

SCIENTIFIC REPORT

Inhibition of experimental diabetic cataract by topical administration of RS-verapamil hydrochloride

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Aim: To investigate the efficacy of verapamil eye drops for inhibition of diabetic cataract in rats.

Methods: Diabetes was induced in 69 male Sprague-Dawley rats by an intraperitoneal injection of streptozotocin (65 mg/kg body weight). One group (DV) of animals was treated by instillation of one drop of 0.2% RS-verapamil hydrochloride in both eyes three times daily for 8 weeks. The placebo treated group (D) received the vehicle solution only. After 8 weeks the lenses were removed, inspected, and photographed using bright and dark field illumination. The transmission of He-Ne laser light was measured in the optical axis of each lens in order to determine the turbidity coefficient (t) as a measure of central lens opacity. Following digital image analysis, the integrated density as a measure of central and mid-peripheral opacities was determined.

Results: Lenses of both groups developed peripheral cortical opacities not affecting the optical axis. Advanced and paracentral cortical opacities were present in 10 (16.7%) of the placebo treated lenses (D) and two (3.8%) of the verapamil treated lenses (DV). Complete corticonuclear cataract developed in four (6.7%) of the lenses from group D but none of the lenses from group DV. The mean lens turbidity t was determined to be 0.019 (SEM 0.002) mm^{-1} ($n = 52$) in the verapamil treated diabetic rats (DV) and 0.042 (0.008) mm^{-1} ($n = 60$) in the placebo treated group (D). This difference was statistically significant ($p = 0.0054$). The mean integrated density was 274.91 (22.5) in group D ($n = 60$) and 196.28 (20.7) in group DV ($n = 37$). This difference was also significant ($p = 0.0037$).

Conclusion: Verapamil eye drops 0.2% administered three times daily are effective in inhibiting the progression of lens opacities in streptozotocin diabetic rats.

Cataract is one of the major causes of blindness worldwide and diabetes is the most important risk factor for cataract in western countries.¹ Possible pathophysiological mechanisms include activation of the polyol pathway causing increased hydration,² non-enzymatic glycosylation with cross linking of proteins,³ and calpain mediated proteolysis.⁴ Duncan and coworkers⁵ have pointed out the key role of calcium in the pathogenesis of cataract.⁶ Increased levels of Ca^{2+} in the lens induce opacification *in vitro*^{7,8} and have been measured in human lenses with age related cataract.⁹ Non-specific cation channels in lens membranes¹⁰ have been suggested to cause the age related increase in calcium.¹¹

The greatest increase in free Ca^{2+} levels has been measured in opaque lens regions.¹² The increased Ca^{2+} concentration leads to formation of high molecular weight proteins with increased light scattering¹³ and reduction of gap junction permeability with disintegration of lens fibres.¹⁴

Many anti-cataractogenic agents, such as aldose reductase inhibitors, have been described so far, but owing to lack of success in patients, no drug has yet been approved for clinical use.^{15,16}

Previously, it had been shown that diabetic^{17,18} or galactosaemic¹⁹ cataract in rats can be prevented by high dose oral or subcutaneous administration of verapamil without changing serum glucose levels. However, a systemic high dose administration would not be applicable in patients because of unacceptable side effects.

Recently, we have demonstrated, that RS-verapamil hydrochloride in aqueous solution readily penetrates into the eye after topical administration in rabbits without producing plasma drug levels that could lead to systemic side effects.²⁰

The purpose of this study was to investigate whether a topical formulation of verapamil may inhibit the progression of diabetic lens opacities.

METHODS

Animals

Eighty male Sprague-Dawley rats initially weighing 150–200 g were randomly assigned to the following groups: C (normal controls), V (healthy rats treated with verapamil eye drops only), D (diabetic rats treated with placebo eye drops—that is, vehicle solution), DV (diabetic rats treated with verapamil eye drops). Slit lamp examinations were performed at 6 weeks. Eight weeks following diabetes induction by an intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg body weight, the eyes were enucleated following cervical dislocation under ether anaesthesia and the lenses were dissected free and kept in a cell culture dish with isotonic Dulbecco's phosphate buffered saline (PBS). Seven non-responders to STZ (blood glucose measured using an enzymatic test <150 mg/100 ml) and six animals that died before the end of the experiment were excluded leaving 67 animals in the investigation. The protocol was in accordance with the ARVO resolution on the use of animals in research.

