Original Paper

Ophthalmic Research

Ophthalmic Res 2002;34:97–103

Received: March 3, 2001
Accepted: October 17, 2001

In vitro Toxicity of Rivastigmine and Donepezil in Cells of Epithelial Origin

David Goldblum Monika Gygax Matthias Böhnke Justus G. Garweg

Department of Ophthalmology, University of Bern, Bern, Switzerland

Key Words

Toxicology · Cholinesterase inhibitor · Rivastigmine · Donepezil · Glaucoma · Neuroprotection

Abstract

Neurospecific acetylcholinesterase inhibitors have been shown to lower intraocular pressure (IOP) in normal rabbits and might have additional neuroprotective effects. This study was set out to explore and compare the toxicity of two selective acetylcholinesterase inhibitors, rivastigmine and donepezil, on two standardized cell lines of epithelial origin. Chang and Vero cells were incubated with various concentrations of rivastigmine or donepezil. Acute toxicity (4 h) was assessed by monitoring the permeability of cells to propidium iodide. Chronic toxicity (7 days) was determined by monitoring the effect of the two drugs on esterase activity and cell proliferation. The viability of cells was also assessed morphologically by microscopic inspection. Signs of acute toxicity became manifest at a rivastigmine concentration of 50 mg/ml in both Chang and Vero cells. Indications of chronic toxicity became obvious at concentrations of as low as 1×10^{-5} mg/ml. In contrast, degenerative morphological changes became manifest only at a concentration of as high as 1 mg/ml. In donepezil-treated cells, acute toxicity was not observed in the concentration range tested, whereas chronic toxicity was detected at 1×10^{-1} mg/ml in both Chang and Vero cells, a concentration at which degenerative morphological changes became evident as well. In contrast to rivastigmine, donepezil elicited no signs of acute or chronic toxicity in either Chang or Vero cells at the IOP-lowering concentration of 1×10^{-4} mg/ml. At this dose, the drug is therefore unlikely to evoke deleterious effects on ocular surface tissues in the clinical setting.

Introduction

Cholinergic drugs (parasympathetics) have been used in the treatment of glaucoma since the 1870s, making them the oldest of the antiglaucomatous drugs [1]. These drugs fall into two categories. They act by stimulating acetylcholine receptors at the neuroeffector junction (as in the case of direct-acting cholinergic agonists) or inhibiting acetylcholinesterase (AChE), thereby potentiating the action of endogenous acetylcholine (cholinesterase inhibitors). Actually, only three unspecific cholinesterase inhib-

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–3747/02/0342–0097$18.50/0

Accessible online at:
www.karger.com/journals/ore

David Goldblum, MD
Laboratory of Ocular Pharmacology, Department of Ophthalmology
University of Bern, Inselspital
CH–3010 Bern (Switzerland)
Tel. +41 31 632 25 01, Fax +41 31 382 47 79, E-Mail goldblum@vsao.ch

itors (demecarium, echothiophate and physostigmine) have been clinically approved for the treatment of glaucoma in various countries. The main ocular side effects associated with their use include fixed small pupils and accommodation-induced myopia. However, these agents can also precipitate cataract formation, and they are therefore indicated primarily for patients who have undergone cataract extraction [2]. Systemic symptoms include diarrhea and abdominal cramps [3], even after local application of the drugs. Such side effects have largely contributed to the unpopularity of these agents.

Recently, new selective and specific AChE inhibitors have been developed and approved for the treatment of Alzheimer's disease.

Rivastigmine (SDZ ENA 713) is a carbamate-type inhibitor of AChE that preferentially inhibits the globular monomer (G1) form of the different molecular enzymatic forms of the AChE [4].

Donepezil (E 2020) is a piperidine-based specific AChE inhibitor, selective for AChE in the central nervous system [5, 6].

The most abundant form of AChE found in the brain is the tetramer G4. G1 is also mainly present in smaller amounts therein, whereas the dimer G2 is found dominantly in human erythrocytes [7]. There is evidence that three different molecular forms of AChE exist in the human ciliary body [8]. The distribution of the different forms of AChE in the rest of the human eye is still unknown.

