Ocular Hypotensive Effects of Sacubitril and Valsartan Eye Drops on Ocular Normotensive and Betamethasone-Induced Ocular Hypertensive Rabbits

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Abstract

Background: Glaucoma is a neurodegenerative disorder affecting the eye and is accompanied by elevated intraocular pressure (IOP). Patients may gradually lose their visual field if neglected and even go entirely blind. treatment of glaucoma may in involve surgical, laser therapy, and pharmacological treatment, regarding last, adrenergic agonists, carbonic anhydrase inhibitors, beta-blockers, and other common topical ocular therapies for POAG lower IOP by inhibiting the frequency of AH production or by accelerating AH draining through an unorthodox (uveoscleral) outflow channel (e.g. prostaglandin analogs).

Aim of the Study: This present study aimed to explore the efficacy of topically applied sacubatril/valsartan 0.5% eye drop on ocular normotensive and betamethasone-induced ocular hypertensive rabbits.

Material and Method: A total of 48 apparently healthy rabbits were included in the present study. They were examined regularly and were kept in a standard laboratory environment. They were provided with fresh water and a diet. The animals were also kept under a light cycle of 12 hours. Animals are equally divided into two major groups the Normotensive group (24 rabbits) and the betamethasone-induced ocular hypertensive group (24 rabbits) each of them is subdivided into four groups each one containing six rabbits.1- vehicle group 20 latanoprost group 3-valsartan group 4- sacubatril/valsartan group The intraocular pressure was measured for both eyes with the aid of a Schiötz tonometer according to the instructions of the manufacturer. The other examined parameters were pupil diameter, light pupillary reflex, corneal sensation reflex, and conjunctival redness. Histopathological examination also will include in this study.

Result: this study showed that valsartan is effective in reducing the IOP of both normotensive and betamethasone-induced ocular hypertensive eye meanwhile still not comparable to the latanoprost efficacy. The study also revealed that sacubatril/valsartan causes a significant IOP lowering effect in both groups compared to the vehicle group and also comparable to latanoprost efficacy. Both sacubatril / valsartan and valsartan alone showed more safety profile than latanoprost. Histopathological examination revealed that the level of the Amyloid beta in the retina has no change and so no degenerative changes showed in the macula after 21 days of treatment with sacubatril/valsartan.

Conclusion: Sacubitril/valsartan eye drops significantly reduced the IOP with minimum side effects and a clinical trial is required to evaluate its efficacy in the treatment of glaucoma.

Keywords: Ocular Hypotensive, Sacubitril, Valsartan Eye Drops, Ocular Normotensive, Betamethasone-Induced, Ocular Hypertensive Rabbits.

DOI: 10.47750/pnr.2022.13.S03.129

INTRODUCTION

Glaucoma is a type of optic neuropathy that occurs when the loss of retinal ganglion cells and their axons leads to the development of a condition known as optic neuropathy. This condition affects the (ONH) optic nerve head and the (RNFL) retinal nerve fiber layer leading to loss of the visual field. (Hood DC, 2017). Elevated intraocular pressure (IOP) is the sole potential risk for glaucoma that can be addressed, despite the fact that the disease's exact pathophysiology is unknown. (Medical treatment of glaucoma, 2019).

IOP-lowering drugs can be divided into two groups: those that inhibit the production of aqueous humor and those that promote its expulsion. Aqueous humor production is

suppressed by substances such as -adrenergic antagonists, Beta 2-adrenergic agonists, and carbonic anhydrase inhibitors. The trabecular meshwork, Schlemm's canal, and distal typical outflow channels all benefit from the use of cholinergic drugs, and more recently, nitric oxide-donating moieties and rho kinase inhibitors. Uveoscleral pathway aqueous outflow is improved by prostaglandin analogs. (Xinghuai Sun, Y, 2015).

Each of these drugs may have local and systemic side effects. Adherence to medication protocol can be confusing and expensive; if side effects occur, the patient must be willing either to tolerate them or to communicate with the treating physician to improve the drug regimen. Poor compliance with medications and follow-up visits are major reasons for vision loss in glaucoma patients (Leffler et al, 2013).

