The Effect of Levamlodipine in Glucose-Induced Acute Model of Glaucoma in Rabbits

Waleed K. Abdulsahib*

Department of Pharmacology and Toxicology, College of Pharmacy, Al-Farahidi University, Baghdad, Iraq

Abstract

BACKGROUND: Loss of vision and irreversible blindness are the main consequences of glaucoma. There are two main types of glaucoma: Chronic and acute.

AIM: This work aimed to evaluate the intracocular effect of levamlodipine on the acute model of glaucoma in rabbits.

METHODS: Eighteen white albino rabbits of both sexes weighing about 2 kg. We divided them into three groups (six animals in each group) used in the experiment. We use the right eye to evaluate the effect of the test drug and used the left eye as a control (vehicle only). We used the first group to evaluate levamlodipine (0.25%), the second group to estimate levamlodipine (0.5%), and the third group to assess pilocarpine 2% (positive control). Drugs were administered 30 min before induction.

RESULTS: Glucose (5%) fluid produces a significant intracocular pressure (IOP) elevation after 30 min of administration in the left eye (p < 0.001). Pre-treatment topical administration of levamlodipine (0.25%) prevents the rise in the IOP significantly (p < 0.001) in the right eye when compared to the control group (left eye). Moreover, compared with the eyes of the control group at all stages of the experiment, the topical administration of levamlodipine (0.5%) has a significant preventable effect (p < 0.001), compared with the control group. The IOP of the local pilocarpine (2%) in the third group was significantly decreased (p < 0.001). Finally, compared with levamlodipine (0.5%), pilocarpine has a more significant effect in preventing a rapid increase in intraocular pressure (p < 0.001).

CONCLUSION: Levamlodipine is a promising therapeutic agent for patients vulnerable to acute glaucoma.

Introduction

Loss of vision and irreversible blindness are the main consequences of glaucoma [1]. There are two main types of glaucoma: chronic and acute [2]. Acute glaucoma resulted from a blockage around the trabecular meshwork that allows aqueous humor (AH) to accumulate, leading to a rise in intraocular pressure (IOP) followed by the death of retinal ganglion cells (RGCs) and retinal ischemia-reperfusion injury [3].

Today, the most common way to treat glaucoma is to lower IOP. It is supposed that the harm to RGCs and axons can be minimal by IOP reduction. If mechanical force is the only pathophysiological mechanism in glaucoma, we can prevent glaucoma progression by IOP reduction. Due to the complexity of glaucoma, about 50% of patients have normal IOP with visual field defects, so there is insufficient attention to IOP reduction strategies [4]. In addition, there are a considerable number of patients with high-tension glaucoma in whom the progression of the disease despite reduced IOP is successful [5]. An essential cell membrane protein complex that facilitates the influx of Ca++ into the cell in response to membrane depolarization is the voltage-gated L-type calcium channel CaV1.2. The increase in intracellular Ca++ can act as a messenger to control many cellular processes, including contraction of muscle, secretion of the hormone, gene expression, and neuronal transmission. These physiological processes depend in part on the action of the CaV1.2 channel categorized as excitation-contraction, excitation-secretion, or excitation-transcription coupling [6], [7].

Amodipine, a fundamental derivative of dihydropyridine, prevents the calcium influx to peripheral vascular and coronary smooth muscle cells through “slow” channels, causing clear vasodilation in peripheral and coronary vascular beds. Amodipine is a racemic mixture of (S)- and (R)-amlodipine, but only the first has therapeutic efficacy [8]. (S)Amodipine, acknowledged as levalmidipine, is pharmacologically identical to amlodipine and has a role in vasodilation and blood pressure drop [9], [10]. In the present study, we investigated the effect of calcium channel blockers (CCBs), levamlodipine effect on IOP, and the possible mechanisms of action of this agent, the possible mechanisms of action of this agent.
Materials and Methods

The research started on approval of the Institutional Animal Ethics Committee of the Faculty of Pharmacy, Farahidi University.

Drug and chemicals

Pure powder of levamlodipine purchased from Selleck Chemicals, and phosphate buffer purchased from Sigma-Aldrich. About 5% glucose in water (Pioneer, Iraq), procaine hydrochloride drops (Alcaine, Belgium), and pilocarpine drops (API, Jordan) were purchased from the private market.

