The Mydriatic Effect of Intracameral Epineine Hydrochloride

Björn Lundberg and Anders Behndig

PURPOSE. To compare the mydriatic effect and the short-term corneal endothelial safety of intracameraly injected N-methyl-3,4-dihydroxyphenylamine (epineine) to phenylephrine in a porcine eye model.

METHODS. One hundred twelve eyes from newly slaughtered pigs were used in this study. After pretreatment with 20 mg intracameral acetylcholine to give miosis, 0.15 mL epineine or phenylephrine 0.3%, 1.5%, or 3.0% was given as an intracamer- 4 4 4 4 al injection. The pupils were filmed during 90 seconds with a video camera connected to an operation microscope, and the mean pupil diameters were measured from the video recordings. In 37 additional eyes, 0.15 mL vehicle, 1.5% epineine, or 1.5% phenylephrine was injected intracameraly, and the eyes were kept on ice overnight. Corneal endothelial morphology was assessed before and after the treatment. Ten eyes were given no injection and served as controls.

RESULTS. Epineine had a significantly larger mydriatic effect than phenylephrine at equal concentrations. Endothelial cell loss was equal with both substances and did not exceed that of the vehicle.

CONCLUSIONS. Epineine was a more potent mydriatic than phenylephrine in this porcine eye model. The porcine eye model appears suitable as a first efficacy screening of substances for intraocular use. Epineine is a promising candidate substance for intraoperative (c., cataract surgery) intracameral use in humans. (Invest Ophthalmol Vis Sci. 2009;50:5336 –5338) DOI: 10.1167/iovs.09-3651

Adequate mydriasis is a prerequisite for intraocular surgery and is traditionally achieved by preoperative topical anti-cholinergic and sympathomimetic mydriatic agents such as cyclopentolate and phenylephrine. A more rapid and logisti-cally more efficient alternative is using intracameral mydriatics such as cyclopentolate and phenylephrine. A more rapid and logis-tically more efficient alternative is using intracameral mydriatics that stimulate the alpha-1-stimulator phenylephrine for intracameral use. Epineine (N-methyl-3,4-dihydroxyphenethyamine) is a substance with both adrenergic and dopaminergic properties. It stimulates all six known receptors—α1, α2, β1, β2, DA3, and DA4—in cardiovascular tissues and was suggested early as a treatment for heart conditions. Numerous studies have been performed with epineine and its orally active 3,4-diisobutyryl ester, ibopamine, in heart conditions. Ibopamine is rapidly hydrolyzed to epineine by esterases and exerts all its phar-macologic effects as epineine. In ophthalmology, ibopamine has been successfully used to dilate the pupil for fundus examinations and surgery. Ibopamine is hydrolyzed to epineine during its passage through the cornea and, analogous to the cardiovascular system, exerts its pharmacological effects within the eye as epineine. Ibopamine has an interesting pharmacologic profile: 2% ibopamine is more potent than 10% phenylephrine in producing mydriasis, and the mydriatic effect is reversed within 4 hours, making ibopamine the most short-acting and most effective topical mydriatic agent studied thus far. Epineine is poorly absorbed through the cornea and is, therefore, not useful for topical application but is likely to have pharmacologic effects identical to those of topical ibopamine if injected directly into the anterior chamber of the eye. Based on this background, we decided to evaluate the mydriatic effect and the corneal endo- thelial safety of intracameral injection epineine 0.3% to 3% and to compare this effect with that of phenylephrine at the same concentrations. For the study, we used a model involving postmortem porcine eyes.

MATERIALS AND METHODS

The research ethics committee of Umeå University (Umeå, Sweden) approved this study. The investigation was performed at the surgical laboratory of Advanced Medical Optics, Inc. (Uppsala, Sweden). One hundred twelve porcine eyes were used for the study of pupil re-sponses. The eyes were kindly provided from a local slaughterhouse within 24 hours of slaughter and were kept on ice until the investiga-tion. All eyes were pretreated with intracameral acetylcholine (0.2 mL 1% Miochol-E; Novartis Ophthalmics, Inc., Täby, Sweden) to contract the pupil. After 1 minute, 0.15 mL of 0.3% epineine (n = 14), 0.3% phenylephrine (n = 24), 1.5% epineine (n = 14), 1.5% phenylephrine (n = 20), 3.0% epineine (n = 20), or 3.0% phenylephrine (n = 20) was injected intracameraly. After this injection, the pupil was filmed for 90 seconds with a video camera connected to an operation microscope. Minimum and maximum pupil diameters were measured by a masked observer in accordance with our previous studies, and the mean pupil diameter was calculated for each eye at 10, 20, 30 60, and 90 seconds after the injection.