Since the ciliary epithelium develops from the neuroectoderm, we assumed that the AChE subtype distribution would most likely resemble that manifested in the brain. On this assumption, we reported that a specific inhibitor of the G1 form, such as rivastigmine or donepezil, would be lowering intraocular pressure (IOP) without inducing the systemic side effects commonly associated with the use of non-selective AChE inhibitors [9, 10].

Even though these two drugs are both selective AChE G1 inhibitors, their pharmacokinetics and chemical structures are not related, and therefore their side effect profiles are likely to be dissimilar. As with any topical treatment, these two substances given locally will mainly affect the most superficial cells of the external eye at the applied concentration. The potential toxic effects of donepezil or rivastigmine on cultured epithelial cells have not been reported yet.

Therefore, the purpose of this study was to explore toxicity of both donepezil and rivastigmine on highly proliferating cells of epithelial origin and to compare the effects.

Material and Methods

Cell Cultures

Human transfected conjunctival Chang P12 cells were purchased from ECACC (Salisbury, UK) and transfected African monkey kidney epithelial Vero cells from ICN Flow (Allschwil, Switzerland). Each cell line was cultured in MEM (Gibco), enriched with 10% fetal calf serum (Gibco) and supplemented with *L*-glutamine (2 m*M*), penicillin (10,000 U/ml), streptomycin (10,000 µg/ml), amphotericin B (25 µg/ml) in a humidified atmosphere containing 5% CO_2 at 32°C.

Drugs

Rivastigmine (SDZ ENA 713) (S)-N-Ethyl-N-methyl-3-[1-(dimethylamino) ethyl]-phenyl carbamate, hydrogen-(2*R*,3*R*)-tartrate (molecular formula: $C_{14}H_{22}N_2O_2 + C_4H_6O_6 = C_{18}H_{28}N_2O_8$, molecular weight: 250.34 + 150.09 = 400.43) was obtained from Novartis (Basel, Switzerland). Donepezil (E 2020) (±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenyl-methyl)-4-piperidinyl]methyl]-1H-inden-1-one-hydrochloride (molecular formula: $C_{24}H_{29}NO_3HCl$, molecular weight: 415.96) was obtained from Eisai Inc. (New York, USA).

Experimental Set-Up

Rivastigmine and donepezil were dissolved in the culture medium and tested on subconfluent cultures in concentrations ranging between 10^{-5} and 50 mg/ml in 10-fold steps. For this, cells were seeded at a density of 30,000 per well in a standard fashion (in 24-well Costar culture plates) and grown to subconfluent monolayers overnight before the addition of one of the drugs. Cell viability was determined with the various methods described below and also assessed by microscopic inspection.

Each experimental condition was tested four times, a negative control being included in all instances.

Tests for Chronic Toxicity

Chronic toxicity was assessed 7 days after continuous exposure of cells to donepezil or rivastigmine using cell viability and cell esterase activity and the estimated cell numbers as parameters for viability. Culture medium including the tested drugs was changed once after 4 days.

Esterase and Viability Testing

Calcein-AM (Molecular Probes Europe) is a fluorogenic esterase substrate which is converted to free calcein intracellularly. Calcein is a membrane-impermeable green fluorescent dye which is retained only by viable cells. It can be quantified by measuring the fluorescence emitted at 485 nm in a Cytofluor™ (Millipore). Monolayers were washed in PBS and then incubated with 200 µl of MEM (w/o Phenol Red) containing 1.5 µ*M* calcein-AM for 30 min. They were then rinsed twice and covered with 500 µl MEM (w/o Phenol Red) for the measurement of fluorescence resulting from calcein uptake and activation.

Cell Counting

After performance of the calcein-AM assay, the monolayers were washed, trypsinized and harvested. The numerical density of cells was determined using a cell counter calibrated for this application (Sysmex, Digitana).