Experimental animals

We divided 19 white albino rabbits of both sexes (weighing about 2 kg) divided into three groups (six animals in each group) used in the experiment. We use the right eye to evaluate the effect of the test drug, and we use the left eye as a control (vehicle only). We use the first group to evaluate levamlodipine (0.25%), use the second group to evaluate levamlodipine (0.5%), and the third group to evaluate pilocarpine 2% (positive control). Drugs were administered 30 min before induction. We perform an acute model of glaucoma using 5% glucose by intravenous injection at the marginal vein of the ear in dose 15 mL/kg. Before starting induction of acute glaucoma (0 times), we measure the IOP using a Schiotz tonometer and subsequently every 15 min until 105 min [2]. We carried the experimental work in the morning to avoid day and night fluctuations of intraocular pressure [11]. We used one or two drops of proparacaine to anesthetize the rabbit’s eye before each reading. We were freshly prepared the levamlodipine solution by diluting the required amount in phosphate buffer [12].

Statistical analysis

We used a paired Student’s t-test to analyze the data with a 95% probability level. We performed a split graph analysis of variance to study the time-dependent interaction between the drug and other drugs.

Results

Glucose (5%) fluid produces a significant IOP elevation after 30 min of administration in the left eye (p < 0.001). The pre-treatment administration of topical levamlodipine (0.25%) prevents the rise in the IOP significantly (p < 0.001) in the right eye when compared to the control group (left eye) at times (30, 45, 60, 75, 90, and 105), as shown in Figure 1 and Table 1.

| IOP (initial) | IOP after distilled water instillation |
|--------------|--------------------------------------|
|              | 30 min | 45 min | 60 min | 75 min | 90 min | 105 min |
| Left eye (control) | 32.8 ± 0.43 | 32.8 ± 0.43 | 32.8 ± 0.43 | 32.8 ± 0.43 | 32.8 ± 0.43 | 32.8 ± 0.43 |
| Right eye (levamlodipine 0.5%) | 30.55 ± 0.91 | 28.46 ± 0.42 | 25.38 ± 0.53 | 21.71 ± 0.64 | 20.33 ± 0.26 | 18.63 ± 0.29 |
| p-value       | 0.68   | 0.91   | 0.51   | 0.81   | 0.75   | 0.60   |

Moreover, compared with the eyes of the control group at all stages of the experiment, the topical administration of levamlodipine (0.5%) has a significant preventable effect (p < 0.001), as shown in Figure 2 and Table 2. Compared with the control group, the IOP of the local pilocarpine (2%) in the third group was significantly decreased (p < 0.001), Figure 3 and Table 3. Finally, compared with levamlodipine (0.5%), pilocarpine has a more significant effect in preventing a rapid increase in intraocular pressure (p < 0.001) as shown in Figure 4.

Figure 1: Impact of levamlodipine (0.25%) on intraocular pressure of acute model of glaucoma in rabbits. Each measurement denotes the mean intraocular pressure ± SDM of six rabbits. *There is a significant difference when compared to the control (p < 0.05)

Discussion

This study shows that dihydropyridine-levamlodipine can effectively prevent the progression of an acute model of glaucoma induced by glucose...
Similarly, the preventive effect of S-amlodipine may be due to the blocking effect of Ca++ entry by activating cell membrane phosphorylation-dependent pathways [20]. Decreasing the influx of Ca ++ by levamlodipine can also increase outflow facilities by relaxing the muscles in trabecular meshwork cells [21]. This finding is consistent with the views of Erickson et al., and Schroeder et al. [22] showed a dose-related increase in the outflow facility after the administration of CCB in the dissected human eyes. The above findings state that topical application of forskolin (1%) causes a decrease in cAMP which reduces IOP in humans, monkeys, and rabbits, which indicates that the net rate of AH inflow decreases when cAMP increases [23].

![Figure 2: Impact of levamlodipine (0.5%) on intraocular pressure of acute model of glaucoma in rabbits. Each measurement denotes the mean ± SDM of six rabbits. *There is a significant difference when compared to the control (p < 0.5)].

There is a close interaction between Ca++ and adenylyl cyclase (AC) [15]. Five types of AC are regulated by Ca ++ [16], stimulated three types, and two are inhibited by it [7]. The cAMP produced, especially in the ciliary body and iris by AC activation. This cAMP can affect the Ca++ exchange, resulted in increasing Ca++ entrance into the cell through the voltage-dependent L-type channels. This will lead to IOP elevation, so levamlodipine administration can prevent glaucoma development [17], [18]. In addition, the lowering influence of levamlodipine attributed to the reduction of AH by reducing cAMP accumulation [19].