The epineine and phenylephrine solutions were prepared from salts of the respective substances using a vehicle of sodium edetate 1 mg/mL, boric acid for isotonity, and deionized water, as previously detailed.

Effects on corneal endothelial cells induced by the two substances studied were assessed in a separate batch of 37 porcine eyes, after which 0.15 mL vehicle (n = 8), 1.5% epineine (n = 10), or 1.5% phenylephrine (n = 9) was injected intracameraly, and the eyes were kept on ice overnight. Before and after treatment, the corneal endo-thelium was photographed with a specular microscope (SP-2000P; From the Department of Clinical Sciences/Ophthalmology, Umeå University Hospital, Umeå, Sweden.

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Corresponding author: Anders Behndig, Department of Clinical Sciences/Ophthalmology, Umeå University, SE-901 87 Umeå, Sweden; anders.behndig@ophthal.umu.se.

Topcon Europe B.V., Capelle a/d IJssel, The Netherlands), and corneal endothelial morphology was calculated from a central cluster of 50 cells from each photo, as previously detailed.²⁰ Ten eyes were given no injection but were otherwise treated identically and served as controls. The endothelial cell count and the hexagon shape factor²⁰ were calculated from the specular microscope (SP-2000P; Topcon Europe B.V.) photographs by a masked observer, and the pretreatment and post-treatment values were compared.

Student’s two-tailed paired t-tests were used as appropriate for statistical comparisons, and all results are presented as means ± SD. When comparing more than two groups, Bonferroni corrections were preformed. P < 0.05 was considered statistically significant.

Results

Both epinine and phenylephrine showed a significant mydriatic effect at 0.3%, 1.5%, and 3.0% from 10 seconds on (P < 0.05). Epinine consequently had a larger mydriatic effect than phenylephrine (Figs. 1A–C). The onset of the mydriatic effect was equally rapid with epinine and phenylephrine, and the maximum effect was seen at 60 or 90 seconds with little or no increase in effect toward the end of the observation period (Figs. 1A–C). From 10 to 30 seconds, both substances showed a larger mydriatic effect at the higher concentrations (1.5% or 3.0%) than at 0.3% (P < 0.05 after Bonferroni correction). No significant differences in effect could be seen between the 1.5% and the 3.0% solutions for either substance (P = NS).

Significant corneal endothelial cell loss was seen in the eyes injected with vehicle (from 3938 ± 171 to 3284 ± 1258 cells/mm²; −6.2% ± 6.0% cell loss; P = 0.017), epinine 1.5% (from 4058 ± 348 to 3855 ± 313 cells/mm²; −4.8% ± 5.4% cell loss; P = 0.021), and phenylephrine 1.5% (from 3923 ± 306 to 3629 ± 238 cells/mm²; −7.3% ± 5.8% cell loss; P = 0.0061), but the cell loss did not differ significantly between these three treatments (P = NS). The untreated eyes showed no significant endothelial cell loss (from 3743 ± 344 to 3741 ± 356 cells/mm²; +0.1% ± 5.7% change in cell count; P = NS). Similarly, deviation from the hexagonal cell shape was seen in eyes injected with vehicle (P = 0.019), epinine (P = 0.041), and phenylephrine (P = 0.026) but not in control eyes (P = NS). No differences were seen between the three treatments (P = NS).