Accordingly, till now much research has been done to seek new drugs with new mechanisms of action and a wide margin of safety.

The role of arterial natriuretic peptide (ANP) on intraocular pressure (IOP) has been studied by many researchers. An experimental animal study suggested that the level of ANP is elevated in the intraocular chamber when the IOP increases (Fernandez-Durango et al, 1999).

Other studies demonstrated that intravenous, intracameral, or intravitreal ANP injection caused a decrease in IOP (Tsukahara *et al*, 1988; Sugrue & Viader, 1986; Samuelsson-Almén, 1991; Nathanson, 1987). Furthermore, natriuretic peptide receptors have been identified on the ciliary epithelium and corneal endothelium (Stone & Glembotski, 1986; Walkenbach *et al*, 1995).

On July 7, 2015, the FDA approved sacubitril and valsartan for the treatment of patients with chronic heart failure. This drug is the first angiotensin receptor neprilysin inhibitor to receive this approval. (East Hanover, NJ: 2015). The primary cause of heart failure is a maladaptive response that occurs when the renin-aldosterone system is activated. This activation leads to various conditions such as high blood pressure and cardiac remodeling. (Osmanska J and Jhund PS 2019).

This research was designed to evaluate the efficacy of topically applied sacubitril / valsartan eye drop on ocular normotensive and betamethasone-induced ocular hypertensive in rabbits and compare the results in IOP reduction obtained with topically applied valsartan and latanoprost eye drop.

MATERIAL AND METHODS

Drugs, Chemical, and instrument

The chemical and drugs that are used in this study are highly purified and include latanoprost eye drop (Pfizer, USA), Sacubitril/ valsartan powder (Shaanxi Jeujon bio- tech LTD /China). Valsartan (Samarra Drug Industry, Iraq). Betamethasone injection (Clint pharmaceuticals m, united States), Benzalkonium chloride (Samarra Drug Industry, Iraq), Sodium phosphate monophasic (Sigma Aldrich, Germany), Sodium phosphate diphasic anhydrous (Sigma Aldrich, Germany), and Congo red stain (Sigma Aldrich, Germany).

Experimental animals

In this study, Forty-eight healthy adult rabbits (Albino type) are involved with body weight (1.5-2 kg). The animals were examined regularly and were kept in a standard laboratory environment. They were provided with fresh water and a diet. The animals were also kept under a light cycle of 12 hours light and 12 hours dark. The experiments were approved by the Animal Ethical Committee, College of Medicine/ Al-Nahrain University, Baghdad, Iraq.

Preparation of sacubitril / valsartan 0.5% eye drops

The ophthalmic solution sacubitril/valsartan 0. 5% was prepared by dissolving 5 mg of sacubitril/valsartan powder in 7 ml of pH 8 phosphate buffer solution (a buffer prepared from NaH2PO4 and Na2HPO4) and mixed well, then benzalkonium chloride solution (to the concentration of 0.01% of the final volume). The final volume was completed to 10 ml with phosphate buffer solution to get 0. 5% (w/v) sacubitril/valsartan solution. Preparation was done in aseptic condition and the final solution was filled in a sterile container. (Upendra. N, 2019).

Preparation of valsartan 0.5% eye drop

Valsartan eye drop was prepared using a procedure prescribed previously by Saydam *et al*, 2007. First, 5 mg of the valsartan powder was dissolved in 7 ml of pH 8 phosphate buffer solution (a buffer prepared from NaH2PO4 and Na2HPO4) and mixed well, then benzalkonium chloride solution (to a concentration of 0.01% of the final volume). The final volume was completed to 10 ml with phosphate buffer solution to get 0. 5% (w/v) valsartan solution. (Upendra. N, 2019).

Measurement parameters

The intraocular pressure was measured for both eyes with the aid of a Schiötz tonometer (Vaishno Medisales, Nepal) according to the instructions of the manufacturer. The other examined parameters were pupil diameter, light pupillary reflex, corneal sensation reflex, and conjunctival redness. These parameters were recorded before starting the experiment and then daily before and after drug application. (Jones MD, et al, 2006).

