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Topical Rivastigmine, a Selective Acetylcholinesterase Inhibitor, Lowers Intraocular Pressure in Rabbits

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ABSTRACT

Non-selective acetylcholinesterase (AChE) inhibitors are known hypotensive agents. The purpose of the present investigation was carried out to ascertain whether rivastigmine, a selective carbamate-type inhibitor of AChE, which inhibits selectively an isoform of this enzyme found almost exclusively in the central nervous system, is able to depress the intraocular pressure (IOP) in normotensive rabbits.

IOP was monitored with a TonoPen XL in conscious adult rabbits before and hourly up to 8 hr after administration of the drug. Baseline measurements without treatment and after one single topical application of rivastigmine [1% (n=8); 2% (n=4); and 5% (n=6)] to the right eye and of the vehicle alone to the left one were performed.

Rivastigmine reduced the IOP of treated eyes significantly ($p<0.05$) in a dose-independent manner. Maximal effects of 23.2% (5% rivastigmine), 19.6% (2% rivastigmine) and 15.2% (1% rivastigmine) were achieved 1, 3 and 5 hr after application of the drug. A non-significant reduction of IOP in the contralateral eye was also observed. Rabbits evidenced no signs of discomfort after administration of rivastigmine. No conjunctival discharge or other signs of drug related local toxicity were found.

Rivastigmine, a selective antagonist of AChE, lowers IOP significantly and may thus be of potential use in glaucoma therapy.

INTRODUCTION

Cholinergic agonist 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 either stimulating acetylcholine receptors at the neuroeffector junction (as in the case of direct acting cholinergic agonists) or inhibiting AChE, thereby potentiating the action of endogenous acetylcholine (cholinesterase inhibitors). Actually, only three unspecific cholinesterase inhibitors (demecarium, echothiophate and physostigmine) have been clinically approved for the treatment of glaucoma. 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, and such side effects have contributed largely to the unpopularity of these agents.

Recently, new selective 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 which preferentially inhibits the globular monomer (G1) form of the different molecular enzymatic forms of the AChE (4-6). The most abundant form of AChE found in the brain is the tetrameric G4. G1 is also present mainly in smaller amounts therein, whereas the dimer, G2, is found dominantly in human erythrocytes (7). It seems that 3 different molecular forms of AChE in the human ciliary body exist (8). The distribution of the molecular 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 mimic that manifested in the brain. On the basis of this tenet, an inhibitor of the G1 form, such as rivastigmine, might logically be expected to depress IOP without inducing the systemic side effects commonly associated with the use of unspecific AChE inhibitors acting on AChE more widely distributed amongst bodily tissues and organs.

The purpose of the present study was to ascertain whether rivastigmine would be able to lower IOP after topical application, and to evaluate its possible acute side effects in normal conscious rabbits.

MATERIALS AND METHODS

Animals

Male and female adult brown burgundy rabbits (2.6 - 4.4 kg) were used in this study. The animals were kept in individual cages under well-defined and standardized conditions (humidity and temperature controlled room, 13 hr light/11 hr dark cycle) with standard dry food and water *ad libitum*. All animals were accustomed to the procedure of IOP measurement, but only those animals that had stable records were included in the study. All experiments were conducted in accordance with the ARVO resolution for the use of animals in ophthalmic and vision research and were approved by the Federal and Local Ethical and Agricultural Committees.

Drugs

Rivastigmine (S)-N-Ethyl-N-methyl-3- [1-(dimethylamino) ethyl]-phenyl carbamate, hydrogen-(2R, 3R)-tartrate was obtained from Novartis Pharma Ltd., Basel, Switzerland. Topical anesthesia of the cornea was induced with Novocaine 0.2% (Inselspital Pharmacy) eye drops.

Solutions of the test drug were freshly prepared for each experiment by dissolution in BSS (Alcon Pharmaceuticals Ltd., Fort Worth, TX, USA) at the required concentrations under sterile conditions. One 50-μL drop of the test solution was applied topically to the right eye whereas the contralateral eye received the carrier BSS only.

IOP Measurements

IOP was measured with a TonoPen XL (Mentor, Norvel, MA), the device being calibrated daily according to the manufacturer's instructions. The first measurement result with a coefficient of variation displayed <5% was noted. Less than 5% of the measurements were repeated until the coefficient of variation displayed was <5%. Corneas were anesthetized by topical application of a 50-μL drop of 0.2% Novocaine prior to each IOP measurement.

Measurements were always initiated at the same time (8 a.m.); a sufficient recovery period of at least 7 days was provided for the animals between the experiments. Control readings were taken 10 minutes before instillation of the test drug to the right eye and of the vehicle to the left one. IOP was recorded at 1 hr intervals for the ensuing 8 hr. Baseline measurements were likewise performed hourly in all animals prior to treatment, for monitoring of diurnal rhythms (9).

Pupil Diameter

Pupil diameter was measured horizontally in both eyes using a pupil gauge closely applied to the cornea under diffuse illumination conditions.

