Oculo Hypotensive Effect of Nitric Oxide Donor in Glaucomatous Male Rabbits

Saja Majeed Shareef¹, Mustafa Ghazi Al-Abbassi²*, Dalia Abd Al Kader Shakur³

Department of Pharmacology and Toxicology, College of Pharmacy, Al-Mustansiriyah University/Iraq.

*Corresponding Authors: Mustafa Ghazi Al-Abbassi

Abstract

Objective: The aim of present study was to evaluate the effect of topical nitric oxide donor in lowering intraocular pressure of glaucomatous male rabbits. Method: Thirty two albino rabbits weighing (2.5-3.5 kg) were used in the study and allocated into four groups include: normal group, glaucoma induced group, sodium nitroprusside (SNP) group and timolol group. Glaucoma induction was done by single intravitreal injection of 50 units of freshly prepared alpha-chymotrypsin in 0.1 ml of sterile saline. The treatment was applied twice daily: at 9:00 a.m. and at 1:00 p.m. during 12 day. The intraocular pressure was recorded two times a day: at 9:00 a.m. and at 12:00 p.m. The parameters that measured include; the intraocular pressure, pupil diameter, cornea lass sensation, light reflex, conjunctival redness and cyclic guanosine monophosphate (cGMP) level in aqueous humor. Results: The results revealed a high significant decrease in intraocular pressure and a high significant increase in cyclic guanosine monophosphate level, also no variation in pupil diameter, positive pupillary response to light, no corneal sensation, and no conjunctival redness were observed. Conclusion: The findings suggest that sodium nitroprusside has oculohypotensive action and it may prove to be an additional effective for the management of glaucoma.

Keywords: Glaucoma, Nitric oxide donor, intraocular pressure.

Introduction

Glaucoma is a multi factorial eye disorder with a several elements responsible to its progression [1]. It is advanced optic neuropathy defined by the damage of ganglion cells with axons, leading to distinctive appearance of the optic disc [2].

An expected 60.5million individuals are affected by primary open angle glaucoma (POAG), and this will rise to 111.8million in 2040[3].

Certain risk factors relevant to glaucoma which include: people above age 40, family history with glaucoma, African heritage, raised eye pressure, cornes which are thin in the center, farsighted /near sighted [4].

The exact path physiology of glaucoma wasn't identified completely; a serious risk factor is the elevation of the intraocular pressure above normal values [5].

The elevation of intraocular pressure (IOP) is a primary factor and a key role in retinal ganglionic cells (RGCs) apoptosis and it is likewise right that reduction of high IOP often aids in reducing the development of deteriorating variations in glaucoma [6]. An ideal balance between the production of aqueous humor (AH) and its outflow keeps IOP in a normal eye.

Usually, the rate of aqueous humor production is same as to the rate of out flow, and it is reserved within a normal range of 10 to 21 mmHg [7].Elevation of IOP can be attributed to either an increase in aqueous humor formation or drop in outflow.

Two main pathways for the out flow of aqueous humor are: trabecular meshwork (TM) out flow (conventional pathway), and uveoscleral out flow (UN conventional pathway) [8].
The trabecular outflow resistance restricted in the inner wall part which consists of the juxtacanalicular tissue (JCT) and the inner barrier endothelium of schlemm’ canal. The outflow resistance in this part is lowered through the relaxation of contractile my fibroblast -like cells in TM and the adjacent scleral, also contraction of the ciliary muscle [9].

Nitric oxide (NO) is a two atomic gas with so short half-life of limited seconds. It acts a key part in numerous organs and participates in smooth muscle vasodilation, cardiovascular, respiratory, gastrointestinal urogenital and even immunes system and has role in angiogenesis [10].

Nitric oxide was formed by group of enzymes, the nitric oxide synthases NOS, by conversion of L- Arginine to NO, L-citrulline [11].

Three main forms of NOS in tissue endothelial e NOS, neuronal n NOS and inducible INOS. The nitric oxide donor release NO when applied to biological systems, where they either similar to endogenous NO linked response or reserve for an endogenous NO absence.