Discussion

We here demonstrate that epinine has a larger mydriatic effect than phenylephrine in this porcine eye model. This finding aligns well with the observation by Marchini et al.¹⁶ who demonstrated that topical ibopamine is a more potent mydriatic agent than topical phenylephrine in humans. However, when comparing a prodrug (ibopamine) to an active drug (phenylephrine), one can assume better bioavailability for the prodrug, analogous to the eightfold higher bioavailability seen with a prodrug to phenylephrine, phenylephrine oxazolidine, in rabbit eyes.²¹ The present study is the first to demonstrate a greater mydriatic effect from the active metabolite of ibopamine, epinine, on intracameral injection. Our findings indicate that increased absorption through the cornea alone does not explain the pronounced mydriasis seen with topical ibopamine but that the active substance itself is more potent than phenylephrine at equal concentrations.

In this model the isolated porcine eyes were pretreated with intracameral acetylcholine to induce miosis, which is necessary because of the absent natural pupil sphincter tonus postmortem. For the sake of reproducibility, a high dose of acetylcholine was given to obtain maximal miosis in all eyes, which explains the rather small dilating effects seen from the subsequent dilating substances compared with what is seen in humans in vivo.¹⁵,¹⁶ Although we tried to make our model resemble the in vivo situation as closely as possible by keeping the postmortem time short and keeping the eyes on ice, some postmortem weakening of the pupil dilator might also have reduced the mydriatic response in this study. Porcine eyes are commonly used in surgical training because of their anatomic resemblance to human eyes. In particular, the anterior segment of the porcine eye has been shown to resemble the human eye both anatomically and ultrastructurally.²² Furthermore, isolated porcine eyes²³,²⁴ or isolated porcine iris muscles²⁵ are established models for investigating ocular adrenoceptors.²³,²⁴ and the data obtained are considered to have an acceptable degree of transferability to the human system.²³–²⁵

In contrast to epinine, which has a broad spectrum of adrenergic and dopaminergic effects, phenylephrine is characterized as a specific α₁-receptor agonist.³ Still, it has been suggested that the mydriatic effect of epinine in humans is mediated primarily by α₁-receptor stimulation.¹⁵ In rats and rabbits, it has been shown that the α₁(1A)-adrenoceptor is the chief mediator of the adrenergic mydriatic effects of the iris dilator muscle.²⁶,²⁷ but the mydriatic effect from an adrenergic substance may still be more complex; as an example, stimulation of iris β₂-receptors can cause relaxation of the porcine pupil sphincter.²⁸ In humans, a recent clinical study indicates that epinephrine may be a more potent mydriatic agent than phenylephrine when injected intracameral.²⁹ and, indeed, epinephrine has a larger affinity for the β-receptor than phenylephrine.²⁵ Epinine stimulation of all adrenergic receptors, including the β₁-receptor,³ might have contributed to the larger mydriatic effect seen in the present study.

Topical ibopamine has been demonstrated to increase intraocular pressure in persons with glaucoma or compromised

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933244/ on 06/26/2017)
aqueous humor outflow facilities. A small part of this effect can be ascribed to an increase in aqueous humor production, but the apparent doubling of aqueous humor flow has been shown to be an artifact primarily of fluorescein loss through the dilated pupil. Therefore, we do not think this calls for precaution if epinine is considered an intracameral mydriatic agent in humans, especially because no increase in intraocular pressure is seen in healthy subjects with topical ibopamine. Furthermore, in subjects with glaucoma, the increase in intraocular pressure after topical ibopamine is no greater than that seen after topical tropicamide.

The level of corneal endothelial cell loss seen in the present study was equal to that of phenylephrine, epinine, and vehicle, indicating that the vehicle used might have been chiefly responsible for the endothelial cell loss. Although intracameral mydriatics with this vehicle do not cause detectable corneal endothelial injury in cataract surgery in humans, a modification of the vehicle composition may be considered based on the present results. It should be emphasized, however, that this model is suited to detect acute effects on the corneal endothelium only; assessment of long-term effects would require an in vivo model.

To summarize, the porcine eye model used in the present study appears suitable as a first efficacy screening of substances for intraocular use, and it can also give an impression of the acute effects on the corneal endothelium. We can here demonstrate that epinine appears safe for the corneal endothelium and that its mydriatic effect is more pronounced than that of phenylephrine in equal doses. We conclude that epinine is a promising candidate substance for intracameral injection to achieve mydriasis in, for example, cataract surgery.

References