Slit-Lamp Examination

Slit-lamp biomicroscopy was performed by a trained ophthalmologist before drug administration and 4 and 8 hr thereafter. Eyes were controlled for conjunctival redness and discharge, for integrity of the corneal epithelium, and for the absence or presence of flare (protein in the anterior chamber being an indication of blood aqueous barrier breakdown).

Statistical Analysis

Data were analyzed according to the Mann-Whitney U-test. IOP values recorded before and after application of rivastigmine or the drug vehicle were compared in the same eye, the differences with a first order error of $P<0.05$ being considered as statistically significant.

RESULTS

Rivastigmine lowered the IOP significantly in the treated eye in a more-or-less dose-independent manner. Maximal mean decreases in IOP were time-staggered according to the concentration of the drug solution used, occurring 1, 3 and 5 hr after application of 5% (3.5 ± 1.2 mm Hg), 2% (2.2 ± 0.8 mm Hg) and 1% rivastigmine (2.6 ± 1.2 mm Hg), respectively. After administration of the 1% rivastigmine solution, the longest significant IOP-lowering effect was observed during 5 hr in the treated eyes (Fig. 1A). In untreated eyes, maximal mean decreases in IOP were likewise time-staggered, occurring 1, 3 and 4 hr after application of 5%, 2% and 1% rivastigmine, respectively, to the contralateral ones. However, only the effect induced by 5% rivastigmine was significant (Fig. 1B). Overall, in the treated and nontreated eyes, the maximal IOP decrease occurred 1 hr after administration of the 5% drug solution.

An insignificant miotic pupil reaction was observed in some drug-treated eyes. No signs of rivastigmine-related local toxicity were manifested.

DISCUSSION

Within human ocular tissues, only true (i.e., acetyl) cholinesterase is found (10). In proteins of rabbit ocular tissue homogenates, true cholinesterase is largely present with a small amount of pseudocholinesterase also present (11).

It seems that 5 of the different isoenzymes could be present in the human ciliary body whereas only 1 to 3 of the AChE subtypes have been detected in any of the ocular tissues that have been subjected to qualitative analysis (sclera, cornea iris, lens, aqueous, vitreous and retina) (10,12). Nothing is known about the different effects of these isoenzymes nor has their quantitative

distribution in the ocular tissue been studied. Since the ciliary body derives embryologically from the neuroectoderm, we thought it not unlikely that the distribution of AChE subtypes therein reflected that, manifested in the brain. On the basis of this assumption, we deemed it possible that rivastigmine – a specific inhibitor of the G1 subtype of AChE, which is found almost exclusively in the brain – might have a depressive action on IOP without eliciting the undesired side effects on other ocular and systemic tissues that are commonly encountered when using less specific AChE inhibitors. To prove a potential relevance of the hypothesis, this preliminary single application study was conducted.