Induction model for Ocular Hypertension in Rabbits

Ocular hypertension induced according to Melana12 and coworkers who found that this model of induction is mimicked human chronic open-angle glaucoma. After proper

anesthetization of the eyes by local instillation of 2% lidocaine HCL, a subconjunctival injection (by using micro-fine syringes, 30 gauge × 1/2 inches) of 0.7 ml of betamethasone suspension containing betamethasone sodium phosphate (3 mg/ml)and betamethasone acetate (3 mg/ml). This formulation provides a readily accessible (sodium phosphate) and a sustained release (acetate) fraction of betamethasone. The value observed at zero time (first betamethasone injection) was considered the starting pressure. The animals received weekly (for 4 weeks) subconjunctival injections of betamethasone in both eyes over a period of 21 days. The instillation of the tested drugs was started at the 24th day of corticosteroid treatment (3 days after the fourth subconjunctival injection), a time at which the betamethasone-induced ocular hypertension turned out to be stable, and was prolonged up to 25 days. (Malena, et al, 1998).

Experimental design in normotensive rabbits

In this part four groups were involved, each group consisting of six rabbits (24 rabbits). All animals had been examined for the studied parameters on the day before the application of the tested agent. On the seven next days, the experiment had been done by instillation of the tested agents into the right eye and distilled water instilled into the left eye twice daily at 10:00 a.m. and at 10:00 p.m. except for latanoprost, which was administered once daily. The tested parameters were recorded 30 minutes before instillation and 30, to 60 minutes after instillation. The parameters were also measured in the left eyes to detect the contralateral effects of the tested agent.

The studied groups were the negative control group (right eye received inactive ingredients while left eye received distilled water), Latanoprost 0.005% group, valsartan 0.5% group, and sacubitril/valsartan 0.5% group.

Experimental design in induced ocular hypertensive rabbits

This part also included four groups, each group of six rabbits (24 rabbits). All animals had been examined for the tested parameters one day before induction. On the 24th day after induction, IOP was measured again to ensure that ocular hypertension was definitely established. Then treatment began by instillation of 1 to 2 drops of the tested agents into the right eye and distilled water instilled in the left eye twice daily at 10:00 a.m. and at 10:00 p.m. for seven days except for latanoprost, which was administered once daily. The tested parameters were recorded 30 minutes before instillation and 30, to 60 minutes after instillation. The treatment period was continuing for seven days. The parameters were also measured in the left eyes to detect the contralateral effects of the tested agent.

The studied groups were the negative control group (right eye received inactive ingredients while left eye received distilled water), Latanoprost 0.005% group, valsartan 0.5% group, and sacubitril/valsartan 0.5% group.

Statistical analysis

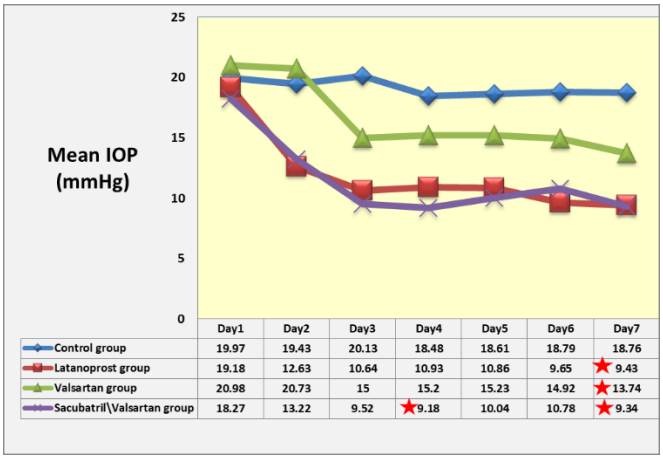
Microsoft Excel 2010 and SPSS version 26 programs were used for data analysis. Numeric variables were expressed as mean ± SD and all statistical comparisons were made by means of an independent t-test and one-way ANOVA t-test. Categorical variables were expressed as numbers and analyzed using the chi-square test. All data is represented by tables and figures. P <0.05 was considered statistically significant. (Daniel, A. and Yu, X. 2008).

