

Effect of Silibinin in Lowering the Intraocular Pressure in Normotensive Rabbits: Interaction with Pilocarpine and Cyclopentolate

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Abstract

Previous data indicated the effectiveness of silibinin as intraocular pressure (IOP) - lowering agent. The present study was performed to evaluate the interaction of silibinin with pilocarpine or cyclopentolate in lowering IOP in normotensive rabbits. The effects of topically instilled silibinin hemisuccinate solution (0.75%) alone or adjunctly combined with 2% pilocarpine or 1% cyclopentolate on the IOP of normotensive rabbits were evaluated using indentation tonometry. The results showed that 0.75% solution of silibinin was found more potent than pilocarpine (2% drops) in lowering IOP of normotensive rabbits, while their combination results in longer duration of action. Moreover, the elevated IOP values produced by cyclopentolate (1% drops) were decreased by silibinin, while prior instillation of cyclopentolate did not interfere with the IOP-lowering effect of silibinin. In conclusion Silibinin lowers IOP in normotensive rabbits more than pilocarpine, and their combination elongates the duration of the IOP-lowering effect. This might be due to interference with aqueous humor formation as a possible mechanism. In addition, cyclopentolate did not significantly alter the effect of silibinin on IOP.

Key words: silibinin, IOP, pilocarpine, cyclopentolate

لقد ثبت في دراسة سابقة فعالية السيليبينين في خفض الضغط الداخلي لعين الارنب، تم تصميم هذه الدراسة لتقييم التداخل بين السيليبينين والبايلوكاربيين أو السايكلوبنتوليت عند استخدامهم موضعياً في العين. تمت دراسة تأثير الاستخدام الموضعي في العين لمحلول 0.75% سيليبينين لوحده أو بوضعه قبل أو بعد 2% قطرة بايلوكاربيين أو 1% سايكلوبنتوليت على خفض الضغط الداخلي لعين الارانب الطبيعية باستخدام تقنية Indentation tonometry. أظهرت النتائج بأن تأثير 0.75% سيليبينين في خفض ضغط العين الداخلي أكبر من 2% بايلوكاربيين وأن استخدامهما معا يؤدي الى اطالة مدى مثل هذا التأثير. كما ثبت بأن للسيليبينين القدرة على تقليل الارتفاع الحاصل في ضغط العين الداخلي الذي أحدثته مادة السايكلوبنتوليت 1%، بينما لم يرتفع ضغط العين عند استخدام الاخير قبل السيليبينين. وعليه يمكن ان نستنتج بأن السيليبينين افضل من البايوكاربيين في خفض ضغط العين الداخلي لعين الارانب وان استخدامهما معا يطيل مدة هذا التأثير، ويمكن أن يعزى هذا التأثير الى التداخل مع عملية تكوين ماء العين الداخلي كيميائية عمل للسيليبينين. اضافة الى ان الساكلوبنتوليت لم يؤثر على فعالية السيليبينين في خفض الضغط الداخلي للعين.

Introduction

Two mechanisms underlay the effectiveness of various drugs implicated in the management of elevated intraocular pressure (IOP), the reduction in aqueous humor (AH) inflow and enhancement of AH outflow ⁽¹⁾. Reduction of aqueous inflow was observed due to the use of β -adrenoceptor blockers ⁽²⁾, carbonic anhydrase inhibitors ⁽³⁾ and others including Forskolin ⁽⁴⁾. However, reduction of AH inflow involves either vascular ^(5, 6) or ionic mechanisms ⁽⁷⁾. In a recent study, we reported on a decrease in IOP of normotensive rabbits after ocular instillation of silybinin solution ⁽⁸⁾. It has been suggested that a direct pharmacological effect on trabecular pathway to be likely involved in pilocarpine-induced fall in IOP ⁽⁹⁾. The role of cAMP in AH regulation is very well explained ^(10, 11). It inhibits AH inflow by

blocking ion transport across ciliary epithelium ⁽¹²⁾. Inhibition of $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ by cAMP decreases the uptake of Cl^- by ciliary epithelium ⁽¹³⁾, and the increase in cAMP level, stimulated by β_2 -adrenergic receptor activation in the trabecular meshwork cells, suggested to be responsible for the increased outflow facility after application of β_2 -agonists ⁽¹⁴⁾. Silibinin hemisuccinate, a powerful antioxidant flavonoid ⁽¹⁵⁾, inhibits cAMP-Phosphodiesterase enzyme, even more potent than theophylline or papaverine ⁽¹⁶⁾, an effect that can be utilized for the interference with AH formation and IOP regulation. The present study was designed to examine the interaction between silibinin and pilocarpine or cyclopentolate in lowering IOP of normotensive rabbits, and to provide preliminary evidence for the mechanism of this effect.