Soluble guanylyl cyclase (s GC) is the principal enzyme in mediating the biological actions of nitric oxide. It is disodium pentacyanonitrosylferrate (2) dehydreate, a hypotensive agent whose structural formula is Na2 [Fe (CN) 5NO] 5 2H2O. The concentrated solution of SNP is stable for more than two years at room temperature. Sodium nitroprusside in dry form is a redddish-brown powder [12].

Its action in the body as a prodrug that binds with sulphhydryl groups on erythrocytes, albumin, and additional proteins and release NO [13]. The goal of the current study was to evaluate the effect of topical sodium nitroprusside in lowering intraocular pressure of glaucomatous rabbits.

Materials and Methods

Chronic Ocular Hypertension Model

Glaucoma was induced in the right eyes of 24 rabbits after sedated through injection of intramuscular ketamine hydrochloride (ROTEX MEDICA GmbH -Germany) 35 mg/kg and xylazine HCL (Kepro. B.V – Holland) 4mg/kg the chronic ocular hypertension model was done by intravitreal insertion of 50 unit of freshly alpha-chymotrypsin (Leurquin-France) in 0.1 ml of saline [14], by needle of gauge (31G, 0.23×9.5 mm) in to the posterior chamber of the right eye only.

The IOP was recorded every week until reach above 30 mmHg. Rabbits with IOP more than 30 mmHg were included in this study, while the rabbits with IOP less than 30 mmHg were excluded.

Experimental Animals

Thirty two albino male rabbits evaluating (2.5-3.5 kg) were utilized in this study, and treated agreeing to the ethics committee of the College of Pharmacy/ Mustansiriyah University.

Rabbits were saved in the animal house of the Iraqi national center for drug control and research. Rabbits were fed commercial pellets and water.

Selected animals were examined before starting study and identified to be normal on ophthalmic and overall examinations. Animals have been divided into four groups and eight rabbits in each group.

Normal group includes: normal rabbits received distilled water (D.W) in right eye twice daily.

Glaucoma group includes: rabbits which were received distilled water (D.W) in right eye only twice daily after induction of glaucoma.

Sodium nitroprusside (SNP) group includes: rabbits which were received sodium nitroprusside (SNP 0.08% w/v) eye drops in right eye only, twice daily after glaucoma induction.

Timolol group includes: rabbits received timolol (0.5%w/v) eye drops in right eye, once time daily after induction of glaucoma. The treatment was given two times at 9:00 a.m. and 1:00 p.m. from day1 to day 12.

The IOP was continuously recorded two times daily, at 9:00 a.m. (t = 0) and at 12:00 p.m. (t=3 hours) to avoid faults that result from diurnal pressure differences.

Preparation of Sodium Nitroprusside (SNP) 0.08% w/v eye Drops

The tested drug used was powder of high pureness (Merck-Germany). Eye drops of sodium nitroprusside (SNP) 0.08% w/v, were prepared via dissolving 80 mg of SNP powder
in 100 ml of isotonic buffer solution, and 1% (w/v) of benzalkonium chloride were liquefied in 100 ml of isotonic buffer solution. The eye drops formed in pure condition and filled in hygienic containers.

**Intraocular Pressure Measurement**

Intraocular pressure was measured by schiotz tonometer. Calibration of the tonometer made first by putting the foot-plate of the tonometer on the curved metallic portion (the synthetic cornea) that supported with box. Correctly calibrated instrument which had scale measured zero.

Following calibration, the foot-plate was sanitized with diethyl ether, then the animals were located in horizontal situation, and the cornea was anesthetized by local anesthetic (2% lidocaine hydrochloride).

A minor weight was used to a central plunger, that cause portion of the cornea under the plunger to move in ward [15].

Conversion table was supported with this tonometry because the schiotz tonometer does not measure pressure directly, and the table used to convert scale- readings in to estimates IOP in mmHg.

Chloramphenicol eye drop was administered in the rabbit’s eye finely to each measurement, to avoid bacterial infection.