RESULTS

Effect of tested drugs on mean IOP in normotensive groups

No significant difference in mean IOP was found among the control group during all days of treatment, P=0.93. Both of Latanoprost treated group and the valsartan treated group shows a significantly higher decline in mean IOP during day 7 of treatment, P<0.001. While Sacubatril\Valsartan treated group shows a significantly higher decline in mean IOP during day 4 of treatment, P<0.001. Table & figures.

Table: Comparison between normotensive groups according to mean IOP and days of treatment for each group



	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Control group	19.97	19.43	20.13	18.48	18.61	18.79	18.76
Latanoprost group	19.18	12.63	10.64	10.93	10.86	9.65	★9.43
Valsartan group	20.98	20.73	15	15.2	15.23	14.92	★13.74
Sacubatril\Valsartan group	18.27	13.22	9.52	★9.18	10.04	10.78	★9.34

Figure: Comparison between normotensive groups according to mean IOP and days of treatment, ★=treated group showed a significant decline in mean IOP during that day

Effect of tested drugs on mean IOP in betamethasone-induced ocular hypertensive

The results of the induced-ocular hypertensive groups showed that topical eye application of sacubitril/valsartan 0. 5% eye drop significantly changed (reduced) the elevated IOP during the course of the experiment (P ≤ 0.01). Latanoprost treated group shows a significantly higher decline in mean IOP from (45.63 ± 7.0) before treatment to (11.42 ± 1.54) on day 7 of treatment (P<0.001). Similarly,

Valsartan treated group shows a significant reduction in mean IOP during day 7 of treatment (P<0.001, table). On the other hand, no significant difference in the mean IOP was found among the Vehicle group during all days of treatment (P value 0.88).

Table: Comparison between betamethasone-induced hypertensive groups according to mean IOP and days of treatment for each group

Days of treatment	Betamethasone induced hypertensive groups							
	Vehicle group		Latanoprost treated group		Valsartan group		Sacubatril \Valsartan group	
	Mean IOP	SD	Mean IOP	SD	Mean IOP	SD	Mean IOP	SD
Day 1	41.68	7.97	45.63	7	39.18	4.6	39.6	4.55
Day 2	45.85	21.04	32.62	4.40	36.35	6.3	34.7	5.31
Day 3	38.4	9.85	28.25	2.97	31.57	2.56	22.52	4.11
Day 4	36.65	6.31	24.73	2.97	30.77	3.23	19.88	4.13
Day 5	39.85	11.09	19.57	3.33	25.35	4.47	13.7	4.4
Day 6	40.02	7.18	15.75	1.80	18.98	2.61	12.52	2.18
Day 7	39.9	9.43	11.42	1.54	18.82	4.29	10.1	1.64
P	0.88		<0.001		<0.001		<0.001	

One-way ANOVA test

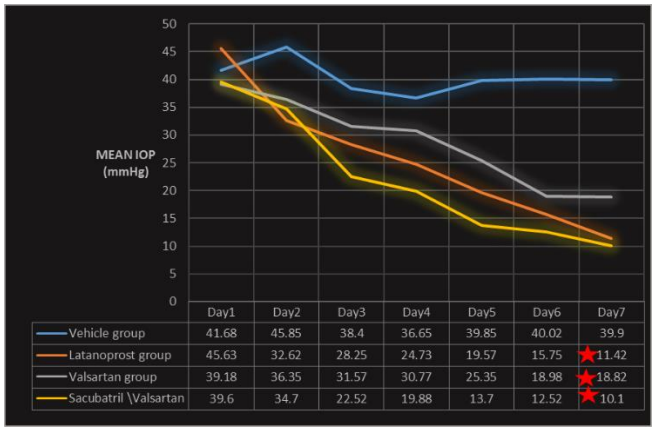


Figure: Comparison between induced groups according to mean IOP and days of treatment, ★=treated group shows a significant decline in mean IOP during that day

All studied groups during all 7 days of treatment have a positive corneal reflex. The pupillary diameter was significantly higher in all studied groups and there when compared to the size diameter before and after treatment with a p-value < 0.001. Regarding the light reflex, all normotensive groups during all 7 days of treatment have the constant pupillary light reflex. Furthermore, no conjunctival redness was found among all animals with normal IOP treated by tested drugs during the course of therapy except those rabbits treated with Latanoprost eye drops, where it was significantly associated with conjunctival redness during days 3, 4, 5, and 7 of treatment with significant p-value < 0.05 when comparing before starting treatment.