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Materials and Methods

Thirty-five New Zealand white rabbits weighing 1.5-2.5 kg were used in this study. Animals were kept in the animal house of the College of Pharmacy, University of Baghdad, under standardized conditions (12 hrs light-dark cycles at room temperature), and were fed a standard diet (Quality Control Laboratories, MOH, Iraq) and water *ad libitum*.

Drugs treatment

Silibinin hemisuccinate in pure form was a gift from Tolbiac SRL, Argentina, and all other compounds used during the study were supplied by the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. Silybinin hemisuccinate was dissolved in arachis oil and used freshly prepared (0.75%) solution. Pilocarpine (2%) and cyclopentolate (1%) were used as commercial eye drop formulas (Alcon Pharmaceuticals LTD, Cham, Switzerland). The animals were allocated into 7 groups (5 rabbits each) for studying the effect of topically instilled silybinin hemisuccinate alone, pilocarpine alone, cyclopentolate alone, the effect of pilocarpine or cyclopentolate instilled 30 min prior to silybinin and the effect of prior instillation of silybinin 30 min before pilocarpine or cyclopentolate.

Measurement of IOP

Indentation tonometry, using Schiøtz tonometer, was utilized in this study for measuring IOP before and after instillation of drugs or drug vehicle (Arachis oil). Thirty minutes before starting drug instillation, the cornea was anesthetized with 0.5% tetracaine HCl (Chauvin Pharmaceuticals Ltd, Surry, England) and baseline IOP was measured using Schiøtz tonometer. After instillation of 1 drop of silibinin hemisuccinate (0.75%), pilocarpine (2%) and cyclopentolate (1%), measurement of IOP was performed every 30 min for 3 hr⁽¹⁷⁾. After each measurement the eyes were washed with normal saline and the instrument was cleaned with diethyl ether. All experiments were conducted in a masked manner, and were performed during a fixed time of the day (from 10:00 AM to 3:00 PM) to exclude the effect of circadian changes in IOP. For assessment of the pre-instillation effect of pilocarpine or cyclopentolate, baseline IOP was recorded before instillation of these drugs to both eyes, and then 1 drop of freshly prepared silibinin hemisuccinate oily solution was applied after 30 min to both eyes. The IOP was measured every 30 min for 3 hr. The same later approach was followed to evaluate the effect of pre-instillation of silibinin on that produced by pilocarpine and

cyclopentolate. All results were expressed as mean \pm S.E. Comparisons with baseline were made using Student's paired *t*-test, while a single-factor analysis of variance (ANOVA) for repeated measurements was used to evaluate differences between groups. *P* values less than 0.05 were considered significantly different.

Results

Effects of 0.75% silibinin, 2% pilocarpine and 1% cyclopentolate

In normotensive rabbits, instillation of 0.75% silibinin decreases IOP significantly for 2.5 hr, and remains significantly different with respect to baseline value at all measured time points (Figure 1). The maximal decrease in IOP was achieved after 1 hr of instillation (38.56 %) compared to baseline ($P < 0.05$). Pilocarpine eye drops (2%) significantly reduces IOP after 30 min of instillation (21.5%, $P < 0.05$), decreased gradually to non-significant value (0.46%, $P > 0.05$) at the end of 3 hr compared to baseline (Figure 1). Instillation of 1 drop cyclopentolate (1%) significantly elevates IOP, reaching maximum value after 0.5 hr (34.76 %); then gradually decreases with time (4.24 %) after 2.5 hr of instillation.

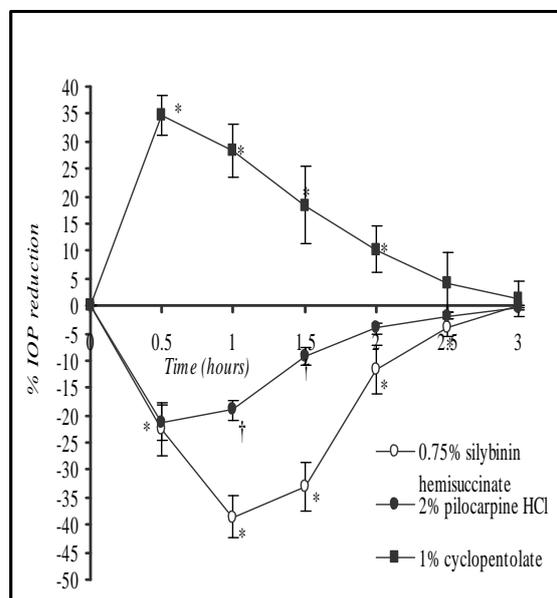


Figure 1: Effect of 0.75% silibinin hemisuccinate, 2% pilocarpine HCl and 1% cyclopentolate HCl on IOP in normotensive rabbits; results are presented as mean of percent changes \pm SEM; n=10 eyes for each group; * $P < 0.05$ with respect to baseline; † $P < 0.05$ with respect to 0.75% silibinin alone.

