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

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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 intraocular 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 intraocular 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]. Amlodipine, 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. Amlodipine is a racemic mixture of (S)- and (R)-amlodipine, but only the first has therapeutic efficacy [8]. (S)Amlodipine, acknowledged as levamlodipine, 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.

Table 1: The preventive effect of levamlodipine (0.25%) 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 (control)						
19.13 ± 0.43	32.98 ± 0.43	33.3 ± 0.54	32.88 ± 0.49	32.9 ± 0.61	33.5 ± 0.54	32.83 ± 0.19
IOP after treatment						
Right eye (levamlodipine 0.25%)						
19.03 ± 0.70	32.25 ± 0.93	30.55 ± 0.68	27.58 ± 0.80	26.33 ± 0.51	22.88 ± 0.61	20.3 ± 0.4
p-value						
0.77	0.12	0.001	0.001	0.001	0.001	0.001

IOP: Intraocular pressure

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.

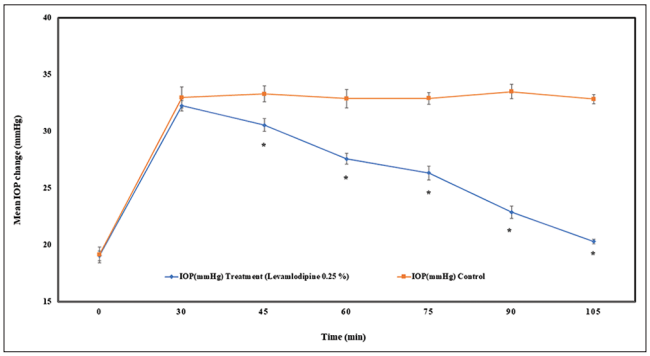

*Figure 1: Impact of levamlodipine (0.25%) on intraocular pressure of acute model of glaucoma in rabbits. Each measurement denotes the mean intraocular pressure ± SDM of six rabbits. *There is a significant difference when compared to the control (p < 0.5)*

Table 2: The preventive effect of levamlodipine (0.5%) 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 (control)						
19.01 ± 0.52	32.83 ± 0.68	33.58 ± 0.91	32.83 ± 0.51	32.66 ± 0.81	33.16 ± 0.75	33.16 ± 0.60
IOP after treatment						
Right eye (levamlodipine 0.5%)						
19.23 ± 0.43	32.5 ± 0.64	28.46 ± 0.42	25.38 ± 0.53	21.71 ± 0.64	20.33 ± 0.26	18.63 ± 0.29
p-value						
0.45	0.40	0.001	0.001	0.0001	0.001	0.001

IOP: Intraocular pressure

Discussion

This study shows that dihydropyridine-levamlodipine can effectively prevent the progression of an acute model of glaucoma induced by glucose

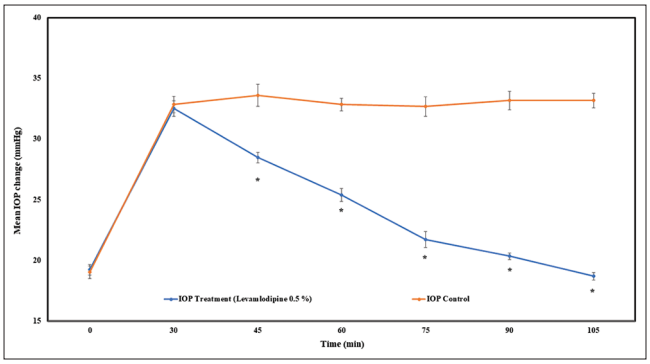

*Figure 2: Impact of levamlodipine (0.5%) on intraocular pressure of acute model of glaucoma in rabbits. Each measurement denotes the mean intraocular pressure ± SDM of six rabbits. *There is a significant difference when compared to the control (p < 0.5)*

and suggest the paramount role of levamlodipine in the regulation of IOP. Irina *et al.* will strengthen this point, demonstrated the benefits of CCB in preventing the progression of adrenaline-induced acute glaucoma, especially in the early stages of the disease [13], [14].

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 (control)						
18.86 ± 0.15	33.56 ± 0.47	33.03 ± 0.82	33 ± 0.89	32.96 ± 0.63	33.25 ± 0.68	33.16 ± 0.5
IOP after treatment						
Right eye (pilocarpine 2%)						
18.65 ± 0.37	33.26 ± 0.44	27.33 ± 1.21	23.05 ± 0.64	19.3 ± 0.40	18.66 ± 0.21	18.35 ± 0.398
p value						
0.21	0.28	0.001	0.001	0.001	0.001	0.001

IOP: Intraocular pressure

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].

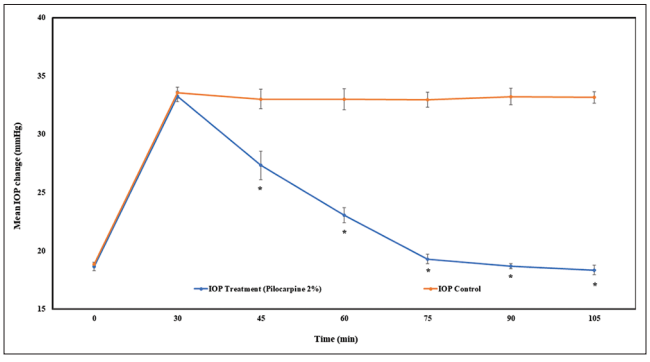

*Figure 3: Impact of pilocarpine (0.25%) on intraocular pressure of acute model of glaucoma in rabbits. Each measurement denotes the mean intraocular pressure ± SDM of six rabbits. *There is a significant difference when compared to the control (p < 0.5)*

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].

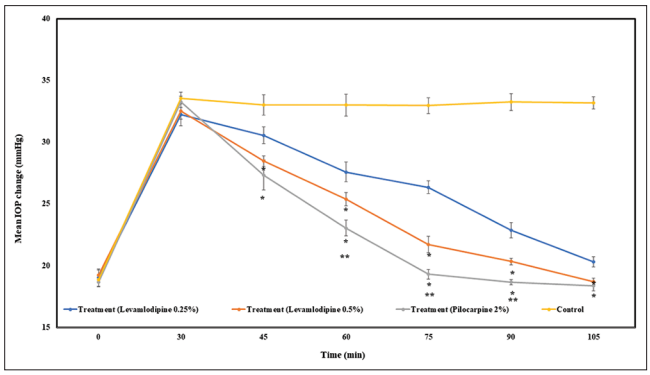

*Figure 4: Impact of levamlodipine (0.25%), levamlodipine (0.5%), and pilocarpine (0.25%) on intraocular pressure of acute model of glaucoma in rabbits. Each measurement denotes the mean intraocular pressure ± SDM of six rabbits. *There is a significant difference when compared to the control (p < 0.5). **Significant different when compare to levamlodipine (0.5%) (p < 0.5)*

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 Waleed *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.

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