Discussion

Many studies have demonstrated the role of ANP in the regulation of intravascular fluid volume and blood pressure. These researches demonstrated that elevation of endogenous ANP within the physiological range by neprilysin inhibition was associated with a significant reduction in IOP in the normal eye. Additional research showed that plasma ANP may have an important role in the normal regulation of IOP (Thomas *et al* 1987; Wolfensberger *et al*, 1994; Janusz *et al*, 2018).

In the current research, sacubitril/valsartan is the tested drug. The drug was formulated as eye drops to examine its topical effect in reduction of intraocular pressure when used as a single dose daily. Following the second administration of the sacubitril/valsartan, a significant decline in the ocular pressure was observed compared to the baseline reading and this reduction continued till the end of the experiment both in normotensive and in betamethasone-induced ocular hypertension. The positive thing in this study is the ability of sacubitril/valsartan eye drops to decrease the IOP comparably (i.e., with no significant differences) to the reference drug, latanoprost eye drops.

In fact, the idea behind the formulation of neprilysin inhibitor (sacubatril) with angiotensin receptor blocker (valsartan) was because neprilysin enzyme has the ability to degrade many vasoactive peptides including angiotensin (Douglas & Jasjit, 2019), Henry *et al*., 2021) consequently, the effect of angiotensin II on normal ocular pressure will be antagonized by valsartan adding synergistic effect to ANP.

In betamethasone-induced ocular hypertensive groups, the reduction in the IOP was significant on the second day with a 24% reduction and continued to reduce toward the normal level during the period of the study. Maximal IOP reduction was in the 7th day with a huge reduction of 75% to low IOP levels. This result is agreed with Ana Marie, *et al.* (2021) who found that the break reduction in the IOP was after 6 hours. The data obtained from this experiment showed a significant reduction in IOP exerted by sacubitril/valsartan treated rabbits which were superior to the effect exerted by valsartan alone and comparable (i.e., with no significant differences) to

that of the latanoprost treated group at the end of the experiment. The explanation for this reduction in the ocular pressure might be a clue to an important role of natriuretic peptides in the regulation of IOP.

The natriuretic peptide system has been mostly documented for mediating circulatory homeostasis by promoting natriuresis, diuresis, and vasorelaxation via intracellular generation of cyclic guanosine monophosphate (cGMP) (Rollin R،2004) (Pandey,2014) CNP preferentially binds to NPR-B, whereas ANP and BNP preferentially bind to NPR-A (Suga et al., 1992). Results from molecular studies demonstrated the expression and function of the natriuretic peptide system in several ocular systems, including in human trabecular meshwork, ciliary epithelial, and ciliary muscle cells (Pang *et al*., 1996; Ortego and Coca-Prados, 1999;). CNP is the most potent natriuretic peptide at lowering IOP in rabbits. Intravitreal injection of CNP to rabbits increased cGMP concentrations in the aqueous humor, resulting in a lowering of IOP (Takashima et al., 1998). The group of rabbits that received sacubitril/valsartan eye drops was also monitored for changes in pupillary diameter, corneal light reflexes, and conjunctival hyperemia. Fortunately, no changes have been observed in the above clinical features in rabbits managed by sacubitril/ valsartan eye drops neither in normotensive experiment nor in betamethasone-induced ocular hypertension experiment. Short-term therapy probably represents the main limitation of this study so there is a need for further prolonged studies in an attempt to elicit the incidence of side effects of this new topically-applied agent.

Conclusion

1. Sacubatril /valsartan is effective in the lowering of intraocular pressure in both normotensive and betamethasone-induced ocular hypertensive rabbits.
2. Sacubatril/valsartan showed a very close effect compared to latanoprost.
3. Sacubatril/valsartan in 0.5% showed a good safety profile regarding using parameters and the clinical trial period.

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