**Pupil Diameter**

Pupil gauge chart was used for measuring the pupil diameter and it was expressed in millimeter units [16].

**Pupillary Reflex to Light**

The pupillary light reflex (PLR) is the constriction of the pupil in the eye which was elicited when concentrating the light to rabbit’s eyes [17].

**Corneal Sensation**

Sensation of cornea was observed with cotton wool which was motivated from side- side. Results were shown whatever there was corneal -sensation found or absent [16].

**Conjunctival Redness**

Conjunctival redness could be noticed by checking up the conjunctiva of both rabbit’s eyes and the effects were shown as either present or not [18].

**Measurement of Cyclic Guanosine Monophosphate (cGMP) Level in Aqueous Humor**

Aqueous humor (AH) sample 200μl to 400μl was collected from the anterior chamber of right eyes of all rabbits.

Then AH samples were collected and putted in sterile eppendorfs and centrifuged at 3000 rpm for approximately 20 min, supernatant was collected and stored in refrigerator at (-40°C) for detection of cyclic guanosine monophosphate (c GMP).

Measurement of CGMP level was based on sandwich enzyme linked immune sorbent assay (ELISA) technology.

At the end of ELISA technique the optical density was read at 450 nm by a micro liter plate- reader after 15 min.

Then the concentration of CGMP was calculated in the sample by comparing the optical density of the samples to standard curve [19]. P value represented not significant if (P> 0.05) and highly significant if (**P <0.01).

**Results**

**Intraocular Pressure (IOP)**

Following distilled water (D.W) instillation to the right eyes of normal group, there was no significant difference (P>0.05) in the IOP at 9 a.m. compared with IOP at 12 p.m.

Along the twelve days of trial period when measured by schiotz tonometer. In addition, there was no significant difference in the IOP of right rabbits’ eyes of glaucoma group at 12 p.m. compared with IOP at 9 a.m.

After D.W instillation along the trial period there was a high significant decrease (P<0.01) in IOP of timolol group at 12 p.m.

Compared with IOP at 9 a.m. (three hrs. after first drops instillation) in the day1 and day2. While no significant difference (P>0.05) was observed in next ten days of study as shown in (Table 1).

There was no significant difference (P>0.05) in the IOP of timolol group when compared with IOP of normal group, excluded from that what was revealed in IOP in day 1 and day 2 of treatment as shown in (Fig. 1) and (Table1).
In addition, a high significant decrease in the IOP of timolol group was elicited when compared with IOP of glaucoma group.

The mean IOP of SNP group at 9 a.m. compared with the IOP at 12 p.m. of the same group (SNP group), and no significant difference was revealed along the study period, except in the day 1. There was no significant change ($P>0.05$) in IOP of SNP group at 9 a.m. and at 12 p.m. compared with IOP of normal group, excluded from that day 1 and day 2. There was highly significant decrease ($P<0.01$) in the IOP of SNP group at different time interval when compared with glaucoma group along the study days. These results were presented in (Table 1) and (Fig.2).

Meanwhile, the IOP of SNP group at 9 a.m. was compared with that of timolol group at 9 a.m. and the results revealed no significant difference ($P>0.05$) along the trial period, also no significant change in the IOP of SNP 0.08% treated group at 12 p.m. when compared with IOP of timolol group at 12 p.m. except for day 2.
Table 1: Mean values of intraocular pressure of study groups at 9 a.m. and at 12 p.m. along twelve day of study period