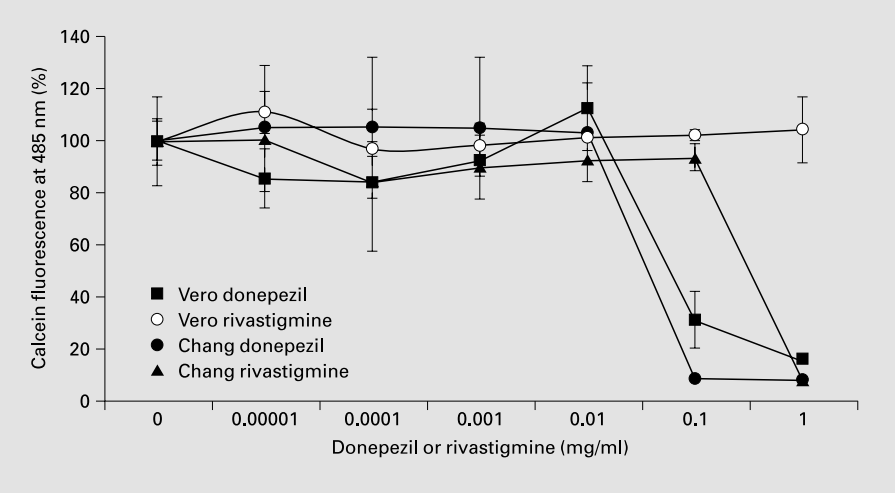

Fig. 1. Calcein-AM assay for chronic toxicity in cultured Chang and Vero cells: the esterase activity of cells after incubation with various concentrations of donepezil or rivastigmine for 7 days is expressed as a percentage of the maximal fluorescence (incubation without drug). All values are presented as the mean (± SD) of the 4 experiments.

Morphological Assessment

The cell morphology was analysed microscopically using phase contrast and a magnification of 40-fold. The presence of morphological changes was assessed by increased pleomorphism, elevated nuclei, shrunken cytosol or by the disruption of intercellular junctional complexes.

Tests for Acute Toxicity

Acute toxicity was assessed according to the propidium-iodide assay described by Ventura and Böhnke [11]. Propidium iodide (Sigma) enters non-viable cells and binds irreversibly to their nucleus, whereupon it acts as a fluorescent stain. The amount of bound propidium iodide can be quantified by measuring the fluorescence in a Cytoflour (Millipore) (excitation/emission wavelengths: 530/645 nm).

Monolayers were incubated with 1 ml of serum-free MEM containing propidium iodide (20 μg/ml) and various concentrations of donepezil or rivastigmine. Fluorescence measurements were performed every 15 min during the first hour and every 30 min during the following 3 h. To determine the maximal endpoint fluorescence, cells were incubated for 1 h with 1 ml of 1.5% Tween 20 (Gibco) to effect complete permeabilization of the cell membranes. Fluorescence micrographs of the nuclei were recorded routinely using a high-resolution camera (type CF 15 MCC, Kappa) after 1 h, and the proportion of necrotic cells estimated.

Statistical Evaluation

The results represent the average of the four experiments in each group. Fluorescence measurements and cell counts were compared statistically using Student's t test (including Bonferroni's correction). Differences with a first-order error of $p < 0.05$ were considered as significant.

Results

Chronic Toxicity

Chronic toxicity was assessed by monitoring the decrease in esterase activity and cell growth. Rivastigmine-incubated Chang cells showed a first significant decrease in esterase activity and cell number at 1×10^{-5} mg/ml ($p < 0.02$), interestingly in the absence of any visible morphological changes and a second highly significant drop at 1 mg/ml ($p < 0.0005$; fig. 1), at which concentration morphological changes also became apparent (fig. 2). Rivastigmine-incubated Vero cells, in contrast, showed a significant reduction in esterase activity only at 10 mg/ml ($p < 0.0005$). However, cell proliferation was inhibited in two steps, first at 1×10^{-4} mg/ml ($p < 0.0005$) and then at 1 mg/ml (fig. 2). Again, degenerative morphological changes were only observed at rivastigmine concentrations above 1 mg/ml in these cells.