![Figure 3: Impact of pilocarpine (0.25%) on intraocular pressure of acute model of glaucoma in rabbits. Each measurement denotes the mean ± SDM of six rabbits. *There is a significant difference when compared to the control (p < 0.5)].

Table 3: The preventive effect of pilocarpine (2%) on elevated IOP of the acute model of glaucoma in rabbits (n = 6)

| IOP (Initial) | IOP after distilled water instillation |
|--------------|---------------------------------------|
|              | 30 min      | 45 min      | 60 min      | 75 min      | 90 min      | 105 min     |
|              |            |            |            |            |            |            |
| Left eye     | 18.86 ± 0.15 | 33.56 ± 0.47 | 33.03 ± 0.82 | 32.96 ± 3.25 | 33.16 ± 0.63 | 33.25 ± 0.68 |
| IOP after treatment | 0.47 | 0.82 | 0.89 | 0.63 | 0.68 | 0.5 |
| Right eye    | 18.65 ± 0.37 | 33.26 ± 0.44 | 27.33 ± 1.21 | 23.05 ± 0.64 | 19.3 ± 0.40 | 18.66 ± 0.21 |
| p value      | 0.21        | 0.28        | 0.001       | 0.001       | 0.001       | 0.001       |
| IOP: Intraocular pressure |            |            |            |            |            |            |

Decreasing episcleral venous pressure by blocking the calcium channel may directly influence the AH outflow. It leads to a predictable hypotensive effect of the tested drug [24]. Non-pigmented and pigmented ciliary epithelial cells contain gap junctions. Moreover, it is partially regulated by Ca ++ [16]. Verapamil interferes with these connections. Verapamil causes permeability of the cell epithelium that inhibiting the formation of AH [25]. Changes in cAMP content in the ciliary zone also affect IOP by enhancing outflow facilities or inhibiting the formation of AH [14].

In addition, levamlodipine may have neuroprotective effects on RGC. Levamlodipine inhibiting glutamate release [26]. The excitatory neurotransmitter glutamate has a significant pathophysiological role in RGC death in the case of glaucoma. Levamlodipine can inhibit the release of glutamate [27] and therefore have a potential role in guard against RGCs in patients with glaucoma. This recommendation is consistent with Carol et al. [28]. In addition, the vasodilation effect of levamlodipine can prevent ischemic damage to eye tissue [29]. Extracellular matrix collagen protein synthesis also could be inhibited by CCBs, suggesting a protective role of levamlodipine in glaucoma [30]. The antihypertensive effect of levamlodipine is consistent with Waled et al. [31] demonstrated the hypotensive effect of topical nimodipine that follows administration.
to the betamethasone model of glaucoma. Andrew et al. [32] proved that twice-daily administration of flunarizine reduces IOP in dogs after 2 days. Rabbit’s experimental studies reported the hypotensive effect of a single topical administration dose of flunarizine after 1 h [33]. Our findings are consistent with Ashutosh et al. [34] and Irina et al. [35] reported a preventable effect of CCBs on glucose- and adrenaline-induced acute glaucoma in rabbits. In addition, the previous studies show that levamlodipine has a good impact on decreasing IOP in rabbits’ chronic glaucoma models [15]. Based on these findings, we suggest that levamlodipine could deuce the AH production and increase outflow facility. These CCBs should be evaluated in larger animal samples to eliminate bias and withdrawal of animal blood samples to measure serum levels of the tested drug. Finally, we recommend the future evaluation of the neuroprotection effect of levamlodipine in the chronic model of glaucoma.

Conclusion

Levamlodipine is a promising therapeutic agent for patients vulnerable to acute glaucoma.

Authors’ Contributions

Author Waleed K. Abdulsahib performed; conceptualization, data curation, manuscript preparation, investigation, methodology, project administration, writing – original draft, and writing – review and editing. The author has read and agreed to the published version of the manuscript.