Treatment

Verapamil eye drops 0.2% (305 μM , pH 7.1) were prepared by dilution of 8 ml of the intravenous solution containing 2.5 mg/ml RS-verapamil hydrochloride and 8.5 mg/ml NaCl (Isoptin, Knoll, Ludwigshafen, Germany) with 1 ml phosphate buffer pH 6.85 (containing 264 mg 4% sodium dihydrogen phosphate, 725 mg of 4% sodium monohydrogen phosphate, and 10 mg water) and 1 ml 2% methylhydroxypropylcellulose (Dispersa, Hettlingen, Germany).

Animals from groups DV and V were treated by instillation of 12 μl of 0.2% verapamil eye drops three times daily for 2 months. Rats from group D were treated by instillation of the vehicle solution only.

Evaluation of opacities

The lenses were inspected for opacities under an operating microscope and photographed using bright field and dark field illumination.

The lenses were centred under the 1 mm wide beam of a He-Ne laser (Laser Components, Groebenzell, Germany) with a power of 0.1 mW. Then, the voltage in a collecting photodiode mounted under the cell culture dish, was measured. The turbidity t as a measure of opacity was calculated for each lens using Lambert-Beer's equation²¹:

$$t \text{ (mm}^{-1}\text{)} = (\ln I_0/I) \cdot d^{-1}$$

where I is the intensity of the laser light transmitted through the lens, I_0 is the intensity of the laser beam transmitted through the cell culture dish without lens, and d is the central thickness of each lens measured using a micrometer eye piece.

Digital image analysis was performed using the software Adobe PhotoShop 5.0 and the public domain software NIH Image 1.62.^{22, 23} The digitised images consisted of pixels of different grey values between zero (white) and 255 (black). Since dark field illumination was used, clear lens areas resulted in white portions on the negatives whereas lens turbidities were seen as dark parts. After spatial calibration and correcting all images for uneven background illumination, the opacities in the central area comprising 70% of the lens diameter were evaluated, thus excluding the circular reflexes of the illumination device near the lens equator. The integrated density (ID)^{22, 23} of each lens was calculated as follows:

$$ID = N * (\text{mean grey value} - \text{background})$$

where N is the pixel number within the central 70% of the lens area, "background" is the most common pixel value after smoothing the histogram.²² The ID was expected to be low in clear lenses and high in opacified lenses.

RESULTS

Microscopic inspection of the lenses revealed that peripheral cortical opacities were present in both placebo treated diabetic lenses (D) and verapamil treated diabetic lenses (DV). Diffuse cortical opacities involving the paracentral area were seen in 10 (16.7%) lenses of group D and in only two (3.8%) lenses of group DV. Complete corticonuclear cataract was observed in four (6.7%) of the placebo treated diabetic lenses but none of the verapamil treated diabetic lenses (fig 1).

The turbidity (mean (SEM)) as a measure of opacity in the optical axis of each lens, was 0.003 (0.001) mm^{-1} in group C (n = 12), 0.002 (0.001) mm^{-1} in group V (n = 10), 0.042 (0.008) mm^{-1} in group D (n = 60), and 0.019 (0.002) mm^{-1} in group DV (n = 52) (fig 2). Mann-Whitney's U test revealed that the turbidity in verapamil treated diabetic lenses was significantly ($p = 0.0054$) lower than in placebo treated diabetic lenses.

The integrated density (mean (SEM)) was 96.89 (12.61) in group C (n = 12), 102.84 (25.6) in group V (n = 10), 274.91 (22.5) in group D (n = 60), and 196.28 (20.7) in group DV (n = 37) (fig 3). The Kruskal-Wallis test showed a significant difference between the four groups ($p < 0.0001$) and Mann-Whitney's U test calculated a significant difference between placebo treated and verapamil treated diabetic lenses ($p = 0.0037$).

The blood glucose levels (mean (SEM)) at 2 months were 373 (12) mg/100 ml in group D (n = 30), 343 (13) mg/100 ml in group DV (n = 23) (no significant difference between D and V), 51.6 (2.2) mg/100 ml in group C (n = 3) and 51.8