At a concentration of 1×10^{-1} mg/ml, donepezil elicited a significant decrease in esterase activity ($p < 0.0005$; fig. 1), as well as on cell growth in human conjunctival Chang cells ($p < 0.0005$; fig. 2), coinciding with the onset of degenerative morphological changes. In monkey kidney Vero cells, donepezil induced a reduction in esterase activity at a concentration of 1×10^{-1} mg/ml ($p < 0.0005$; fig. 1). At the same concentration (1×10^{-1} mg/ml; $p < 0.0005$), a suppressive effect on cell growth and degenerative morphological changes were detectable (fig. 2).

Acute Toxicity

The membrane permeability of human conjunctival Chang cells started to increase 45 min after exposure to

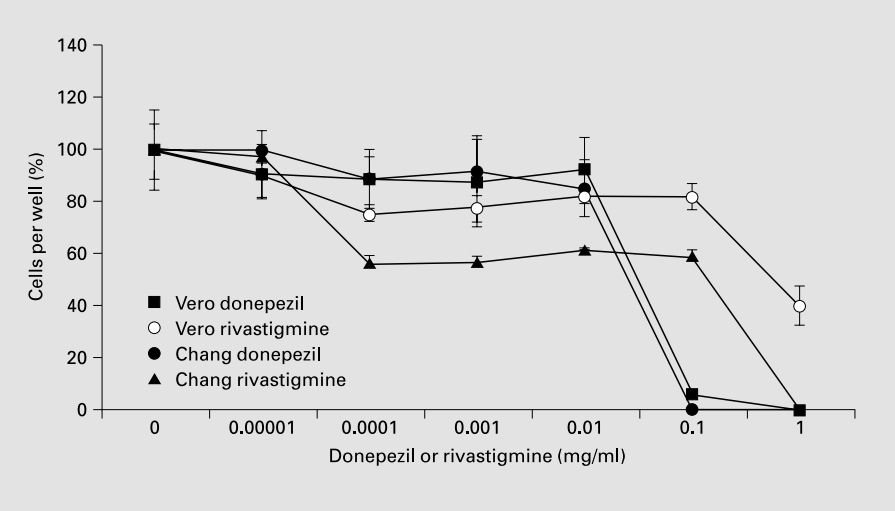

Fig. 2. Proliferation assay for Chang and Vero cells: the numerical density of cells recorded after 7 days of incubation with various concentrations of donepezil or rivastigmine is expressed as a percentage of that obtained in the absence of this drug. Each point represents the mean (± SD) of the 4 experiments.

rivastigmine (fig. 3) only at a concentration of ≥ 50 mg/ml according to the propidium-iodide dead/live-assay ($p < 0.05$). Morphologically, no changes were apparent in either cell type, even after 4 h of exposure to 50 mg/ml rivastigmine.

Donepezil-treated cells showed clear morphological changes and increased membrane permeability at 10 mg/ml (fig. 4).

Discussion

Testing for acute and chronic toxicity of ocular drugs is the basis in pharmacological research to define the range of applicable drug concentrations in humans. This was usually evaluated using animal models. Such tests generally involve monitoring for signs of epithelial irritation in vivo [12]. Similarly, the more or less beneficial effects of drugs applied locally to the eye were assessed by monitoring the epithelial wound-healing response after mechanical or chemical lesions in vivo [13, 14].