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Normal group</th>
<th>Glaucoma Group</th>
<th>SNP group at 9 a.m.</th>
<th>SNP group at 12 p.m.</th>
<th>Timolol group at 9 a.m.</th>
<th>Timolol group at 12 p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+16.30±1.6</td>
<td>+33.41±0.53</td>
<td>+35.83±1.07</td>
<td>+28.26±0.45</td>
<td>+33.31±0.9</td>
<td>+25.2±1.2</td>
</tr>
<tr>
<td>2</td>
<td>+16.41±1.4</td>
<td>+33.23±0.50</td>
<td>+29.21±0.89</td>
<td>+28.56±0.45</td>
<td>+28.02±0.88</td>
<td>+22.92±1.37</td>
</tr>
<tr>
<td>3</td>
<td>+16.13±1.2</td>
<td>+34.6±0.48</td>
<td>+22.90±0.71</td>
<td>+20.43±0.58</td>
<td>+23.41±1.1</td>
<td>+18.58±0.56</td>
</tr>
<tr>
<td>4</td>
<td>+15.57±1.2</td>
<td>+35.37±0.74</td>
<td>+19.76±0.44</td>
<td>+17.7±0.55</td>
<td>+20.68±0.86</td>
<td>+16.78±0.42</td>
</tr>
<tr>
<td>5</td>
<td>+15.57±1.2</td>
<td>+35.55±0.67</td>
<td>+19.27±0.55</td>
<td>+16.10±0.41</td>
<td>+16.98±0.71</td>
<td>+15.68±0.97</td>
</tr>
<tr>
<td>6</td>
<td>+16±1.3</td>
<td>+35.96±0.71</td>
<td>+16.90±0.78</td>
<td>+15.30±0.53</td>
<td>+17.7±0.94</td>
<td>+15.15±1.08</td>
</tr>
<tr>
<td>7</td>
<td>+15.82±1.3</td>
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<td>+18.20±0.75</td>
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<tr>
<td>8</td>
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<td>+15.86±0.58</td>
<td>+14.27±0.59</td>
<td>+15.94±0.45</td>
<td>+13.91±0.24</td>
</tr>
<tr>
<td>9</td>
<td>+15.99±1.1</td>
<td>+36.97±0.54</td>
<td>+15.85±0.74</td>
<td>+13.85±0.31</td>
<td>+15.84±0.87</td>
<td>+13.17±0.40</td>
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<tr>
<td>10</td>
<td>+16.16±1.3</td>
<td>+37.85±0.66</td>
<td>+17.11±0.63</td>
<td>+14.03±0.58</td>
<td>+15.58±0.62</td>
<td>+13.57±0.40</td>
</tr>
<tr>
<td>11</td>
<td>+16.13±1.2</td>
<td>+37.85±0.66</td>
<td>+16.81±1.01</td>
<td>+13.26±0.48</td>
<td>+16.12±0.46</td>
<td>+13.75±0.51</td>
</tr>
<tr>
<td>12</td>
<td>+16.27±1.3</td>
<td>+37.85±0.66</td>
<td>+15.91±0.25</td>
<td>+13.55±0.42</td>
<td>+15.97±0.44</td>
<td>+13.4±0.28</td>
</tr>
</tbody>
</table>

Pupil Diameter

In addition the result revealed that, SNP (0.08%) eye drops had no significant effect on the pupil diameter of right rabbits’ eyes and the mean of pupil diameter was (7.42 ± 0.36 mm) and remained same along the trial period.

Pupillary Reflex to Light

The pupils of rabbits’ eyes had noticeable reflex, in response to the intensity of light at 9 a.m. (t=0) and after three hours (t=3hrs.) of SNP 0.08% eye drops instillation.

Corneal Sensation

Rabbits’ eyes of SNP treated group had positive corneal sensation at zero time and after three hours of eye drops instillation and these results were same until the end of trial period.

Conjunctival Redness

During all the days of trial period, there was no conjunctival redness was observed in the right rabbits’ eyes of SNP group at 9 a.m. and after three hours of SNP drops instillation.

The Level of Cyclic Guanosine Monophosphate (CGMP) in Aqueous Humor

There was a high significant elevation (P<0.01) in the mean concentration of cGMP of SNP group when compared with glaucoma group (6.61 ± 0.18 nmol/L vs. 3.50 ± 0.44 nmol/L, respectively; P=0.0001). The mean concentration of cGMP was highly significant in the SNP group when compared with cGMP level of normal group (6.61± 0.18 nmol/L vs. 5.03 ± 0.24 nmol/L, respectively; P=0.003). The results presented in (Fig.4).