Effects of pre-treatment with 2% pilocarpine and 1% cyclopentolate

The IOP-lowering effect of pilocarpine was enhanced when silibinin instilled 30 min latter, 26.87% compared to 19.0% produced by pilocarpine alone ($P<0.05$). The reduction in IOP observed in this procedure remains significantly high (40.88%, 49.5%, 43.34%, and 26.87%) compared to that produced by pilocarpine alone (9.23%,4.16%,1.81% and 0.46%) for the time intervals 1.0, 1.5, 2.0, and 2.5 hr respectively ($P<0.05$) (Figure 2). Moreover, this level reduction in IOP was significantly different compared to that produced by silibinin alone along the entire period beyond the addition of silibinin. When cyclopentolate instilled 30 min prior to silibinin, the latter still have the ability to reverse the elevation of IOP produced by cyclopentolate (from 34.76% to 17.3%) after 30 min of instillation ($P<0.05$); these changes are not significantly different at the time intervals 1.0, 1.5, and 2.0 hr after instillation of silibinin, $P>0.05$ (Figure 3).

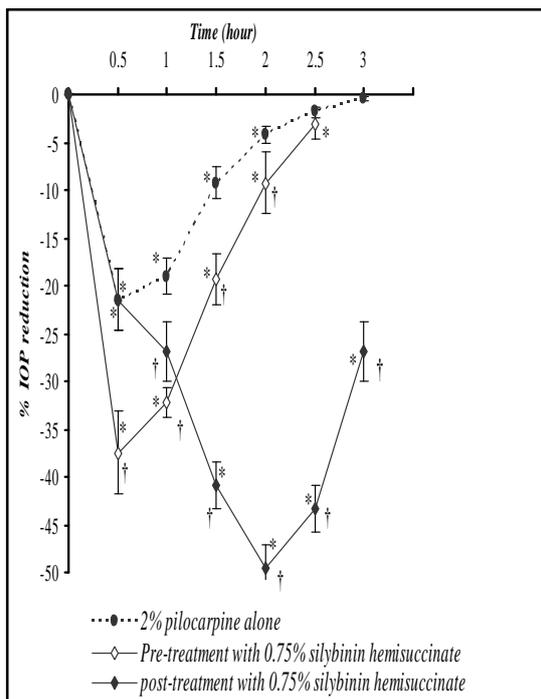


Figure 2: Effect of pre- and post treatment with 0.75% silibinin hemisuccinate on IOP lowering effect of 2% pilocarpine in normotensive rabbits; results are presented as mean of percent changes \pm SEM; n=10 eyes for each group; * $P < 0.05$ with respect to baseline; † $P < 0.05$ with respect to 2% pilocarpine alone.

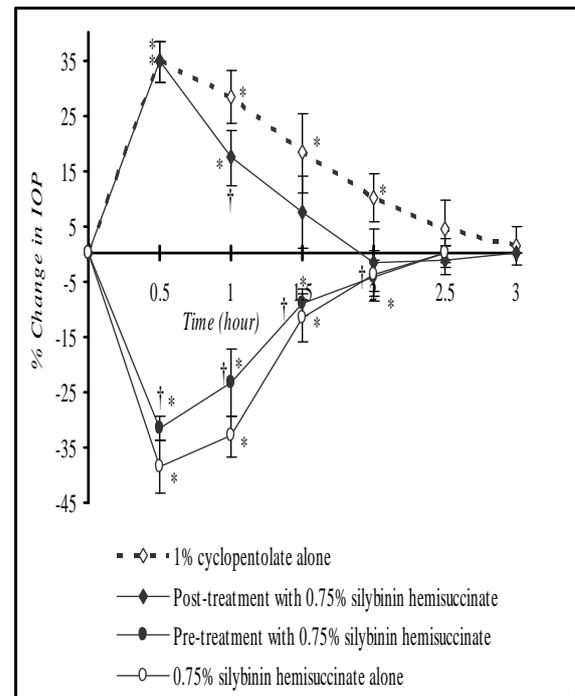


Figure 3: Effect of pre- and post treatment with 0.75% silibinin hemisuccinate on IOP rising effect of 1% cyclopentolate in normotensive rabbits; results are presented as mean of percent changes \pm SEM. n=10 eyes for each group; * $P < 0.05$ with respect to baseline; † $P < 0.05$ with respect to 1% cyclopentolate alone; Pre-treatment with silibinin hemisuccinate did not significantly differ from silibinin hemisuccinate alone $P > 0.05$.