Nowadays, however, the availability of well-defined cell lines and standardized monolayers permits an evaluation of drug toxicity in vitro, thereby dramatically cutting down the number of animal experiments. Such in vitro models are already being employed in some medical fields [15–17] and have been suggested for pharmacological testing of ocular drugs [18–22]. Long-term use of topical antiglaucomatous drugs has been shown to induce ocular surface inflammation [23] and fibrosis [24]. Therefore, in vitro toxicity testing of potential new topical antiglaucomatous drugs is crucial before running into clinical testing. In the present study, concentrations of rivastigmine (1 mg/ml) and donepezil (1×10^{-1} mg/ml) that apparently failed to induce membrane damage in Chang and Vero cells (fig. 3, 4) nonetheless decreased their viability, as determined by intracellular esterase activity (fig. 1) and cell density (fig. 2). Donepezil had equal, chronically toxic effects on Chang and Vero cells with inhibition of their proliferation as well as reduced esterase activity at 1×10^{-1} mg/ml. In contrast, rivastigmine led already to a mild but significant reduction in Chang cell proliferation at the lower concentration of 1×10^{-5} mg/ml. A second more important decrease appeared at 1 mg/ml. Rivastigmine-incubated Vero cells proliferation was inhibited moderately at 1×10^{-4} mg/ml, whereas again the important decrease was found at 1 mg/ml. Additionally, esterase activity only decreased at the high concentration of 10 mg/ml. Therefore, donepezil elicited no signs of acute or chronic toxicity in either Chang or Vero cells at IOP-lowering concentration of 1×10^{-4} mg/ml. Rivastigmine expressed a comparable decrease of cell proliferation only at a ten times higher concentration but, in contrast, tested slightly toxic even at low doses. Nevertheless, we assume that, due to a further local dilution by the tear film, doses of both drugs below 0.1 mg/ml applied topically would be unlikely to have deleterious acute or long-term effects on ocular surface cells.

Only 9.7% of the cholinesterase in the human cornea is attributable to 'true' AChE. The rest is butyrylcholinesterase (BuChE) [25]. In intraocular tissues, in contrast, only AChE is found [26]. It seems that up to 5 different iso-

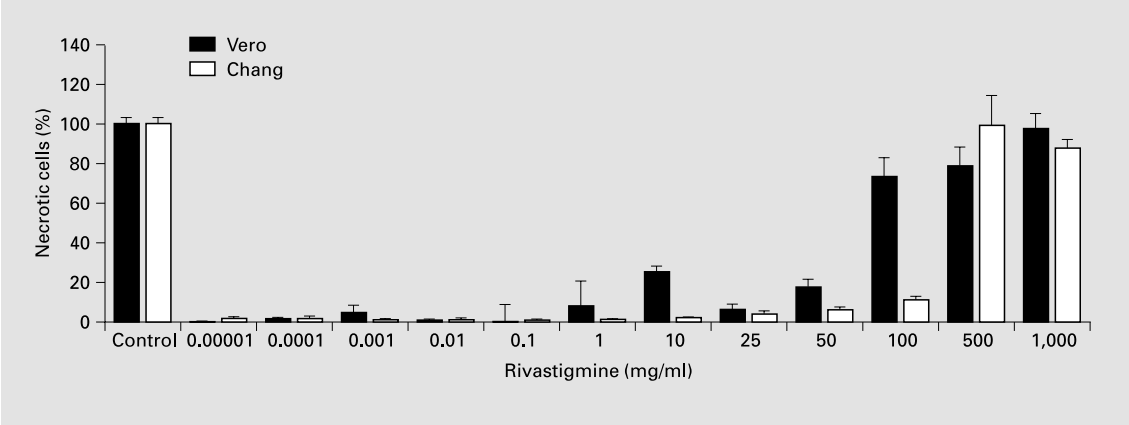

Fig. 3. Propidium-iodide assay for acute toxicity of rivastigmine in Chang and Vero cells: the permeability of cells to propidium iodide – an index of necrosis – when exposed to different concentrations of propidium iodide is expressed as a percentage of maximal fluorescence. Maximal fluorescence (control) was established by completely permeabilizing cell membranes with the detergent Tween 20, thereby inducing necrosis of all cells. Each bar represents the mean of the 4 experiments. The membrane permeability of Chang and Vero cells increased significantly at a rivastigmine concentrations above 50 mg/ml ($p < 0.05$).