Fig.4: Bar chart represents the mean values of cyclic guanosine monophosphate (CGMP) concentration (NMOL/L) in the aqueous humor of different groups. The results represented as means SEM

Statistically high significant difference (**P<0.01) when: A: Compared with normal group. B: Compared with glaucoma group. C: Compared with timolol group
Discussion

The concentration (0.08% w/v) of SNP revealed a gradually and advisable decreased in the IOP which was persist without fluctuation and no signs effects were obvious on rabbits' eyes until the end of study. Previous study was exposed that, the SNP effect in reducing the IOP was peaked at 0.1% and a greater doses were detected to be less effective. [20] A high significant decrease in IOP of timolol treated group in the present study, belong to its ability in reducing the formation of aqueous humor through its action on epithelial of ciliary body and beta-1 and beta-2 receptors inhibition with duration of action higher than 8hours [21].

Additionally, SNP 0.08% revealed a reduction in IOP tended to be similar to IOP reduction that observed with timolol 0.5% treated group. In deed the IOP reduction by sodium nitroprusside SNP may be explained by its role in trabecular meshwork relaxation and perhaps an increase in the permeability of the schlemm's canal. [20, 22, 23] At that point enhancement of aqueous humor out flow through the conventional pathway was predictable as a result from cGMP activation by exogenous NO that released from SNP. It is well known that, in physiological conditions AH outflow is primarily achieved through the trabecular meshwork and Schlemm's canal that accounts (60%–90% of outflow in humans and nonhuman primates), and a minor contribution is from the uveoscleral or nonconventional pathway [24].

While under pathological condition of elevated IOP, there is increasing evidence that the conventional pathway becomes the limiting factor in AH outflow, with the uveoscleral pathway becoming a more significant contributor, this is due to increased rigidity in the TM and its extracellular matrix [25].

Trabecular meshwork cells are known to be highly contractile in nature, analogous to vascular smooth muscle cells (VSMC), in which the role of nitric oxide-CGMP signaling in endothelium dependent relaxation is well understood [26]. At the cellular level, relaxation of the TM is thought to be analogous to the well-characterized NO/ sGC/cyclic guanosine monophosphate (cGMP) signaling cascade delineated in blood vessels. Schlemm's canal cells, similar to endothelial cells lining the blood vessels, Increase endogenous no production in response to shear stress supporting a role for NO in IOP homeostasis [27]. Nitric oxide can diffuse into neighboring cells, namely the vascular smooth muscle cells in the blood vessel and TM cells in the eye, where it binds to and activates the sGC enzyme, resulting in increased production of cGMP and activation of protein kinase G (PKG).[22,27].

Activation protein kinase G in turn, leads to inhibition of Rho A and thus Rho kinase, activation of K+ channels, inhibition of L-type Ca2+ channels, and increased uptake of calcium into the sarcoplasmic reticulum. These signaling pathways, together with the direct action of PKG, result in activation of myosin light chain phosphatase, while lower intracellular Ca2+ levels result in inhibition of myosin light chain kinase. Subsequent de phosphorylation of the regulatory light chain of myosin prevents actin–myosin interaction, promoting cell relaxation [23, 27, 28-31] Thus facilitating the aqueous humor out flow pathway.

Conclusion

The present study indicates the beneficial effect of cyclic GMP elevation in aqueous humor of rabbits that received SNP 0.08% eye drops in decreasing IOP. Several trials revealed the effect of NO-cGMP signaling pathway and it was found that impaired NO signaling may contribute to IOP deregulation in POAG. In previous study mice deficient in the α1 subunit of sGC-1 exhibited an elevation in IOP as they aged from 19 to 37 weeks, in contrast to wild-type mice in which IOP was unchanged over time. Additionally sGC-1-deficient mice showed a decrease in AH outflow and exhibited both optic neuropathy and retinal vascular dysfunction [32].

Furthermore, it was observed previously that inhibition of SGC in TM and schlemm's canal cells abolished the NO-induced decreases in TM and schlemm's canal cell volume [33] suggesting a functional role for SGC in regulating conventional aqueous humor outflow and IOP.

Acknowledgments

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