References

1. Twa MD. Intraocular pressure and glaucoma. In: Optometry and Vision Science. Vol. 95. United States: American Academy of Optometry; 2018. p. 83-5. https://doi.org/10.1097/ opx.0000000000001183
2. Abdulsahib WK, Al-Zubaidy A, Sahib HB, Kathem SH. Tolerable ocular hypotensive effect of topically applied sildenafil in ocular normotensive and betamethasone-induced hypertensive rabbits. Int J Pharm Sci Rev Res. 2015;35(1):96-102.
3. Mead B, Tomarev S. Evaluating retinal ganglion cell loss and dysfunction. Exp Eye Res. 2016;151:96-106. https://doi.org/10.1016/j.exer.2016.08.006
PMId:27523467
4. Wolvaardt E, Stevens S. Measuring intraocular pressure. Community Eye Health. 2019;32(107):56-7.

PMId:32123477
5. Cho H, Kee C. Population-based glaucoma prevalence studies in Asians. Surv Ophthalmol. 2014;59(4):434-47.
PMId:24837853
6. Striesnig J, J Ortner N, Pinggera A. Pharmacology of L-type calcium channels: novel drugs for old targets? Curr Mol Pharmacol. 2015;8(2):110-22. https://doi.org/10.2174/1874467208666150507105845
PMId:25966690
7. Hofmann F, Flockerzi V, Kahl S, Wegener JW. L-type CaV1.2 calcium channels: from in vitro findings to in vivo function. Physiol Rev. 2014;94(1):303-26. https://doi.org/10.1152/physrev.00016.2013
PMId:24382889
8. Bulsara KG, Cassagnol M. Amlodipine. Treasure Island, FL: StatPearls Publishing; 2020.
9. Plummer CE, Bras D, Grozdanic S, Komáromy AM, McLellan G, Miller P, et al. Prophylactic anti-glaucoma therapy in dogs with primary glaucoma: A practitioner survey of current medical protocols. Vet Ophthalmol. 2021;24(Suppl 1):96-108. https://doi.org/10.1111/vop.12820
PMId:32920915
10. Lu Y, Yin J, Wu X, Fan Y, Liu F. Comparative effects of 2.5mg levamlodipine and 5mg amlodipine on vascular endothelial function and atherosclerosis. Pak J Pharm Sci. 2019;32(5(Special)):2433-6.
PMId:31894030
11. Abdulsahib WK, Abood SJ. Effect of digoxin ophthalmic solutions on the intraocular pressure in rabbits. Drug Invent Today. 2020;14(1):5-9.
12. Hussein MQ, Kadim HM, Abdulsahib WK. Effect of telmisartan on intra-ocular pressure in induced open angle glaucoma in rabbits. Int J Sci Res. 2015;6:2319-7064.
13. Arai M, Mayama C. Use of calcium channel blockers for glaucoma. Prog Retin Eye Res. 2011;30(1):54-71. https://doi.org/10.1016/j.preteyeres.2010.09.002
PMId:20933604
14. Abdulsahib WK. Future therapeutic strategies in the glaucoma management. J Adv Med Pharm Sci. 2020;22(7):40-9.
15. Abdulsahib WK and AJS. The effect of calcium channel blocker in the betamethasone-induced glaucoma model in rabbits. J Adv Pharm Educ Res. 2021;11(1):135-40. https://doi.org/10.51847/2d3w8vfsvt
16. Fadheel OQ, AL-Jawad FH, Abdulsahib WK, Ghazi HF. Effect Felodipine against Pilocarpine induced seizures in rats. Int J Pharm Sci Res. 2018;52(1):54-60.
17. Baumann L, Gerstner A, Zong X, Biel M, Wahl-Schott C. Functional characterization of the L-type Ca2+ channel Cav1.4 from mouse retina. Invest Ophthalmol Vis Sci. 2004;45(2):708-13. https://doi.org/10.1167/iovs.03-0937
PMId:14749418
18. Shim MS, Kim KY, Ju WK. Role of cyclic AMP in the eye with glaucoma. BMB Rep. 2017;50(2):80. https://doi.org/10.5391/BMBR.2017.50.2.080
PMId:27916026
19. Sena DF, Lindskog K. Neuroprotection for treatment of glaucoma in adults. Cochrane database Syst Rev. 2017;1(1):CD006539.
PMId:28122126
20. Reves JG, Kissin I, Lell WA, Tosone S. Calcium entry blockers: Uses and implications for anesthesiologists. J Am Soc Anesthesiol. 1982;57(6):504-18. https://doi.org/10.1097/00005422-198212000-00013
PMId:6756213
21. Li X, Wang C, Li T, Liu Y, Liu S, Tao Y, et al. Bioequivalence of levamlodipine besylate tablets in healthy Chinese subjects:
A single-dose and two-period crossover randomized study. BMC Pharmacol Toxicol. 2020;21(1):80. https://doi.org/10.1186/s40360-020-00459-6
PMid:33213527