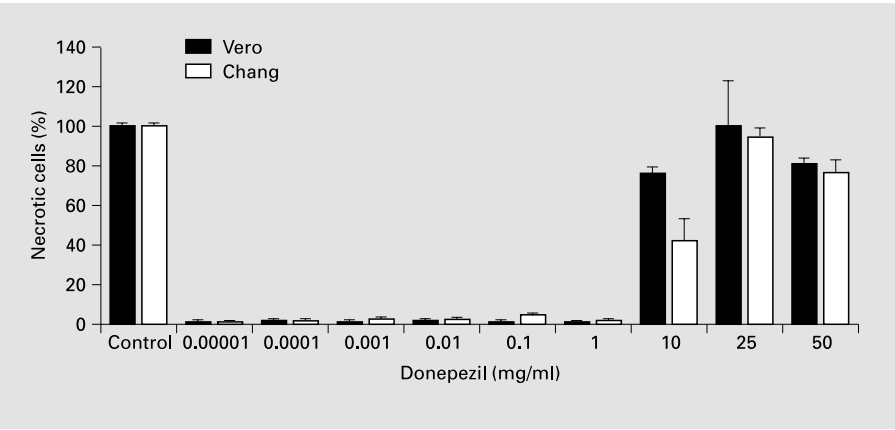

Fig. 4. Propidium-iodide assay for acute toxicity of donepezil in Chang and Vero cells: the permeability of cells to propidium iodide is expressed as a percentage of maximal fluorescence. Maximal fluorescence (control) was established by completely permeabilizing cell membranes with the detergent Tween 20. Each bar represents the mean of the 4 experiments. The membrane permeability of Chang and Vero cells increased significantly at 10 mg/ml.

enzymes could be present in the human ciliary body, whereas only 1–3 of the AChE subtypes have been detected in other ocular tissues that have been subjected to qualitative analysis (iris, lens, aqueous, vitreous and retina) [25]. Nothing is known about the different effects of these isoenzymes, nor have their distribution principles and patterns been studied in ocular tissues. According to in vitro data, donepezil is highly specific for AChE as opposed to BuChE. The ratio of donepezil IC_{50} values for BuChE/AChE was 1,252 compared with a ratio of 12 for physostigmine [6]. Rivastigmine is also a potent, selective inhibitor of brain AChE. In animal studies, rivastigmine produced a 10-fold greater inhibition of AChE in the hippocampus and cortex than on BuChE and AChE in the heart, skeletal muscle and other peripheral tissues [27]. This lower inhibition of the non-central ChE explains the reduced severity of the peripheral side effects (gastrointestinal, cardiac, neuromuscular).

Donepezil and rivastigmine are both used in the treatment of Alzheimer's disease. It seems, therefore, highly worth noting that the cellular mechanisms underlying neuronal degeneration in glaucoma and the corresponding clinical findings parallel those described for Alzheimer's disease [28–31]. Furthermore, donepezil and rivas-

tigmine were found to have neuroprotective effects on neurological and motor functions [32–35]. Acetylcholine has been assumed by Kashii and Honda [36] to protect retinal neurons against glutamate neurotoxicity by stimulating the nicotinic receptors, which release dopamine. Therefore, any drug acting by either stimulating acetylcholine receptors at the neuroeffector junction (as in the case of direct-acting cholinergic agonists, e.g. pilocarpine) or inhibiting AChE, thereby potentiating the action of endogenous acetylcholine (cholinesterase inhibitors), might have neuroprotective effects. Such neuroprotective effects on the retina and optic nerve, if present after topical application, would broaden its therapeutic use and have an additive important effect above its direct IOP-lowering potency.

In conclusion, donepezil did not show a clinically relevant toxicity in concentrations from 1×10^{-5} to 1×10^{-2} mg/ml, whereas rivastigmine showed only minor, incongruent changes at concentrations between 1×10^{-5} and 1×10^{-1} mg/ml. Therefore, in a next step, both drugs should be tested in vivo to prove the ocular hypotensive potency of this obviously well-tolerated new category of antiglaucomatous agents. Additionally, they are assumed to have neuroprotective properties, which suggests further therapeutic benefit from these drugs in glaucoma treatment in the future. However, further investigations and clinical studies are urgently required to evaluate their clinical application and possible long-term side effects.

