermore, deficient autoregulation has been demonstrated in the retinal vasculature of patients with glaucoma.40 In the ET-1 administered model, an approximately 38% reduction of the optic nerve blood flow in the ET-1 administered eyes was demonstrated, as compared with the fellow control eyes. This partial reduction of optic nerve blood closely simulates the compromised microcirculation present in glaucoma. In view of this information, it may be reasonable to interpret our results as suggesting that memantine may be useful as an additional agent in the management of glaucoma as well as other ischemic conditions of the optic nerve.

In conclusion, we demonstrated that memantine has a neuroprotective effect in a rabbit model of optic nerve ischemia. Our data suggests that memantine may eventually be beneficial in the future treatment of various ischemic disorders of the optic nerve.
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