22. Erickson KA, Schroeder A, Netland PA. Verapamil increases outflow facility in the human eye. Exp Eye Res. 1995;61(5):565-7. https://doi.org/10.1016/s0014-4835(05)80050-8
PMid:8654499

23. Majeed M, Nagabhushanam K, Natarajan S, Vaidyanathan P, Karri SK, Jose JA. Efficacy and safety of 1% forskolin eye drops in open angle glaucoma an open label study. Saudi J Ophthalmol. 2015;29(3):197-200. https://doi.org/10.1016/j.sjopt.2015.02.003
PMid:26155078

24. Göbel K, Rüfer F, Erb C. Physiology of aqueous humor formation, diurnal fluctuation of intraocular pressure and its significance for glaucoma. Klin Monbl Augenheilkd. 2011;228(2):104-8.
PMid:21328169

25. Peracchia C. Calmodulin-mediated regulation of gap junction channels. Int J Mol Sci. 2020;21(2):485.
PMid:31940951

26. Plumbly W, Brandon N, Deeb TZ, Hall J, Harwood AJ. L-type voltage-gated calcium channel regulation of in vitro human cortical neuronal networks. Sci Rep. 2019;9(1):13810. https://doi.org/10.1038/s41598-019-49226-9
PMid:31554851

27. Yang Y, Yu L, Wu Z, Yu J, Zhang J, Chen Q, et al. Synergic effects of levamlodipine and bisoprolol on blood pressure reduction and organ protection in spontaneously hypertensive rats. CNS Neurosci Ther. 2012;18(6):471-4. https://doi.org/10.1111/j.1755-5949.2012.00323.x
PMid:22672299

28. Toris CB, Gleason ML, Camras CB, Yablonski ME. Effects of brimonidine on aqueous humor dynamics in human eyes. Arch Ophthalmol. 1995;113(12):1514-7. https://doi.org/10.1001/archopht.1995.01100120044006
PMid:7487618

29. Yamada H, Chen Y-N, Aihara M, Araie M. Neuroprotective effect of calcium channel blocker against retinal eect of levamlodipine of calcium channel blocker against retinal eect of levamlodipine
PMid:10.1016/j.brainres.2005.11.072
PMid:11163049

30. Quill B, Irnaten M, Docherty NG, McElnea EM, Wallace DM, Clark AF, et al. Calcium channel blockade reduces mechanical strain-induced extracellular matrix gene response in lamina cribrosa cells. Br J Ophthalmol. 2015;99(7):1009-14. https://doi.org/10.1136/bjophthalmol-2014-306093
PMid:25795916

31. Abdulsahib WK, Fadhil OQ, Tizkam HH. Effect of topically applied nimodipine on the intraocular pressure on ocular normotensive and betamethasone-induced hypertensive eyes in rabbits. Int J Res Pharm Sci. 2019;10(4):2727-32. https://doi.org/10.26452/ijrps.v10i4.1537
PMid:11163049

32. Greller AL, Hoffman AR, Liu C, Ying G, Vudathala DK, Acland GM, et al. Effects of the topically applied calcium-channel blocker flunarizine on intraocular pressure in clinically normal dogs. Am J Vet Res. 2008;69(2):273-8. https://doi.org/10.2460/ajvr.69.2.273
PMid:18241026

33. Maltese A, Bucolo C. Pharmacokinetic profile of topical flunarizine in rabbit eye and plasma. J Ocul Pharmacol Ther. 2003;19(2):171-9. https://doi.org/10.1089/108076803321637708
PMid:12804062

34. Ganekal S, Dorairaj S, Jhanji V, Kudlu K. Effect of topical calcium channel blockers on intraocular pressure in steroid-induced glaucoma. J Curr Glaucoma Pract. 2014;8(1):15-9. https://doi.org/10.5005/jp-journals-10008-1155
PMid:26997802

35. Mikheytevskaya IN, Kashintseva LT, Krizhanovsky GN, Kopp OP, Lipovetskaya EM. The influence of the calcium channel blocker verapamil on experimental glaucoma. Int Ophthalmol. 2004;25(2):75-9. https://doi.org/10.1023/b:inte.0000031737.08988.b0
PMid:15290885