Acknowledgements

The authors would like to thank A. Enz (senior research scientist, nervous system, at Novartis Pharma Inc., Switzerland) for providing rivastigmine and J.R. Ieni (director, medical affairs, at Eisai Inc., USA) for providing donepezil. They are also indebted to Barbara Lévy for her helpful collaboration and technical assistance.

References

1 Kronfeld PC: Eserine and pilocarpine: Our 100-year-old allies. Surv Ophthalmol 1970;14:479–485.
2 De Roetth A Jr: Lens opacities in glaucoma patients on phospholine iodide therapy. Am J Ophthalmol 1966;62:619–628.
3 Hiscox PE, McCulloch C: The effect of echothiophate iodide on systemic cholinesterase. Can J Ophthalmol 1966;1:274–282.
4 Enz A, Floersheim P: Cholinesterase inhibitors: An overview of their mechanisms of action; in Becker R, Giacobini E (eds): Alzheimer Disease: From molecular Biology to Therapy. Boston, Birkhäuser, 1996, pp 211–215.
5 Rogers SL, Yamanishi Y, Yamatsu K: E2020. The pharmacology of a piperidine cholinesterase inhibitor; in Becker RE, Giacobini E (eds): Cholinergic Basis for Alzheimer Therapy. Boston, Birkhäuser, 1991, vol 3, pp 314–320.
6 Sugimoto H, Iimura Y, Yamanishi Y, Yamatsu K: Synthesis and structure-activity relationships of acetylcholinesterase inhibitors: 1-Benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl) methyl]piperidine hydrochloride and related compounds. J Med Chem 1995;38:4821–4829.
7 Massoulié J, Bon S: The molecular forms of cholinesterase and acetylcholinesterase in vertebrates. Annu Rev Neurosci 1982;5:57–106.
8 Lachman C, Wilson IB: Multiple forms of acetylcholinesterase from the ciliary body of the human eye. Exp Eye Res 1974;19:195–199.
9 Goldblum D, Garweg JG, Böhnke M: Topical rivastigmine, a selective acetylcholinesterase inhibitor, lowers intraocular pressure in rabbits. J Ocul Pharmacol Ther 2000;16:29–35.
10 Goldblum D, Garweg JG, Böhnke M: Topical donepezil, a specific acetylcholinesterase inhibitor, lowers intraocular pressure in rabbits. Ophthalmic Res 1999;31(suppl 1):130.
11 Ventura AC, Böhnke M: Toxicity of pentoxifylline on monolayers of highly proliferative cells of epithelial origin. J Ocul Pharmacol Ther 1999;15:525–535.
12 Draize JM, Woodard G, Calvery HO: Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Therap 1944;82:377–390.
13 Boisjoly HM, Sun R, Giasson M, Beaulieu A: Topical fibronectin and aprotonin for keratectomy wound healing in rabbits. Arch Ophthalmol 1990;108:1758–1763.
14 Cejkova J, Lojda Z, Salonen EM, Vaheri A: Histochemical study of alkali-burned rabbit anterior eye segment in which severe lesions were prevented by aprotinin treatment. Histochemistry 1989;92:441–448.
15 Wenzel DG, Cosma GN: A quantitative metabolic inhibition test for screening toxic compounds with cultured cells. Toxicology 1983;29:173–182.
16 Gunther K, Scharf HP, Puhl W: In vitro toxicity tests of bioceramics and bone transplants in fibroblast culture (transl from German). Biomed Tech (Berl) 1993;38:249–254.
17 Barile FA, Dierickx PJ, Kristen U: In vitro cytotoxicity testing for prediction of acute human toxicity. Cell Biol Toxicol 1994;10:155–162.
18 Cottin M, Zanvit A: Fluorescein leakage test: A useful tool in ocular safety assessment. Toxicol in vitro 1997;11:399–405.
19 Wilson SE, He YG, Weng J, Zieske JD, Jester JV, Schultz GS: Effect of epidermal growth factor, hepatocyte growth factor, and keratinocyte growth factor, on proliferation, motility and differentiation of human corneal epithelial cells. Exp Eye Res 1994;59:665–678.
20 De Saint Jean M, Debbasch C, Brignole F, Rat P, Warnet JM, Baudouin C: Toxicity of preserved and unpreserved antiglaucoma topical drugs in an in vitro model of conjunctival cells. Curr Eye Res 2000;20:85–94.
21 Tripathi BJ, Tripathi RC, Kolli SP: Cytotoxicity of ophthalmic preservatives on human corneal epithelium. Lens Eye Toxic Res 1992;9:361–375.
22 Lin CP, Böhnke M: Influences of methylcellulose on corneal epithelial wound healing. J Ocul Pharmacol Ther 1999;15:59–63.
23 Mietz H, Niesen U, Krieglstein GK: The effects of preservatives and antiglaucomatous medication on histopathology of the conjunctiva. Graefes Arch Clin Exp Ophthalmol 1994;232:561–565.
24 Broadway DC, Grierson I, Hitchings R: Adverse effects of topical antiglaucoma medications on the conjunctiva. Br J Ophthalmol 1993;77:590–596.
25 Harris LS, Mittag TW, Shimmyo M: Properties of cholinesterases of human ocular tissues. Ophthalmologica 1972;165:153–160.
26 Leopold IH, Furman M: Cholinesterase isoenzymes in human ocular tissue homogenates. Am J Ophthal 1971;72:460–463.