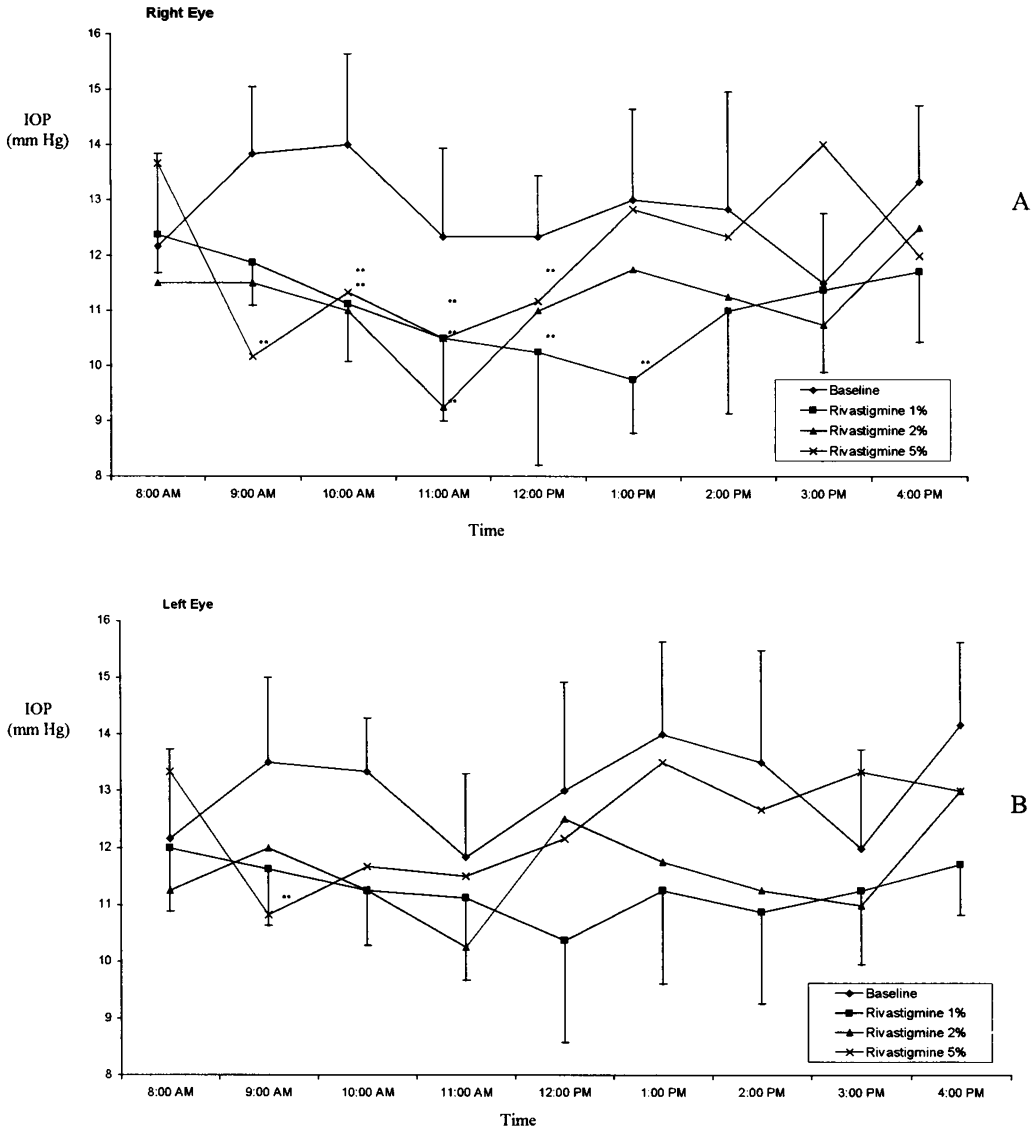

FIGURE 1. Baseline Measurements (Positive SD) and Mean IOP Measurements Recorded after Topical Application of a Single 50-µL Drop of 1% (N=8) (Negative SD), 2% (N=4) and 5% (N=6) Rivastigmine to the Right Eye (**A**) and of a Similar Volume of the Drug Vehicle to the Left (**B**) of Normotensive Adult Rabbits. In the drug-treated group, maximal effects occurred after 1 hr (5% rivastigmine), 3 hr (2% rivastigmine) and 5 hr (1% rivastigmine). In the untreated eyes, maximal IOP reductions occurred 1, 3 and 4 hr after administration of 5%, 2% and 1% rivastigmine, respectively, to the partner ones (**$P<0.05$).

A single topical application of rivastigmine to pigmented normotensive rabbits' eyes reduced IOP in a dose-independent manner. Although the measurements were not performed in a 'blind' fashion, use of the automated TonoPen XL, and recording only the first measurement with coefficient of variation displayed as <5%, reduced any influence of the investigator to a minimum. The TonoPen XL has been shown to be one of the tonometers of choice available for measuring IOP in rabbits (13).

The acceptability of topically applied rivastigmine was high, no significant ocular side effects having been observed. The profile of the systemic effects of rivastigmine found in toxicologic studies suggests that relevant side effects are not likely to occur. Furthermore, the degree to which the substance is metabolized by the cytochrome-P450-isoenzymes is negligible. Hence, the chances of its interacting systemically with other drugs that are metabolized *via* this P450 system are remote. Nevertheless, the hypotensive effects observed in untreated contralateral eyes suggest that rivastigmine was conveyed systemically thereto, although other mechanisms also could have contributed to the response.

As has been postulated for other cholinergic agents, the IOP-lowering effect of rivastigmine probably results from contraction of the ciliary body, which, by pulling on the scleral spur, opens up the trabecular meshwork and thereby enhances aqueous humor outflow (14). Because we have only found a mild insignificant pupil constriction in the treated eye, alternatively, rivastigmine might act by inhibiting aqueous humor secretion, which has already been demonstrated in the case of pilocarpine (15,16). Lachmann and Wilson found three molecular forms of AChE with sedimentation coefficients of 7 S, 11 S and 15 S in the human ciliary body. The 7 S form was found to be globular and had a molecular weight of about 110000. This might correspond with the so-called G1 form. Still, nothing is known about the actions of these different molecular forms in the eye. It could be possible that selectively inhibiting only one isoenzyme leads to an isolated inhibition of aqueous humor secretion without affecting the ciliary muscle.

Given that rivastigmine is used in the treatment of Alzheimer's disease and that it has now been shown to have an IOP-lowering effect in rabbits, it is interesting to note that clinical findings pertaining to, and the cellular mechanisms underlying, neuronal degeneration in glaucoma parallel those described for Alzheimer's disease (17-19). Furthermore, rivastigmine has been recently shown to have a neuroprotective effect on neurological and motor functions in a closed-brain murine injury model (20). Such neuroprotective effect, if present after topical application, would broaden the therapeutic application to normotensive glaucoma and would have an additive effect above its direct IOP lowering potency.

In conclusion, rivastigmine may prove to be a potent and well-tolerated ocular hypotensive agent. It could also have a neuroprotective effect, and therefore have a therapeutic benefit in a future treatment of glaucoma. However, further investigations and clinical studies are required to evaluate its clinical utility and possible long-term side effects.

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