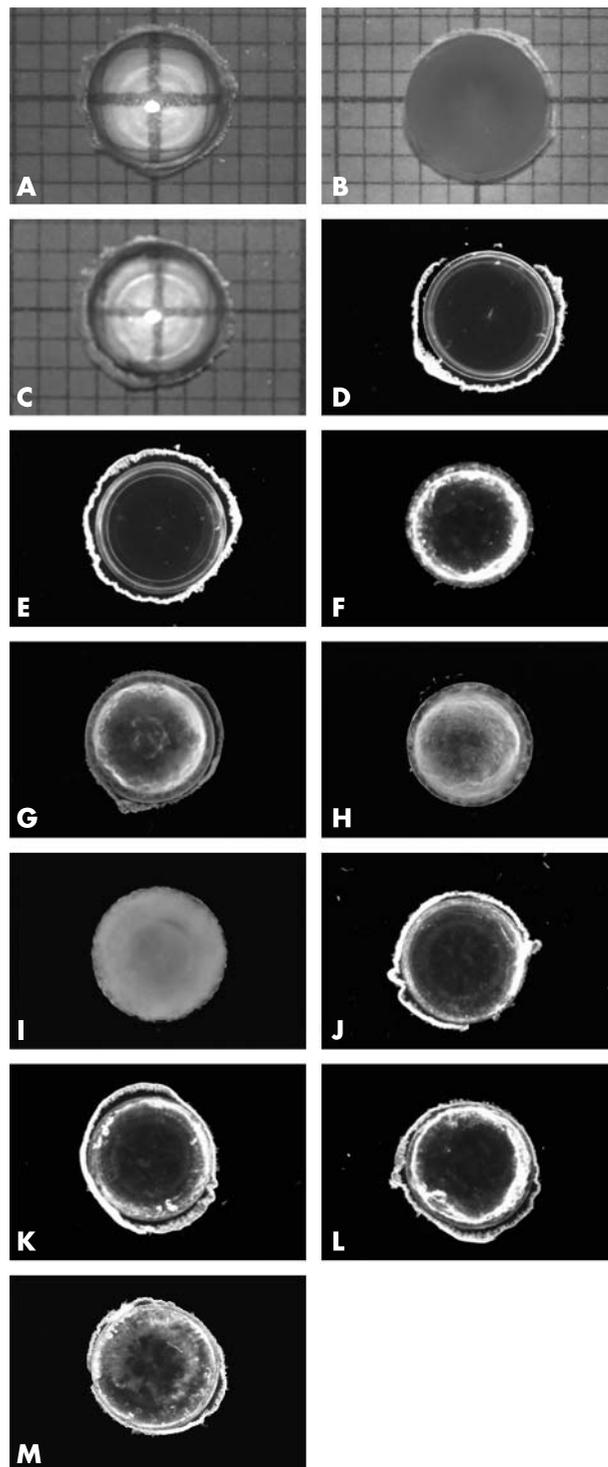


Figure 1 Bright field (A–C) and dark field (D–M) microphotographs of rat lenses. (A) Untreated normal lens (group C); (B) placebo treated diabetic lens (group D) showing total corticonuclear cataract; (C) verapamil treated diabetic lens (group DV); (D) untreated normal lens; (E) verapamil treated normal lens; (F, G) placebo treated diabetic lenses showing mainly peripheral cortical opacities; (H) placebo treated diabetic lens showing diffuse cortical opacities; (I) placebo treated diabetic lens showing total corticonuclear opacity; (J) verapamil treated diabetic lens showing mild peripheral cortical opacities; (K, L) verapamil treated diabetic lenses showing dense peripheral cortical opacities; (M) verapamil treated diabetic lens with diffuse cortical opacities representing one of the most opaque lenses of group DV.

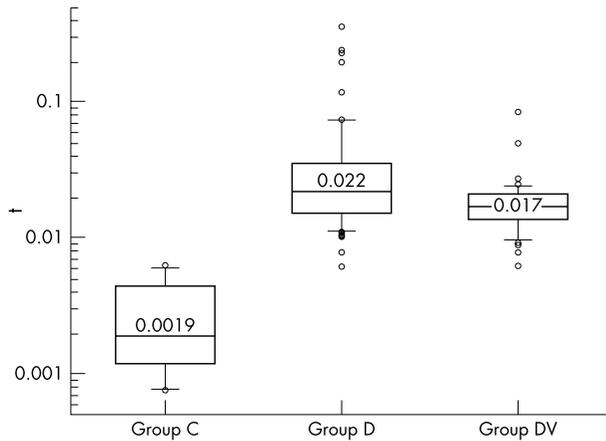


Figure 2 Box plots of the t values (mm^{-1}) from untreated, normal lenses (C), placebo treated diabetic lenses (D), and verapamil treated diabetic lenses (DV). The horizontal line inside the box represents the median (50th percentile), the borders of the box the 25th and the 75th percentile and the ends of the bars the 10th and the 90th percentile of the t values. Mann-Whitney's U test yielded a significant difference between group D and DV. Note the logarithmic scale of the y axis.