27 Polinsky RJ: Clinical pharmacology of rivastigmine: A new-generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. Clin Ther 1998;20:634–647.
28 McKinnon SJ, Kerrigan-Baumrind LA, Pease ME, Mitchell R, Quigley HA, Zack DJ: Caspase-3 is activated in experimental rat glaucoma and cleaves amyloid precursor protein. Invest Ophthalmol Vis Sci 2000;41:S536.
29 Jonas JB, Schmidt AM, Müller-Bergh JA, Schlötzer-Schrehardt UM, Naumann GO: Human optic nerve fiber count and optic disc size. Invest Ophthalmol Vis Sci 1992;33:2012–2018.
30 Vickers JC: The cellular mechanism underlying neuronal degeneration in glaucoma: Parallels with Alzheimer's disease. Aust N Z J Ophthalmol 1997;25:105–109.
31 Tsai CS, Ritch R, Schwartz B, Lee SS, Miller NR, Chi T, Hsieh FY: Optic nerve head and nerve fiber layer in Alzheimer's disease. Arch Ophthalmol 1991;109:199–204.
32 Chen Y, Shohami E, Bass R, Weinstock M: Cerebro-protective effects of ENA713, a novel acetylcholinesterase inhibitor, in closed head injury in the rat. Brain Res 1998;16;784:18–24.
33 Chen Y, Shohami E, Constantini S, Weinstock M: Rivastigmine, a brain-selective acetylcholinesterase inhibitor, ameliorates cognitive and motor deficits induced by closed-head injury in the mouse. J Neurotrauma 1998;15:231–237.
34 Svensson AL, Nordberg A: Tacrine and donepezil attenuate the neurotoxic effect of A beta(25–35) in rat PC12 cells. Neuroreport 1998;11;9:1519–1522.
35 Taverni JP, Seliger G, Lichtman SW: Donepezil mediated memory improvement in traumatic brain injury during post acute rehabilitation. Brain Inj 1998;12:77–80.
36 Kashii S, Honda Y: How does one choose which drugs to study for ocular disease neuroprotection? 3rd International Symposium on Ocular Pharmacology and Pharmaceutics (ISOPP), Lisbon, 2000.