Effects of pre-treatment with silibinin

Pre-treatment with silibinin, 30 min before instillation of pilocarpine resulted in additive effect on the IOP lowering activity of pilocarpine (37.5%), which was significantly different, compared to the effect of pilocarpine alone (21.5%). This reduction in IOP remains significantly different with respect to that produced by pilocarpine after 1.0, 1.5 and 2.0 hr. However, the effect of pre-treatment with silibinin did not significantly differ compared to that produced by silybinin alone during all time intervals ($P>0.05$) (Figure 1). Even when cyclopentolate instilled 30 minutes latter, pre-treatment with silibinin results in highly significant reduction in IOP (31.47% $P<0.05$), and remains significantly high 1.0, 1.5, and 2.0 hr after instillation of cyclopentolate (-20.08%, -8.92%, -4.12% respectively) compared with the rise in IOP (28.36%, 18.29%, 10.18% and 10.18%) produced by cyclopentolate alone ($P<0.05$). Cyclopentolate appeared to slightly reverse the action of silibinin when instilled 30 min latter; however, such effect did not significantly differ from that produced by silibinin alone ($P>0.05$).

Discussion

It has been reported previously in our laboratory that corneal instillation of silibinin lowers IOP in normotensive rabbits in a dose dependent manner. It also delays IOP recovery rate after I.V infusion of 20% sodium chloride solution⁽⁸⁾. Although inhibition of cAMP-phosphodiesterase is proposed as a suspected mechanism for the action of silybinin on IOP⁽¹⁸⁾, interference with the cholinergic influence in this respect was evaluated. In the present study, the effect of silibinin on IOP was higher than that produced by pilocarpine, and their combination results in an additive effect. Muscarinic agonists, including pilocarpine, lower IOP through enhancing AH outflow due to contraction of the iris sphincter⁽¹⁹⁾. According to the reported mechanisms of action of silibinin, targeting AH formation and interference with ion transport can be suggested as possible mechanisms⁽²⁰⁾. Consequently, the mechanisms through which pilocarpine and silibinin produce their effects can be utilized for explaining the additive effect reported when both of them are used at the same time. To confirm the idea that silibinin lowers IOP through a mechanism not related to the cholinergic system, the interaction of silibinin with anticholinergic agent like cyclopentolate was evaluated. Although there is no practical evidence on elevation of IOP in rabbits due to instillation of cyclopentolate, the results reported in this study demonstrated such effect, which can be attributed to the abnormal sensitivity of the locally bred strain of rabbits to the effect of cyclopentolate. In the present work, the rise in IOP produced by instillation of cyclopentolate was effectively reversed by silibinin; meanwhile, the IOP lowering effect of silibinin was not affected by postinstillation of cyclopentolate. Based on these data, one can postulate that silibinin interferes with IOP regulation through reduction of AH inflow. Taken together with the data obtained in previous study⁽⁸⁾ about the effect of silibinin on IOP recovery rate and its contralateral effect, one can suggest the interference with AH inflow as a mechanism involved in pilocarpine-silibinin and cyclopentolate-silibinin interactions. The IOP-lowering effect of cholinomimetics is mediated via the activation of the inositol triphosphate (IP₃) pathway that linked to M₃-receptors in the ciliary and iris-sphincter muscles⁽²¹⁾. Silibinin, on the other hand, strongly inhibits cAMP phosphodiesterase with consequent elevation of cAMP levels⁽¹⁶⁾. The later mediates many biological effects including the inhibition of ion transport by Na⁺-K⁺- 2Cl⁻ co-transporter

across ciliary epithelium and trabecular meshwork^(13, 22), which is similar to that produced by activation of the IP₃ pathway initiated by pilocarpine. Regulation of cell volume is very important phenomenon that involved during exposure to hypo- or hypertonic environment, and Na⁺ - K⁺- 2Cl⁻ co-transporter is the system responsible for such regulation^(23, 7, 24). Infusion of hypertonic sodium chloride solution resulted in shrinkage of ciliary epithelium, and to retain the original volume, ion transporters should be activated to transport Na⁺ and Cl⁻ across ciliary epithelium⁽²⁵⁾. Inhibition of this co-transporter by cAMP delayed osmotic recovery rate, an effect reported after silibinin administration. In conclusion, corneal instillation of silibinin lowers IOP in normotensive rabbits, probably through a mechanism not related to the interference with cholinergic influence on IOP.

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