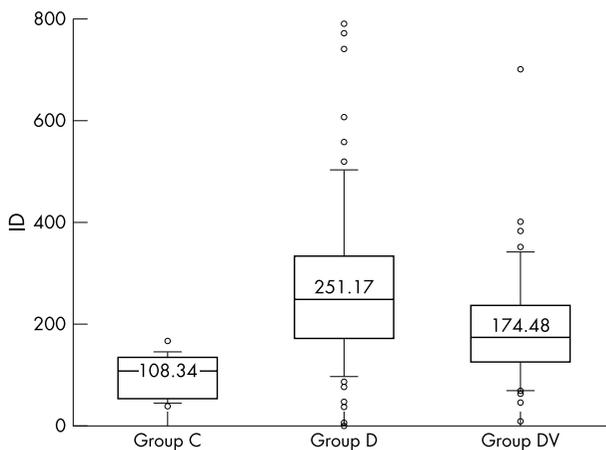


Figure 3 Box plots of the integrated density (ID) values from untreated, normal lenses (C), placebo treated diabetic lenses (D), and verapamil treated diabetic lenses (DV). The horizontal line inside the box represents the median, the borders of the box the 25th and 75th percentile and the bars the 10th and the 90th percentile of the integrated density values. Mann-Whitney's U test yielded a significant difference between group D and DV.

(1.6) mg/100 ml in group V ($n = 4$). On slit lamp examinations at 6 weeks, there were no signs of conjunctival or corneal side effects.

DISCUSSION

This investigation demonstrated that a topical administration of verapamil inhibited cataract formation in streptozotocin diabetic rats. A complete prevention of cortical opacification was not possible in this diabetes model where severe lens opacities developed within a short time. However, verapamil prevented the formation of opacities in the optical axis and the progression to total corticonuclear cataract. The mode of action of verapamil as an anti-cataractogenic drug is possibly related to an inhibition of lenticular calcium overload.^{17, 18} However, its effect may not entirely be ascribed to changes in lens calcium because it not only inhibited cortical opacities

(with known high calcium levels) but also nuclear cataract (with known low calcium levels).⁹ Although electrophysiological studies have revealed that lens cells contain Ca^{2+} channels with similar properties as in excitable tissues,²⁴ the pharmacological profile of these ion channels has not yet been characterised. It has been suggested that the inhibition of Ca^{2+} induced damage of lens fibres by verapamil is mediated by L-type calcium channels.²⁵⁻²⁷ However, in experiments using the L-type calcium channel selective radioligands $(-)-(^3\text{H})$ -desmethoxyverapamil and $(+)-(^3\text{H})$ -isradipine, we had no evidence of saturable, high affinity binding sites in membrane preparations from bovine lens epithelial cells indicating that L-type calcium channels either occur at a much lower density than in electrically excitable cells or are absent (unpublished results).

Calcium channel blockers are known to interact with a variety of other calcium transport structures including non-L-type calcium channels.²⁸ Some of these structures, such as Ca^{2+} ATPase,²⁹⁻³¹ $\text{Na}^+/\text{Ca}^{2+}$ transporter^{32, 33} and receptor mediated calcium entry pathways³⁴ have been identified in lens membranes. Acetylcholine induced lens membrane oscillations, for example, can be blocked by the L-type Ca^{2+} channel blocker nifedipine.³⁵ D 600, the methoxy analogue of verapamil, has been suggested to block calcium influx into lens cells.³⁶ Possibly, the anti-cataractogenic action of verapamil is related to blockage of non-L-type receptor operated calcium channels.

Verapamil also prevents the accumulation of sorbitol in rat lenses and has therefore been suggested to act as an aldose reductase inhibitor (ARI)³⁷ (despite a low inhibition constant³⁸). Interestingly, ARIs may also have actions not related to the polyol pathway.³⁹

Further investigations using a variety of other compounds including pure stereoisomers of calcium antagonists are needed to identify the pharmacological profile of the anti-cataractogenic effect of verapamil. Since many pathophysiological aspects of diabetic cataract^{12, 40} resemble the changes in senile cataract, further evaluation of verapamil for the prevention of senile cataract is warranted. In patients suffering from cataract and glaucoma, verapamil eye drops may be beneficial for both diseases since they have also been demonstrated to lower intraocular pressure.⁴¹

CONCLUSION

A topical administration of the calcium antagonist RS-verapamil in a concentration of 2 mg/ml inhibits cataract formation and progression in a diabetic animal model.

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